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Obesity and Lipotoxicity

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Preface

Obesity and its associated diseases are a major and growing worldwide public health problem of our century. Recent studies have suggested that obesity prevalence is seriously increasing mostly due to depression- and anxiety-related eating disorders, which may deeply affect quality of life.

Although behavioral improvements are aimed at promoting lifestyle changes, multidisciplinary interventions should be based on biochemical and immunological pathways. Either only conservative methods or only surgical approaches have limited efficacy, in part due to the involvement of counter-regulatory multiple metabolic pathways in obesity. Consequently, multidimensional analysis of adipose tissue-derived signaling would provide greater benefit. In this context, the book covers many critical and complex topics that trigger obesity. We are aware that many subjects have not yet come to light about obesity. Nevertheless, this book may encourage and further the research of scientists and practitioners who are interested in obesity.

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Increase in prevalence of obesity has become a worldwide major health problem in adults, as well as among children and adolescents. Furthermore, total adiposity and truncal subcutaneous fat accumulation during adolescence are positively and independently associated with atherosclerosis at adult ages. Centrally accumulation of body fat is associated with insulin resistance, whereas distribution of body fat in a peripheral pattern is metabolically less important. Obesity is associated with a large decrease in life expectancy. The effect of extreme obesity on mortality is greater among younger than older adults. In this respect, obesity is also associated with increased risk of several cancer types. However, up to 30% of obese patients are metabolically healthy with insulin sensitivity similar to healthy normal weight individuals, lower visceral fat content, and lower intima media thickness of the carotid artery than the majority of metabolically "unhealthy" obese patients.

Abdominal obesity is the most frequently observed component of metabolic syndrome. The metabolic syndrome; clustering of abdominal obesity, dyslipidemia, hyperglycemia and hypertension, is a major public health challenge. The average prevalence of metabolic syndrome is 31%, and is associated with a two-fold increase in the risk of coronary heart disease, cerebrovascular disease, and a 1.5-fold increase in the risk of all-cause mortality.

Keywords

Metabolic syndrome • Body mass index • Metabolically healthy obese • Insulin resistance • Obesity-paradox • Prevalence of obesity

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1 Introduction

Increasing prevalence of obesity is a worldwide health concern because excess weight gain causes an increased risk for several diseases, most notably cardiovascular diseases, diabetes, and cancers (Wang et al. 2011). The global food system drivers interact with local environmental and genetic factors to create a wide variation in obesity prevalence between populations. Epidemiologically, in low-income countries, obesity mostly affects middle-aged adults, whereas in high-income countries it affects both sexes and all ages (Swinburn et al. 2011). On the other hand, increased obesity rates lead to a large health and economic burden in all countries (Rtveladze et al. 2014). According to Keaver et al. overweight and obesity are proposed to reach levels of 89% and 85% in males and females, respectively by 2030. This will result in an increase in the obesity-related prevalence of coronary heart disease (CHD) by 97%, cancers by 61% and type 2 diabetes by 21%. Thereupon, the direct healthcare costs will increase significantly. A 5% reduction in population body mass index (BMI) levels by 2030 is estimated to result in €495 million decrease in the expenditures in obesity-related direct healthcare over 20 years (Keaver et al. 2013). Additionally, after adjustment for significant maternal and sociodemographic characteristics, healthcare costs of children with obesity are 1.62 times higher than those of children with healthy weight (Hayes et al. 2016). Cost-effective strategies targeted at reducing the prevalence of obesity during the early years of life can significantly reduce both healthcare and non-healthcare costs over the lifetime (Sonntag et al. 2016). In this respect, the obesity related disease burden on health care expenses should be evaluated with the epidemiological data considering whether the obese population is metabolically healthy or not.

2 Definition and Prevalence of Obesity

Obesity is usually classified by BMI. It is calculated as body weight in kilograms divided by the height in meters squared (kg/m^2). Other methods, including waist circumference (WC) and central and peripheral fat mass, have also been used, but currently BMI is continued to be used for the classification of obesity. However, BMI does not give a precise idea about the body composition which affects the health risks of excess weight such as the proportion of body weight which consists of fat or the distribution of fat. These discrepancies will be discussed in later sections. Nevertheless, BMI is now the internationally accepted standard method used by researchers and the others dealing with human health, in spite of its alternatives. According to BMI; individuals are allocated to five different categories as 18.5–24.9 kg/m^2: normal range, 25.0–29.9 kg/m^2: overweight, 30.0–34.9 kg/m^2: class 1-obesity, 35.0–39.9 kg/m^2: class 2-obesity, equal or greater 40 kg/m^2: class 3-obesity. Morbid obesity is considered to be grade 3 obesity or grade 2 obesity plus significant obesity-related co-morbidities (Ashwell et al. 2014; Dixon et al. 2011). In the past 33 years, 1769 studies from 104 different centers indicated that the established health risks and substantial increase in prevalence of obesity has become a major worldwide health problem. The proportion of adults with a BMI of 25 kg/m^2 or greater increased between 1980 and 2013 from 28.8% (95% Uncertainty intervals (UI): 28.4–29.3) to 36.9% (36.3–37.4) in men, and from 29.8% (29.3–30.2) to 38.0% (37.5–38.5) in women (Ng et al. 2014). Moreover, population-based studies in different countries showed that obesity will continue to be a serious health-risk in future. Between 1985 and 2011, the prevalence of adult obesity in Canada increased from 6.1% to 18.3%. Furthermore, since 1985, the prevalence of obesity in classes 1, 2 and 3 increased from 5.1% to 13.1%, from 0.8% to 3.6%, and from 0.3% to 1.6%, respectively. It has been predicted that, by 2019, the prevalence of obesity in classes 1, 2 and 3 will increase to 14.8%, 4.4% and 2.0%, respectively (Twells et al. 2014). By 2030, in the USA up to 86% of adults will be overweight or obese (Ginter and Simko 2014). In Australia, approximately 63% of adults were overweight and obese in between the years 2011–2012. The proportion of the Australian population who are overweight and obese is expected to

increase to approximately 66% in 2017 (Sassi et al. 2009; Statistics 2012).

On the other hand the prevalence of overweight and obesity among children and adolescents has also increased worldwide (Ebbeling et al. 2002; Lissau et al. 2004). Thirty-nine articles and one national health report that were undertaken to consideration; in 16 of the 23 countries with national representative data using the International Obesity Task Force (IOTF) cut-off, over-weight and obesity prevalence were found to be higher than 20%, five countries showed prevalence above 30%, and only in two countries prevalence was lower than 10%. Data from the National Health and Nutrition Examination Survey 2009–10 (NHANES) indicated a prevalence of overweight and obesity among 4111 adolescents aged 12 through 19 years of 15.2% and 18.4%, respectively (Bibiloni et al., 2013). IOTF versus BMI cut-offs are widely used to assess the prevalence of child overweight, obesity and thinness. IOTF defines the revised international child cut-offs and they are available corresponding to the following BMI cut-offs at 18 years. 16 kg/m^2: thinness grade 3, 17 kg/m^2: thinness grade 2, 18.5 kg/m^2: thinness grade 1, 23 kg/m^2: overweight (unofficial Asian cut-off), 25 kg/m^2: overweight, 27 kg/m^2: obesity (unofficial Asian cut-off), 30 kg/m^2: obesity, 35 kg/m^2: morbid obesity (Cole and Lobstein 2012).

The prevalence has increased substantially in children and adolescents in developed countries; 23.8% (BMI; 22.9–24.7) of boys and 22.6% (BMI; 21.7–23.6) of girls were overweight or obese in 2013. The prevalence of overweight and obesity has also increased in children and adolescents in developing countries, from 8.1% (min; 7.7–max; 8.6) to 12.9% (min; 12.3–max; 13.5) for boys and from 8.4% (min; 8.1–max; 8.8) to 13.4% (min; 13.0–max; 13.9) for girls during the same time period (Ng et al. 2014). In an another European country, Spain, the prevalence of overweight and obesity in children was determined by using Spanish reference tables (SPART), IOTF reference values, and The World Health Organization (WHO) growth standards. The average prevalence of overweight in boys ranged from 14.1% to 26.7%, and in girls from 13.8% to 25.7% (Pérez-Farinós et al. 2013). The WHO Regional Office for Europe analyzed 168,832 children from 12 countries in the context of WHO European Childhood Obesity Surveillance Initiative (COSI). Indeed, COSI routinely measures overweight and obesity prevalence of primary-school children aged 6–9 years. According to COSI data, the prevalence of obesity ranged from 6.0% to 26.6% among boys and from 4.6% to 17.3% among girls. Consequently, overweight among 6–9-year-old children is identified as a serious public health concern (Wijnhoven et al. 2013). BMI is correlated with visceral adipose tissue (VAT) in pediatric populations. Furthermore, BMI may also be converted to BMI percentiles by using Centers for Disease Control and Prevention (CDC) growth charts. BMI percentile is a sensible and useful tool for the prediction of VAT mass, fat mass and cardiovascular disease (CVD) risk in children and adolescents (Harrington et al. 2013). In a series of 14,493 children, increasing waist-to-height ratio (WHtR) was significantly associated with increased cardiometabolic risk in overweight and obese subjects. Obese subjects with WHtR higher than 0.6 should undergo a further cardiometabolic risk assessment (Khoury et al. 2013). However, in 3–5 years old overweight/obese children, WHtR is not found to be superior to WC or BMI in estimating body fat percentage (BF%) and cardiometabolic risk (Sijtsma et al. 2014). Regardless of weight and length at birth, the correlation of higher BMI at preschool age with the ratio of weight gain per cm gain in height may be a better indicator of risk for overweight and obesity (Nascimento et al. 2011). Actually, overweight children often become overweight adolescents and adults later. A child or adolescent with a high BMI percentile may have a high risk of being overweight or obese at 35 years of age, and this risk increases with age (Guo et al. 2002). Furthermore in men, total adiposity and truncal subcutaneous fat accumulation during adolescence, are positively and independently associated with atherosclerosis at age 36 (Ferreira et al. 2004). Indeed, accumulation of body fat centrally is associated with insulin resistance (IR), whereas distribution of body fat in a peripheral pattern is

metabolically less important. Thus, increased visceral or intra-abdominal fat are more insulin resistant than those who have increased quantities of centrally located subcutaneous fat (Kahn et al. 2001). Body fat content and the fat distribution or adiposity is considered as important indicators of health risk. Body adiposity index (BAI) based on the measurements of hip circumference and height is suggested as a useful predictor of obesity (Bergman et al. 2011). Melmer et al. observed that BAI is significantly associated with leptin and hip circumference in 1770 patients from the Salzburg Atherosclerosis Prevention Program in Subjects at High Individual Risk (SAPHIR) study (Melmer et al. 2013). However, a cross-sectional study was conducted in 29,214 men and 21,040 women Spanish Caucasian participants revealed that, WHtR and WC are better adiposity indexes than BAI and BMI (Bennasar-Veny et al. 2013).

Moreover, the connection between BMI and body fat in young people differs among ethnic groups and childhood. CDC and IOTF thresholds showed low sensitivity or low specificity for predicting excess percentage body fat in different ethnic groups. Overweight may not represent an equivalent level of adiposity and ethnic-specific BMI cut-off points (Duncan et al. 2009). The anthropometric results of COSI Round 2 (2009/2010) and changes in BMI showed that the highest significant decrease in BMI-for-age anthropometric-Z-scores was found in countries with higher absolute BMI values and the highest significant increase in countries with lower BMI values. Changes in BMI and prevalence of obesity over a 2-year period varied significantly among European countries (Wijnhoven et al. 2014). As mentioned above the same anthropometric obesity measures cannot be used across all ethnic groups for the assessment of obesity complications. Overall, it is claimed that the central obesity measures, WC and waist-hip ratio (WHpR) are better predictors of cardiovascular risk. Inspite of that WHpR is reported to have a stronger predictive ability than WC and BMI in Caucasian women. However, BMI in Northern European women is a better indicator of risk using in the general CVD and Framingham risk score models. Nonetheless, it has been suggested that WC is the most predictive tool for cardiovascular risk among Asian women (Goh et al. 2014). In fact, Framingham data suggest that obesity and physical inactivity exert adverse effect on development of CHD through the major risk factors. Indeed, the increased risk associated with metabolic syndrome (MS) is reflected by the Framingham scores primarily through high-density lipoprotein (HDL) cholesterol, blood pressure, and diabetes mellitus. However, some other risk factors for CHD such as triglycerides, low-density lipoprotein (LDL) particles, lipoprotein (a), coagulation factors, and homocysteine are not taken into account. Moreover, the impact of these risk factors may vary in various geographic and ethnic groups (Grundy et al. 1998).

Obesity is associated with large decreases in life expectancy approximately by 3.3–18.7 years and also large increases in healthcare expenditures (Leung et al. 2015). Above BMI 25, each 5 kg/m^2 higher BMI is on average associated with about 30% higher overall mortality, which is mainly due to increased risk of cardiovascular death as 40%. While BMI at 30–35 kg/m^2, median survival is reduced by 2–4 years; at 40–45 kg/m^2, it is reduced by 8–10 years (Prospective Studies Collaboration et al. 2009). However, it is claimed that WHtR is a better predictive risk measure of mortality than BMI (Ashwell 2012; Ashwell et al. 2012). Optimal values of WHtR are calculated as 0.5 for males and 0.46 for females. The years of life lost (YLL) for the different values of WHtR are positively correlated with the life expectancy. In a 30-year-old male non-smoker with a WHtR of 0.7, life expectancy is 7.2 years less than a 30-year-old male with a WHtR of 0.5. The corresponding figure is also valid for a 30-year-old female with WHtR of 0.7 compared to female with WHtR of 0.46. Life expectancy is 4.6 years shorter in patient with higher WHtR. YLL increases dramatically from categories in excess of WHtR 0.52 for both males and females (Ashwell et al. 2014). A pooled data from 11 prospective cohort studies with 650,386 adults aged 20–83 years showed that higher WC was positively associated with higher mortality at all levels of BMI from 20 to 50 kg/m^2. In this

study median follow-up was 9 years and 78,268 participants died. Life expectancy for highest versus lowest WC was approximately 3 years less for men (WC of ≥110 vs <90 cm) and approximately 5 years less for women (WC of ≥95 vs <70 cm) (Cerhan et al. 2014).

The effect of extreme obesity on mortality is greater among younger than among older adults, greater among men than women, and greater among whites than blacks (Hensrud and Klein 2006). Excess weight related deaths increased by 31% and associated years of potential life loss and quality adjusted life years lost by about 37%, respectively, between 2002 and 2008 in Germany. About 73% of total excess weight related costs are attributable to obesity. The main drivers of direct costs are endocrinological (44%) and cardiovascular (38%) diseases (Lehnert et al. 2015). Amongst 1799 patients with BMI more than or equal to 30 kg/m^2, those with either nonspecific or protein-energy malnutrition have increased mortality relative to well-nourished patients, while the odds ratio of 90-day mortality is 1.67 (95% confidence interval (CI), 1.29–2.15; $p < 0.0001$) (Robinson et al. 2015). In a series of 154,308 intensive care unit patients, Pickkers et al. asserted that hospital mortality risks quickly increase in underweight critically ill patients with BMI < 18.5 kg/m^2, whereas obese patients with a BMI of 30–39.9 kg/m^2 have the lowest risk of death. Because of the well-known obesity-associated decrease in overall life expectancy, these results could not be explained (Pickkers et al. 2013). Even though the incidence of hypertension, dyslipidemia, type 2 diabetes mellitus, CVD and mortality are directly proportional with the BMI, obese individuals may have better outcomes compared to lean counterparts. This condition is termed as the obesity paradox (Goyal et al. 2014). Obese and morbidly obese patients more frequently develop intensive care unit-acquired infections than patients in lower BMI categories. However, no significant differences were observed among the groups in intensive care unit or hospital mortality rates (Sakr et al. 2008). Indeed multiple statistical reviews have suggested improved outcomes for obese intensive care unit patients. Many articles highlight potential confounders related to metabolic problems that may cause misleading results. Thus, BMI has been traditionally used to stratify risk in obese populations (Kiraly et al. 2011). For instance, obese patients may be at greater risk of developing acute respiratory distress syndrome (ARDS) than normal weight patients (Stapleton and Suratt 2014). Obesity-related factors cause 11% of heart failure cases in men and 14% in women by inducing haemodynamic and myocardial changes due to an increased cardiac lipotoxicity that lead to cardiac dysfunction (Ebong et al. 2014). In 97 studies providing more than 2.88 million individuals and more than 270,000 deaths, relative to normal weight, both obesity (all grades) and grades 2 and 3 obesity are associated with significantly higher all-cause mortality. Grade 1 obesity overall is not associated with higher mortality. Furthermore, overweight is associated with significantly lower all-cause mortality (Flegal et al. 2013). The relative risks of all-cause mortality in overweight and obese patients with type 2 diabetes were 0.81 and 0.72, respectively, compared with the normal or non-overweight patients out of 161,984 participants from nine studies of 13 cohorts (Liu et al. 2015).

Epidemiological studies have shown that obesity is also associated with increased risk of several cancer types. Recently the protein kinase B/phosphatidylinositol 3-kinase/mammalian target of rapamycin (Akt/PI3K/mTOR) cascade has become a focus of the obesity and cancer connection (Vucenik and Stains 2012). On the one hand leptin is positively correlated with adipose stores. On the other hand, it induces cancer progression by activation of PI3K/Akt, mitogen-activated protein kinases (MAPK), mTOR and signal transducer and activator of transcription 3 (STAT3) pathways as a potential mediator of obesity-related cancer (Chen 2011; Drew 2012). It has been suggested that a close relationship between the metabolic health status and obesity-related cancer mortality. While the risk of cancer mortality decreases depending on the obesity status, it increases depending on the metabolic health status. The mortality rate from cancer rises with the progress of metabolic dysfunction (Oh et al. 2014). Although BMI had no impact on

recurrence-free survival in obese women in the low−/intermediate-risk groups, severe obesity (BMI ≥ 35) negatively impacts recurrence-free survival in women with high-risk endometrial cancer (Canlorbe et al. 2015). Eighty-two studies, including 213,075 breast cancer survivors with 23,182 deaths from breast cancer showed that relative risks of mortality are 1.75 for premenopausal and 1.34 for post-menopausal breast cancer for obese women. For each 5 kg/m^2 increment of BMI before and after 1 year of cancer diagnosis increases risks by 18% and 29% for breast cancer mortality, respectively. In this case obesity is associated with poorer breast cancer survival regardless of BMI ascertainment period (Chan et al. 2014). Similarly, a trend of increased colorectal cancer risk was observed with longer duration of obesity (Peeters et al. 2015), whereas, no association was found between high BMI and risk of prostate cancer incidence in a series of 904 cases (Grotta et al. 2015).

3 Mortality Risk of Metabolically Healthy Obese Individuals

Although obesity significantly increases the risk of developing metabolic disorders, hypertension, CHD, stroke, and several types of cancer, up to 30% of obese patients are metabolically healthy with insulin sensitivity similar to healthy normal weight individuals, lower visceral fat content, and lower intima media thickness of the carotid artery than the majority of metabolically "unhealthy" obese patients (Blüher 2012). These individuals do not display the "typical" metabolic obesity-associated complications. Severity of IR as well as subclinical inflammation, type 2 diabetes, dyslipidemia, hypertension and cardiovascular disease differentiates the metabolically non-healthy obese from metabolically healthy obese (MHO) (Blüher and Schwarz 2014). MHO approximately consists of 10–25% of the obese (Blüher 2010). Systematic review of the prevalence of MHO in database revealed that 30 different forms of metabolic health have been identified in 27 different publications based on four common criteria; blood pressure, HDL cholesterol, triglycerides and plasma glucose. BMI ≥30 kg/m^2 is the main indicator used to define obesity in two thirds of the studies. In these cases, estimated metabolically healthy obesity prevalence is between 10% and 51% (Rey-López et al. 2014). In total of 881 obese subjects, a more detailed MHO was defined by using six sets of criteria including different combinations of WC, blood pressure, total HDL cholesterol or LDL-cholesterol, triglycerides, fasting glucose, homeostasis model, high-sensitivity C-reactive protein (hs-CRP), and personal history of cardiovascular, respiratory or metabolic diseases (Marques-Vidal et al. 2012). Actually the MHO phenotype frequently refers to obese individuals with a favorable metabolic profile. In Whitehall II study, 657 individuals out of 7122 participants were obese and 42.5% of these were classified as MHO. Over the median follow-up of 17.4 years, 828 incident cases of CVD or stroke and 798 incident cases of type 2 diabetes were diagnosed. MHO subjects were at increased risk for CVD (Hazard Ratio (HR): 1.97, 95% CI: 1.38–2.80) and type 2 diabetes (HR: 3.25, 95% CI: 2.32–4.54) when compared with metabolically healthy normal weight individuals. For type 2 diabetes, the MHO phenotype is associated with lower risk than the metabolically unhealthy obese, but CVD risk was high in both obesity phenotypes (Hinnouho et al. 2015). Additionally, MHO individuals have higher prevalence of subclinical coronary atherosclerosis than metabolically-healthy normal-weight subjects (Chang et al. 2014). There are no clear accepted criteria on the definition of MHO, as well as the biological mechanisms to explain this phenotype. Several prospective studies suggested that the MHO individual has been associated with a similar risk of developing type 2 diabetes, CVD and mortality when compared to healthy normal weight subjects (Plourde and Karelis 2014). Thus, Phillips et al. showed that prevalence of metabolically healthy individuals was 6.8–36.6% among the obese, whereas prevalence of metabolically unhealthy subjects was 21.8–87% among the non-obese subjects (Phillips et al. 2013). Moreover, eight studies with 61,386 participants were evaluated for all-cause mortality and/or cardiovascular events. MHO individuals had increased risk for events compared with metabolically healthy normal-weight

individuals. Compared with metabolically healthy normal-weight individuals, obese persons are at increased risk for adverse long-term outcomes even in the absence of metabolic abnormalities, suggesting that there is no healthy pattern of increased weight (Kramer et al. 2013). In a population-based study among Mexican Americans and non-Hispanic whites, type 2 diabetes mellitus and CVD were evaluated in 2814 and 3700 participants aged 25–64 years, respectively. The risk of developing type 2 diabetes mellitus and CVD is increased in both metabolically unhealthy normal weight and MHO individuals (Aung et al. 2014). At any time of life all metabolically unhealthy groups; whether normal weight or overweight and obese had a similarly elevated risk (Kramer et al. 2013). Several factors may participate in these discrepancies. In contrast to MHO subjects, elderly individuals with the metabolically obese normal-weight phenotype exhibit greater all-cause mortality during 10 years of follow-up (Choi et al. 2013). Although the obese population defines as being metabolically healthy or not depending on the method used to ascertain metabolic health, obese individuals carry an excess risk of mortality irrespective of their metabolic status (Hinnouho et al. 2013). Consequently, MHO participants were not significantly different from healthy lean individuals by any definition. Nevertheless, they have an intermediate level of risk between healthy lean and unhealthy obese groups (Durward et al. 2012). Since the incidence of obesity continues to rise, importance of MHO phenotype is increasing (Roberson et al. 2014). Furthermore, some evidences indicate that less than 15 years of follow-up data in population based cohort may underestimate the overall mortality risk associated with the metabolic disorders (Sundström et al. 2006).

4 Prevalence of Metabolic Syndrome

In epidemiological studies, MS occurrence varies between 20% and 45% of population. Abdominal obesity is the most frequently observed component of MS. The incidence of MS is expected to increase to approximately 53% at 2035 (Gierach et al. 2014). Abdominal obesity, nuclear peroxisome proliferator-activated (PPAR) modulation, IR with or without glucose intolerance, atherogenic dyslipidemia, elevated blood pressure and proinflammatory states are included in the principal components of MS (Tenenbaum et al. 2004). Adipocyte size of both subcutaneous and omental fat are increased with higher body fat mass. Hyperplasia takes place primarily in the subcutaneous fat tissue, whereas fat cell hypertrophy occurs both in the omental and subcutaneous compartments. Eventually, fat accumulation is progressively increases in the subcutaneous tissue than in the visceral fat compartment (Drolet et al. 2008). It has been suggested that WC and BMI are the most accurate surrogate markers of visceral adiposity in young adults, and are good indicators of IR and powerful predictors of the presence of hepatic steatosis (Borruel et al. 2014). However, overweight individuals with similar BMI values have markedly different levels of VAT (Cartier et al. 2008). Actually high amount of VAT has significantly higher concentrations of soluble tumor necrosis factor-alpha (TNF-alpha) receptor 2 compared with obese men with low VAT and with lean controls (Cartier et al. 2010). Although both subcutaneous abdominal adipose tissue and VAT are correlated with metabolic risk factors, VAT remains more strongly associated with an adverse metabolic risk profile (Fox et al. 2007). Indeed, increased VAT is strongly associated with incident hypertension, but not total or subcutaneous adiposity (Chandra et al. 2014). The rate of visceral fat accumulation is different according to the individuals' gender and ethnic background. Furthermore, the connection between visceral adiposity and obesity-related cardiometabolic problems has been shown to be independent of age, overall obesity or the amount of subcutaneous fat (Hamdy et al. 2006). Because of the significant contribution of visceral fat accumulation to the development of metabolic disorders, VAT is determined through different imaging techniques (Iannucci et al. 2007). Thus, computerized tomography or magnetic resonance imaging recognizes ethnic differences in susceptibility to visceral adiposity and related metabolic abnormalities. Actually clinical diagnosis of visceral

obesity alone, IR, or of the MS is not sufficient to assess global risk of CVD (Alberti et al. 2005; Després et al. 2008). Analysis of visceral adiposity by body composition analyzer and single-scan computerized tomography revealed that 130 cm^2 of VAT in both sexes and/or 27 kg of fat mass in women are useful cutoff values. In this respect visceral adiposity in men and body fat mass in women seem to be of greater relevance in cardiometabolic risk (Onat et al. 2010). Even so, independent data analysis of 24,670 individuals from ten autonomous communities aged 35–74 years displayed that the average prevalence of MS was 31% (in women 29% and in men 32%). In this study, while the high blood glucose and triglycerides were more frequent in men with MS, abdominal obesity and low HDL cholesterol was predominant in women. Eventually the increase in coronary risk was larger in women than in men (Fernández-Bergés et al. 2012). Abdominally obese and obese, each have a progressive increase in the odds ratio for hypertension when compared with individuals who had a normal BMI or no abdominal obesity based on the US NHANES 2007–2010 (Ostchega et al. 2012).

In 1998, WHO firstly reported an accepted definition of the MS. Within the scope of this guideline, in addition to diabetes or IR any two of the following criteria; obesity, lipid abnormalities, microalbuminuria and hypertension should be covered (Alberti and Zimmet 1998). Subsequently, visceral adiposity, hypertriglyceridemia, HDL-cholesterol, hypertension and more than 110 mg/dL of fasting blood plasma glucose level were accepted as criteria for the MS by the National Cholesterol Education Program-Adult Treatment Panel III (NCEP-ATP III) (Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults 2001). Later, The European Group for the Study of Insulin Resistance (EGIR) proposed an alternative definition for only non-diabetic subjects with hyperinsulinaemia in 2002. Although this definition refers to the same syndrome, in addition to IR there may be two or more of the other components; hyperglycemia, hypertension, dyslipidaemia or central obesity (Balkau et al. 2002).

In April 2005, International Diabetes Federation (IDF) proposed a new definition of the metabolic syndrome (Ford 2005). This latest concept represents a modification of the WHO and NCEP-ATP III, however the main focus is central adiposity. IDF lists the various ethnic group–specific thresholds for WC to define central adiposity. Essentially, to have the MS, an individual should have two or more of the following four criteria in addition to central adiposity; elevated concentrations of triglycerides, reduced concentrations of HDL-cholesterol, elevated blood pressure, and dysglycemia (Ford 2005). According to the American Heart Association/National Heart, Lung, and Blood Institute (AHA/NHLBI) criteria (Grundy et al. 2005), MS is diagnosed when three or more of the following risk factors are present: abdominal obesity (>102 cm in men, and >88 cm in women), hypertension ≥130/≥85 mmHg or specific medication, level of triglycerides ≥150 mg/dL (1.7 mmol/L) or specific medication, low HDL cholesterol: in men <40 mg/dL (1.03 mmol/L), and in women <50 mg/dL (1.29 mmol/L) or specific medication, and fasting plasma glucose ≥100 mg/dL (5.6 mmol/L) or history of diabetes mellitus or taking antidiabetic medications.

According to the 2005 IDF criteria of MS, subsequently revised in 2009, abdominal obesity is identified as the WC >94 cm in men, and >80 cm in women. MS is responsible for the development of IR which decreases the levels of the HDL-cholesterol fraction, increases the levels of triglycerides, and leads to the development of arterial hypertension. Abdominal obesity plus any two of the same risk factors as in NHLBI/AHA criteria: hypertension ≥130/≥85 mmHg or antihypertensive therapy, level of triglycerides ≥150 mg/dL (1.7 mmol/L) or specific medication, low HDL cholesterol: in men <40 mg/dL (1.03 mmol/L), and in women <50 mg/dL (1.29 mmol/L) or specific medication, and fasting plasma glucose ≥100 mg/dL (5.6 mmol/L) or history of diabetes mellitus or taking antidiabetic medications. Diabetes was defined as fasting blood glucose levels of ≥7 mmol/L and/or treatment with antidiabetic medications (Gierach et al. 2014). Based on the results from 16 cohorts

relative risk and incident diabetes were 5.17 (95% CI 3.99–6.69) for the 1999 WHO definition (ten cohorts); 4.45 (2.41–8.22) for the 1999 EGIR definition (four cohorts); 3.53 (2.84–4.39) for the 2001 NCEP definition (13 cohorts); 5.12 (3.26–8.05) for the 2005 AHA/NHLBI definition (five cohorts); and 4.42 (3.30–5.92) for the 2005 IDF definition (nine cohorts) (Ford et al. 2008).

Although not all overweight or obese individuals are metabolically disturbed, the majority are insulin-resistant. Metabolically benign obesity in humans is not accompanied by IR and atherosclerosis (Stefan et al. 2008). Indeed IR significantly increases the risk of developing diabetes mellitus type 2 (Kahn et al. 2001). Abdominal adiposity and IR appear to be central to the MS. In the presence of IR, non-esterified free fatty acids mobilization is accelerated from stored adipose tissue triglycerides. Consequently glucose, triglyceride and very low-density lipoprotein productions are increased (Cornier et al. 2008).

As mentioned above despite of the continuous increase in obesity incidence and paralleled rising rates of MS prevalence, no ideal diagnostic criteria are defined for MS, yet (Tenenbaum and Fisman 2011). Homeostasis Model Assessment (HOMA)-IR scores are higher in MS patients than in subjects without the MS (Vonbank et al. 2013). A relationship between the severity of dysglycemia and long-term mortality is also associated with the highest prevalence of the MS (Bergman et al. 2015).

There is no agreement between the criteria for diagnosis of MS. In 644 consecutive patients with verified carotid disease anthropometric parameters blood pressure, fasting plasma glucose and lipoproteins were measured in order to investigate agreement between AHA/NHLBI and IDF definitions of MS in patients with symptomatic carotid disease and to compare the frequency of cardiovascular risk factor in patients with MS diagnosed by these two sets of criteria. Thus the MS prevalence in patients with symptomatic carotid disease was high regardless of criteria used for its diagnosis (Maksimovic et al. 2012). According to the MS definition of the AHA/NHLBI 2009 Joint Scientific Statement, approximately one-third of the adult U.S. population has MS in 36% of women and 34% of men (Heiss et al. 2014). MS was diagnosed according to IDF and AHA/NHLBI criteria at a rate of 67.9% and 64.9%, respectively. 119 patients out of 644 were categorized differently by the two definitions. The overall agreement of IDF and AHA/NHLBI criteria is 81.5%. Actually IDF and AHA/NHLBI vary in two important aspects. First, in the IDF criteria cut-off values for WC are lower than in modified NCEP criteria (NHLBI/AHA), and the second and crucial, abdominal obesity is required as a prerequisite for diagnosis of MS (Maksimovic et al. 2012). Currently, several different definitions of MS exist, causing substantial confusion as to whether they identify the same individuals or represent a surrogate of risk factors. Therefore, diagnosis, prevention and treatment should better focus on established risk factors rather than the diagnosis of MS (Kassi et al. 2011).

The prevalence of MS in 614 obese children (307 male, 307 female; mean age: 11.3 ± 2.5 years) was found to be 39% and 33% according to the modified WHO and the IDF consensus criteria, respectively (Sangun et al. 2011). In a total of 133 patients, 67 males (50.4%) and 66 females (49.6%) with a mean age of 12.17 ± 3.27 years, the overall prevalence of MS was 19.6%, arterial hypertension and hypertriglyceridemia are the most prevalent metabolic changes. It was recommended that early intervention to control childhood obesity is essential to prevent cardiovascular morbidity and mortality in this series of patients (Guijarro de Armas et al. 2012).

Among the respondents 20 years and older, 3790 women and 4057 men from Turkey, age-adjusted overweight prevalence was 48.4% for women and 46.1% for men (Ergin et al. 2012). The overall prevalence of MS in Turkey was 34.6% (male, 31.2%; female, 37.3%) and 28.8% (male, 23.1%; female, 33.5%) according to IDF criteria and ATP III, respectively. The highest prevalence of MS was detected in 60–69 years of obese people (43.2%) in south district. The prevalence of MS criteria are as follows: type 2 diabetes mellitus, 15%; hypertension, 41.4%; obesity, 44.1%; abdominal obesity, 56.8%; low HDL-cholesterol, 34.1%; hypertriglyceridemia, 35.9%;

and high LDL-cholesterol, 27.4% (Gündogan et al. 2009).

In PubMed database and the Cochrane Library originated eight prospective cohort studies, seven cross-sectional studies, and a case-control study, WHO and NCEP are the most popular definitions to describe MS experienced by the elderly. The prevalence of MS varied from 11% to 43% (median 21%) according to the WHO, and 23% to 55% (median 31%) according to NCEP. Obesity and hypertension are the most prevalent individual components of MS. Cardiovascular morbidity is the most serious risk factor in elderly population (Denys et al. 2009).

The Third Report (ATP III) of the NCEP Expert Panel highlighted the importance of identifying and treating patients with the MS and its complications. The US NCEP's Adult Treatment Panel III requires at least three of five characteristics for MS: (1) abdominal obesity given as WC greater than 102 cm in men and greater than 88 cm in women; (2) hypertriglyceridemia with triglyceride concentration (150 mg/dL or 1.7 mmol/L); (3) abnormal cholesterol profile with HDL cholesterol less than 40 mg/dL or 1 mmol/L in men and less than 50 mg/dL or 1.3 mmol/L in women; (4) blood pressure: 130/85 mm Hg or more; (5) impaired glucose tolerance, i.e. elevated fasting plasma glucose 100 mg/dL or 5.5 mmol/L or more (National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) 2002). A cross-sectional analysis of 4153 Greek adults older than 18 years displayed the age-standardized prevalence of the MS was 23.6%. 61% of those had three components of the MS, 29% had four and only 10% had all five components. Abdominal obesity and arterial hypertension were the most common abnormalities in both sexes with 82% and 78%, respectively (Athyros et al. 2005).

In a multi-stage complex cross-sectional survey designed according to WHO guidelines by using the revised 2005 IDF definition of MS, Davila et al. showed that 64% of 3000 participants from Colombian region had abdominal obesity. Among those, 41% had MS (Davila et al. 2013). The prevalence of MS is 35.73% among all adults in Morocco. MS in women is 2.16 times more frequent than in men. Abdominal obesity is the most common abnormality with 49.15 per centile (El Brini et al. 2014).

The diagnosis of MS by WHO criteria could be made on the basis of IR plus at least two additional risk factors including obesity, hypertension, high triglyceride level, reduced HDL cholesterol level, or micro-albuminuria (Alberti and Zimmet 1998). The IDF dropped the WHO requirement for IR but made abdominal obesity necessary as one of five factors as described above required in the diagnosis (Alberti et al. 2005). Actually WC and insulin resistance estimated by HOMA-IR increases with age. Prevalence of IDF-MS and the Japanese Society of Internal Medicine (JSIM)-MS also increase with age at least until the age of 80, whereas the incidence of MS according to AHA/NHLBI does not show any apparent age changes. Abdominal obesity and IR are mentioned within the sets of criteria by all three institutions. However, significantly elevated linear association between WC and HOMA-IR overlaps only with IDF's and JSIM's MS definitions (Sakurai et al. 2010).

34,821 subjects from 12 cohorts from ten European countries and one cohort from USA in the Metabolic syndrome and Arteries REsearch (MARE) Consortium were investigated in accordance with the ATP III criteria (MS was defined as an alteration three or more of the following five components: elevated glucose, fasting glucose $\geq$110 mg/dL; low HDL cholesterol, <40 mg/dL for men or <50 mg/dL for women; high triglycerides, $\geq$150 mg/dL; elevated blood pressure, $\geq$130/$\geq$85 mmHg; abdominal obesity, WC >102 cm for men or >88 cm for women). MS has a 24.3% prevalence (8468 subjects: 23.9% in men vs. 24.6% in women, $p < 0.001$) with an age-associated increase in its prevalence in all the cohorts. The analysis of the distribution of MS suggested that MS is not a unique entity rather a constellation of cluster of MS components (Scuteri et al. 2015). Prevalence of MS is found to be different according to selected definition and components. In 2051 participants, prevalence of MS was significantly greater

when using AHA and IDF compared to the NCEP-ATP III definition (Mancia et al. 2010). Similarly, the prevalence of MS in 867 adults aged 25 years and older from an urban population of Karachi, Pakistan, according to the IDF definition and modified ATP III criteria was 34.8% and 49%, respectively (Hydrie et al. 2009). In 3914 adults aged 35–74 years in Jiangsu province, China, age-standardized prevalence of MS was 30.5% according to the modified NCEP-ATP III. In these patients, high blood pressure was the most prevalent component of MS (45.2%), followed by elevated triglycerides (40.1%) and low HDL cholesterol (40.1%). Multivariate ordinal regression analysis revealed that women had significantly higher risk of MS than men (Zuo et al. 2009).

As appears from the above data, the MS "clustering of abdominal obesity, dyslipidaemia, hyperglycaemia and hypertension" is a major public health challenge worldwide (Eckel et al. 2005). The prevalence of MS increases even more dramatically as BMI increases. More than 6 and 5.5 times more frequently MS occurs in overweight males and females, respectively, compared to under-weight and normal-weight individuals (Ervin 2009). In a total of 21 studies including 372,411 participants with MS, 18,556 deaths from any cause occurred during a mean follow-up of 11.5 years (Wu et al. 2010). Indeed, evaluation of 87 studies including 951,083 patients according to the third NCEP definition showed that MS was associated with a two-fold increase in the risk of coronary heart disease, cerebrovascular disease, all cardiovascular lethal and nonlethal CVD, and a 1.5-fold increase in the risk of all-cause mortality (Cicero and Derosa 2014). Over 12-year follow-up of 2051 individuals, 179 cardiovascular events and 233 deaths were determined for any cause. Risks of fatal and nonfatal cardiovascular events, diabetes mellitus, hypertension and left ventricular hypertrophy were similar for the three definitions of MS. However, the AHA and IDF definitions are more sensitive than that of ATP III in identifying MS condition (Mancia et al. 2010). Nonalcoholic fatty liver disease (NAFLD) and MS frequently coexist and 90% of NAFLD patients have more than one manifestation of the MS. Actually, CVD is the leading cause of death in patients with NAFLD and the MS (Almeda-Valdes et al. 2014).

5 Conclusion

Obesity is associated with large decreases in life expectancy and, also large increases in healthcare expenditures, but the mechanism of obesity-paradox is still needed to be clarified. Although metabolically benign obesity in humans is not associated with IR and atherosclerosis, weight loss is an appropriate treatment outcome in MHO individuals. Thus, from a clinical perspective, MHO is located in the intermediate-risk group. Additionally, there is no agreement between the criteria for diagnosis of MS. Different definitions of MS cause serious confusion as to whether the same individuals are identified. In this context, further epidemiological analysis and investigations are necessary to enlighten the pathological progress of obesity.

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Circadian Rhythms in Diet-Induced Obesity

2

Atilla Engin

Abstract

The biological clocks of the circadian timing system coordinate cellular and physiological processes and synchronizes these with daily cycles, feeding patterns also regulates circadian clocks. The clock genes and adipocytokines show circadian rhythmicity. Dysfunction of these genes are involved in the alteration of these adipokines during the development of obesity. Food availability promotes the stimuli associated with food intake which is a circadian oscillator outside of the suprachiasmatic nucleus (SCN). Its circadian rhythm is arranged with the predictable daily mealtimes. Food anticipatory activity is mediated by a self-sustained circadian timing and its principal component is food entrained oscillator. However, the hypothalamus has a crucial role in the regulation of energy balance rather than food intake. Fatty acids or their metabolites can modulate neuronal activity by brain nutrient-sensing neurons involved in the regulation of energy and glucose homeostasis. The timing of three-meal schedules indicates close association with the plasma levels of insulin and preceding food availability. Desynchronization between the central and peripheral clocks by altered timing of food intake and diet composition can lead to uncoupling of peripheral clocks from the central pacemaker and to the development of metabolic disorders. Metabolic dysfunction is associated with circadian disturbances at both central and peripheral levels and, eventual disruption of circadian clock functioning can lead to obesity. While CLOCK expression levels are increased with high fat diet-induced obesity, peroxisome proliferator-activated receptor (PPAR) alpha increases the transcriptional level of brain and muscle ARNT-like 1 (BMAL1) in obese subjects.

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Consequently, disruption of clock genes results in dyslipidemia, insulin resistance and obesity. Modifying the time of feeding alone can greatly affect body weight. Changes in the circadian clock are associated with temporal alterations in feeding behavior and increased weight gain. Thus, shift work is associated with increased risk for obesity, diabetes and cardio-vascular diseases as a result of unusual eating time and disruption of circadian rhythm.

Keywords

Obesity • Circadian rhythm • Clock genes • Suprachiasmatic nucleus (SCN) • N-methyl-D-aspartate receptors (NMDAR) • Brain and muscle ARNT-like 1 (BMAL1) • Cryptochrome circadian clock 1 (CRY1) • Peroxisome proliferator-activated receptor (PPAR) • Adenosine monophosphate-activated protein kinase (AMPK) • Nicotinamide phosphoryl-transferase • Mammalian target of rapamycin (mTOR) • Resistin • Calorie restriction

1 Introduction

The circadian system is a complex feedback network that is closely linked to metabolic homeostasis and involves interactions between the central nervous system and peripheral tissues (Green et al. 2008). Actually the circadian clock controls food processing by regulating the expression of enzymes and hormones which exhibit circadian oscillation (Froy 2007). The circadian clock is generally reset by environmental time cues. The central clock controls peripheral clocks directly and indirectly by virtue of neural, humoral, and other signals in a coordinated manner (Hirota and Fukada 2004). Actually the mammalian circadian system consists of two major oscillators; primarily the central clock mediates synchrony to daily light-dark cycles, whereas food-entrainable circadian oscillator generates activity rhythms by food and are synchronized with regular daily mealtimes (Smit et al. 2013). Nevertheless, central clock entrains peripheral clocks which can be synchronized by non-photic environmental cues (Pardini and Kaeffer 2006). Food processing is controlled through overlapping transcriptional networks that are tied to the clock and are thus time sensitive (Kohsaka and Bass 2007). Moreover, food anticipatory rhythms are under the control of a food-entrainable clock. The mutations of clock genes cannot impair expression of food anticipatory components (Feillet et al. 2006). Initially undifferentiated stem cells do not possess a functioning canonical molecular clock. Nevertheless, undifferentiated stem cells express a self-sustained rhythm in glucose uptake that is not coincidential with rhythmic expression of clock genes. Thereby rhythmic expression of glucose transporter genes has been thought to be rhythmic transcriptional regulator of glucose utilization (Paulose et al. 2012). Indeed, a large number of nuclear receptors involved in lipid and glucose metabolism has been found to exhibit circadian expression (Yang et al. 2006). Since pancreatic islets possess self-sustained circadian gene and protein oscillations of the transcription factors CLOCK and brain and muscle ARNT-like protein 1 (BMAL1), the beta-cell clock coordinates insulin secretion according to the sleep-wake cycle (Marcheva et al. 2010). In particular disrupted environmental light-dark cycles abolish the normal oscillation of peripheral clocks and induce internal de-synchrony in mammals (Oishi et al. 2015). Furthermore disruption of the traditional sleep/wake cycle is coupled with a tendency to eat at irregular times (Marcheva et al. 2013). Nutritional status is sensed by nuclear receptors and co-receptors, transcriptional regulatory proteins, and protein kinases, which synchronize metabolic gene expression and epigenetic modification, as

well as energy production and expenditure with behavioral and light-dark cycle (Mazzoccoli et al. 2012). Eventually, circadian disruption alters the metabolic hormone levels and increases weight gain by changing the morphology of medial prefrontal neurons (Karatsoreos et al. 2011). However, mammalian central oscillators are regulated differently from peripheral oscillators (Glossop and Hardin 2002). Feeding and meal timing are potent regulators of circadian rhythm in peripheral tissues. Temporal feeding restriction under light-dark or dark-dark conditions can change the phase of circadian gene expression in peripheral cells by leading to an uncoupling of peripheral oscillators from the central pacemaker (Damiola et al. 2000).

Actually circadian rhythms in gene expression synchronize biochemical processes and metabolic fluxes with the external environment, allowing the organism to function effectively in response to physiological challenges (Mazzoccoli et al. 2012). Even though the biological clocks of the circadian timing system coordinate cellular and physiological processes and synchronizes these with daily cycles, feeding patterns also regulates circadian clocks in mammals. While acute food restriction promotes arousal and food seeking behavior, chronic food restriction induces physiological adaptations to facilitate the extraction and storage of energy from ingested nutrients and to reduce energy expenditure (Patton and Mistlberger 2013). All transcript levels of the clock genes and adipocytokines such as adiponectin, resistin, and visfatin show circadian rhythmicity. The rhythmic expression of these genes is mildly attenuated in obesity (Ando et al. 2005). Consequently, it may be asserted that dysfunctions of molecular clock genes and these adipokines are involved in the development of obesity (Kaneko et al. 2009).

2 Master Circadian Clock System: The Suprachiasmatic Nucleus and Related Network

In mammals, master circadian clock is located in the suprachiasmatic nucleus (SCN) of the anterior hypothalamus as the central circadian pacemaker (Welsh et al. 2010). Circadian oscillations in expression of mammalian "clock genes" are detected not only in the SCN but also in peripheral tissues (Welsh et al. 2004). The SCN are distinguished from those in other brain regions and peripheral tissues regarding the capacity to generate coordinated rhythms and driven oscillations in other cells (Farnell et al. 2011). Although the individual cells of the SCN are capable of functioning independently from one to another, the SCN leads to coordination of circadian rhythms among its neurons and neuronal subpopulations by forming a circadian network through intercellular coupling (Mohawk and Takahashi 2011). Thereby a large population of circadian oscillator cells of the SCN are entrained to daily light-dark cycles via a direct input from intrinsically photoreceptive retinal ganglion cells (Dibner et al. 2010). Light-evoked information is perceived primarily by melanopsin-expressing retinal ganglion cells and these signals are transmitted via the retinohypothalamic tract (RHT) to the SCN (Gooley et al. 2001).

The efferent SCN projections mainly target neurons in the medial hypothalamus surrounding the SCN. The activity of these pre-autonomic and neuro-endocrine target neurons is controlled by differentially timed waves of vasopressin, gamma-aminobutyric acid (GABA), and glutamate release from SCN terminals (Kalsbeek et al. 2006a). Control of the pre-autonomic and neuroendocrine target neurons by vasopressin, GABA, and glutamate substantially depends on the light-dark cycle. Furthermore, four different phenotypic subpopulations are defined among SCN neurons which contain the same neurotransmitters (Kalsbeek et al. 2006b). Both sympathetic and parasympathetic pre-autonomic neurons also receive excitatory inputs, either from the biological clock or from non-clock areas, but the timing information is mainly provided by the GABAergic outputs of the biological clock (Kalsbeek et al. 2008). Under reverse light/dark conditions, responses to suprachiasmatic afferents of thalamic paraventricular nucleus neurons are in accordance with their membrane potential-dependent properties. This indicates the existence of glutamatergic and GABAergic neurotransmission from the suprachiasmatic nucleus to its target neurons (Zhang et al. 2006).

In this manner exposure to light synchronizes the circadian clock to the environmental light-dark cycle through the release of glutamate into the SCN. Hence N-methyl-D-aspartate (NMDA)-type glutamate receptors play a critical role in mediating the phase shifting effects of light (Novak and Albers 2002). N-methyl-D-aspartate receptors (NMDARs) located at glutamatergic synapses, which are formed between retinal ganglion afferents and SCN neurons, partly mediate light-induced phase resetting (Clark and Kofuji 2010). NMDA-evoked currents in SCN neurons also peaks during the night. Meanwhile the synaptic release of glutamate will always move cells toward the glutamate equilibrium potential (Colwell 2001). Thus activation of NMDARs is a critical step in the transmission of photic information to the SCN (Mintz et al. 1999). NR2B is a major NMDAR subtype within the SCN and is known to be sensitive to modulation (Clark and Kofuji 2010). Indeed, NR2B subunit of NMDAR-mediated responses within SCN neurons contribute to light-induced phase shifts of the mammalian circadian system (Wang et al. 2008). Moreover GABAergic transmission-related synaptic communication has a critical role in the synchronization of circadian rhythms in individual SCN neurons (Shirakawa et al. 2000). In this case GABA regulates the phase of the circadian clock through both pre- and postsynaptic mechanisms (Mintz et al. 2002). Presynaptically, spontaneous postsynaptic GABAergic current frequency varies with the length of the day, whereas postsynaptically, the photoperiod affects GABAergic activity within the SCN by changing the equilibrium of GABA-evoked current. The ratio of GABAergic excitation to inhibition determines the photoperiod-induced phase distribution in the SCN network (Farajnia et al. 2014). Furthermore, terminals of the retino-hypothalamic tract (RHT) terminate not only on peptidergic SCN cells but also on gastrin-releasing peptide (GRP) cells. Expression of chemical messengers released by these retinorecipient cells results from an interaction of GRP with other transmitter substances, such as GABA, glutamate, the neuropeptide vasoactive intestinal polypeptide (VIP), and substance P (Antle et al. 2005; Antle and Silver 2005). The primary neurotransmitter in the ventral SCN is VIP. VIP is expressed at high levels in the neurons of the SCN and regulates the long-term firing rate of SCN neurons through a VIP receptor 2-mediated increase in the cAMP pathway. VIP-containing neurons process light information received from the RHT and then transfer this information to the dorsal SCN (Antle et al. 2009; Kudo et al. 2013). However, Atkinson et.al have argued that cAMP-mediated signaling is not a principal regulator of cyclic nucleotide-gated channel function in the SCN (Atkinson et al. 2011). Synchronisation of cellular clocks by VIP in the SCN is paracrine and is mediated via the cytosolic pathways upstream of the intracellular transcriptional/translational feedback loops (Hastings et al. 2014). Lacking VIP or its receptor in SCN, damps and desynchronizes cryptochrome circadian clock 1 (CRY1) expression in cells (Maywood et al. 2013).

On the other hand, the amplitude of calcium flux rhythm is involved in both the circadian rhythms of the input and output signals. Therefore, the difference in amplitude could reflect the different roles in circadian oscillation between clock gene and calcium (Enoki et al. 2012). Lowering the extracellular concentration of potassium or blocking Ca^{2+} influx in SCN causes membrane hyperpolarization and reversibly abolishes the rhythmic expression of period circadian clock 1 (PER1). Transmembrane Ca^{2+} flux is necessary for molecular rhythmicity in the SCN. Periodic Ca^{2+} influx due to circadian variations in membrane potential is a critical process for circadian pacemaker function (Lundkvist et al. 2005). Additionally, calcium-activated potassium channels in the SCN is controlled by the intrinsic circadian clock and regulates daily oscillation of spontaneous firing rate (Meredith et al. 2006). In mammals, there is increasing evidence that voltage-dependent calcium channels (VDCCs) may contribute to the clock function of SCN cells. Circadian regulation of calcium channels in SCN cells is compatible with their potential involvement in intercellular coupling and coordination of molecular oscillations between SCN clock cells (Nahm et al. 2005).

The mechanisms other than Ca^{2+}-dependent synaptic transmission can also synchronize neurons in the mammalian hypothalamus (Bouskila and Dudek 1993). In this respect the inhibitory/excitatory ratio of GABAergic activity indicates the phase-synchronization of individual SCN neurons. The protein connexin-36 (Cx36) interconnects between gap junctions of the excitatory projection neurons of the inferior olivary nucleus and inhibitory interneurons of the neocortex, hippocampus, and thalamus (Connors and Long 2004). Moreover, many SCN neurons are self-sustained oscillators that have the intrinsic capacity to generate circadian rhythms in electrical activity. During the night, the SCN neuron populations are electrically inactive and are most responsive to excitatory or depolarizing stimulation (Colwell 2011). Actually in the absence of chemical synaptic transmission, many neurons in the SCN communicate via electrical synapses. However, synchronization is achieved in pairs of electrically coupled neurons only (Long et al. 2005). Surprisingly, Pfeuty et al. showed that blocking electrical synapses may increase the synchrony of neuronal activity (Pfeuty et al. 2003). In this case electrical and inhibitory synapses may cooperate, both promote synchrony or may compete. Eventually combining electrical synapses with inhibition amplifies synchrony, whereas electrical synapses alone desynchronizes the activity of the neurons (Pfeuty et al. 2005). It is well known that the most important stimulus for the SCN is light. In contrast to a long photoperiod, in a short photoperiod electrical activity rhythm of the SCN is robust due to highly synchronized single-cell activity patterns. Photoperiod-induced changes in the expression of clock genes coincide with the photoperiod-induced changes in the electrical rhythm of the SCN (Ramkisoensing and Meijer 2015). Gap junction-mediated coupling improves the connectivity of neuronal networks. Thus electrical synapses containing Cx36 are critical for the generation of synchronous inhibitory activity (Deans et al. 2001).

Photic resetting of the SCN pacemaker involves induction of PER1 and PER2 and the subsequent communication among distinct cell populations. Activation of the 3′5′-cyclic adenosine monophosphate (cAMP) response element binding protein (CREB) pathway and PER1 are key steps in mediating downstream events of SCN neurons (Gamble et al. 2007). The transcriptional feedback loops of the SCN are supported by cytoplasmic cAMP signaling, which determines their canonical properties of amplitude, phase and period. Daily activation of cAMP signaling is driven by the transcriptional oscillator, in turn regulates progression of transcriptional rhythms. Thus, output from the current cycle constitutes an input into subsequent cycles (O'Neill et al. 2008).

While the photic information received by classical rod/cone photoreceptors and intrinsically photoreceptive retinal ganglion cells influence phase and period of circadian rhythms, the median raphe serotonergic pathway and the neuropeptide Y (NPY)-containing pathway from the thalamic intergeniculate leaflet (IGL) contributes to circadian rhythm regulation (Morin 2013). Subsequent to light information reaches the SCN through the RHT, axons of retinal ganglion cells release glutamate and pituitary adenylate cyclase-activating polypeptide (PACAP) at synaptic contacts with SCN neurons (Hannibal 2002, 2006). Furthermore, PACAP enhances alpha-amino-3-hydroxy-5-methyl-4-isoxazolepro-pionic acid (AMPA)- and NMDA-evoked calcium transients. Actually PACAP is a potent modulator of glutamatergic signalling within the SCN in the early night (Michel et al. 2006). Upon light stimulation, photoentrainable cells exhibit calcium/CREB protein phosphorylation that leads to temporally gated acute induction of the PER2 gene, followed by the phase-dependent changes in PER2 circadian rhythm. CREB activating stimuli can affect amplitude as well as phase of cellular rhythms (Pulivarthy et al. 2007). The net result of RHT stimulation is an increase in firing rate of SCN neurons. These retinal-evoked excitatory postsynaptic responses in the SCN are mediated by NMDA and AMPA/kainate (KA) iontotropic glutamate receptors (Michel et al. 2002). PACAP acts presynaptically to regulate the release of glutamate onto SCN neurons. Hence PACAP enhances both NMDA-evoked and AMPA-evoked

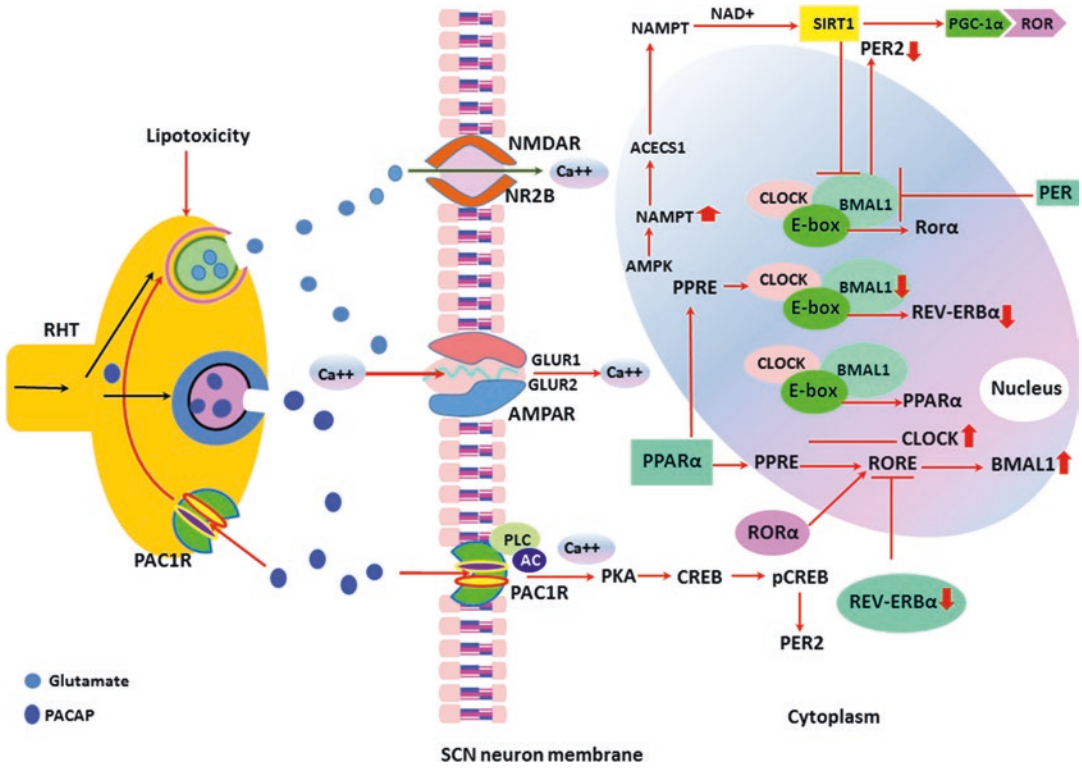

Fig. 2.1 Light-evoked signals are transmitted via the RHT to the SCN. Exposure to light synchronizes the circadian clock to the environmental light-dark cycle through the release of glutamate into the SCN. Activation of NMDARs is a critical step in the transmission of photic information to the SCN. NMDAR-mediated responses within SCN neurons contributes to light-induced phase shifts of the mammalian circadian system. Upon heterodimerization, CLOCK and BMAL1 bind to E-boxes in the promoter region of multiple target genes. In first negative feedback loop upon accumulation of their translation products in the cytosol PER and CRY isoforms heterodimerize and translocate back into the nucleus, subsequently inhibit the transcriptional activity of CLOCK–BMAL1. In second negative feedback loop, CLOCK and BMAL1 induces the transcription of REV-ERBα and RORα. RORα and REV-ERBα regulate lipid metabolism and adipogenesis. RORα stimulates and REV-ERBα inhibits Bmal1 transcription. Accumulation of REV–ERBα protein results in repression of BMAL1 transcription, through binding of REV–ERBα to the RORE within the BMAL1 promoter (*RHT* Retino-hypothalamic tract; *RORα* Retinoic acid-related orphan receptor α; *SCN* suprachiasmatic nuclei; *PER* Period protein; *CREB* adenosine 3′5′ monophosphate (*cAMP*) response element binding protein; *PACAP* Pituitary adenylate cyclase-activating polypeptide; *NAMPT* Nicotinamide phosphoribosyltransferase; *CLOCK* Circadian locomotor output cycles kaput; *NAD+* Nicotinamide adenine dinucleotide; *PLC* Phospholipase C; *AC* Adenyl cyclase; *PKA* Protein kinase A; *pCREB* Phosphorylated CREB; *AMPK* AMP-Activated protein kinase; *PPARα* Peroxisome proliferator-activated receptor α; *PPRE* Peroxisome-proliferator response element; *PGC-1α* PPAR-gamma coactivator; *SIRT1* Silent information regulator 1; *BMAL1* Brain and muscle ARNT-like 1; *PAC1R* PACAP type I receptor; *RORE* Retinoic acid-related orphan receptor response element; *ACECS1* Acetyl-CoA Synthetase 1; *NMDAR* N-methyl-D-aspartate (*NMDA*)-type glutamate receptor; *AMPAR* Amino-methyl proprionic acid (*AMPA*) iontotropic glutamate receptor)

currents in SCN neurons (Michel et al. 2006) (Fig. 2.1).

In fact, homeostatic systems have been adapted to respond to diurnal light/dark cycle (Kitazawa 2013). Peripheral clock mediated circadian expression of muscarinic acetylcholine receptor proteins, and parasympathetic signaling are essential in conferring circadian time information (Bando et al. 2007). Sympathetic efferents from the SCN can substitute for light cycle information, while other external cues may reach tissues through other efferents or non-neural

pathways (Vujovic et al. 2008). Consequently, the SCN imposes its rhythm onto the body via the secretion of hormones besides the parasympathetic and the sympathetic autonomous nervous systems. A reciprocal connection between the arcuate nucleus (ARC) and the SCN is used to transmit feeding related signals to the SCN (Buijs et al. 2006). More often non-photic inputs to the clock may be used to reset or strengthen circadian rhythms in humans (Webb et al. 2014). Two features of the mammalian circadian system provide flexibility in circadian programming to utilize casual regularities, which are social stimuli or food availability. In particular latter is sensitive to stimuli associated with food intake which is a circadian oscillator outside of the SCN. Its circadian rhythm arranges with the predictable daily mealtimes (Mistlberger and Antle 2011). On the other hand, food anticipatory activity is driven by a food-entrainable oscillator which does not require a functional molecular clock (Mohawk et al. 2012). Thus, food anticipatory activity is mediated by a self-sustained circadian timing and its principal component is food entrained oscillator (Mistlberger 2009). Nevertheless, the hypothalamus has a crucial role in the regulation of energy balance rather than food intake (Berthoud 2002). Thereby hypothalamic neurons have the capacity to sense and alter their activity in response to fluctuations in local nutrient concentrations (Moran 2010). The dorsomedial hypothalamic nucleus (DMH) and other brain regions express circadian clock gene rhythms which are sensitive to daytime feeding schedules (Moriya et al. 2009). In fact, the dorsomedial hypothalamus is not essential for the expression of the food-entrainable oscillator. However under conditions of food restriction, food anticipatory behavior originates from a neuronal network comprising an interaction between the DMH and SCN (Acosta-Galvan et al. 2011). Furthermore, daily variations in plasma fatty acid concentrations might be detected by the hypothalamus and brain stem. Thus, fatty acids or their metabolites can modulate neuronal activity by brain nutrient-sensing neurons that are involved in the regulation of energy and glucose homeostasis (Migrenne et al. 2011). Another valid signal on timing of the food-entrained oscillator is insulin. The timing of three-meal schedules indicates close association with the plasma levels of insulin and preceding food availability (Dailey et al. 2012). Fatty acid overload impairs neural control of energy homeostasis and contributes to obesity (Migrenne et al. 2011). In this case hypothalamic "metabolic-sensing" neurons respond to oleic acid by using the fatty acid translocase/receptor (FAT/CD36) (Le Foll et al. 2009). CD36-mediated ventromedial hypothalamic neuronal fatty acid sensing ability is important in the physiological regulation of both energy and glucose homeostasis (Le Foll et al. 2013). Impairment of lipid metabolism and accumulation of specific lipid species in the hypothalamus play a major role in hypothalamic lipotoxicity by integrating peripheral signals with classical neuropeptide-based mechanisms (Martínez de Morentin et al. 2010). In this manner the circadian clock controls acetyl-CoA levels and fatty acid synthesis. Acetylation of acetyl-CoA Synthetase 1 (AceCS1) is cyclic and that its rhythmicity requires a functional circadian clock and the Nicotinamide adenine dinucleotide$^+$ (NAD^+)-dependent deacetylase silent mating type information regulation 2 homolog 1 (SIRT1) (Sahar et al. 2014). Fasting increases hypothalamic SIRT1 expression and decreases forkhead box O1 (FOXO1)acetylation. Thus SIRT1 regulates the central melanocortin system in a FOXO1 dependent manner. Whereas inhibition of the fasting induced SIRT1 activity results in up-regulation of the S6K pathway. By this way hypothalamic SIRT1 regulates the food intake and body weight (Cakir et al. 2009). Actually SIRT1 is required for the circadian transcription of several core clock genes, including BMAL1, RAR-related orphan receptor gamma (ROR gamma), PER2, and CRY1. SIRT1 binds CLOCK-BMAL1 and promotes the deacetylation and degradation of PER2 (Asher et al. 2008). Nicotinamide phosphoribosyltransferase (NAMPT) biosynthesis and NAD^+ levels display circadian oscillations. Inhibition of NAMPT promotes oscillation of the clock gene PER2 by releasing CLOCK: BMAL1 from suppression by the NAD^+-dependent deacetylase, SIRT1 (Ramsey et al. 2009) (Fig. 2.1).

3 Food-Entrainable Circadian Rhythm

Ongoing meal or food availability-dependent circadian timing system is called food-entrainable system which is characterized by food-anticipatory processes depending on a circadian clock (Challet et al. 2009). Desynchronization between the central and peripheral clocks by altered timing of food intake and diet composition can lead to uncoupling of peripheral clocks from the central pacemaker and to the development of metabolic disorders (Oosterman et al. 2015). Actually meal time is a potent synchronizer for peripheral oscillators with no clear synchronizing influence on the suprachiasmatic clock. Therefore, food anticipatory rhythm is under the control of a food-entrainable clock (Feillet et al. 2006). For light entrained rhythms, constant conditions mean constant light or constant darkness. Whereas for food anticipatory activity constant condition is food deprivation (Carneiro and Araujo 2012). Hence, the changes in the metabolic activity can lead to an uncoupling of peripheral oscillators from the central pacemaker (Damiola et al. 2000). Food-entrainable oscillators locate elsewhere that generate rhythms of food-anticipatory activity and synchronizes to daily feeding schedules (Landry et al. 2006). It is known that the DMH is critical for the expression of circadian rhythms and in any way it receives input from systems that monitor food availability. However, DMH is not the site of oscillators or entrainment pathways necessary for food-anticipatory activity, but may participate in this circadian function (Gooley et al. 2006). The rhythmicity in the SCN remained phase-locked to the light-dark cycle, whereas feeding cycles can entrain the peripheral oscillator independent of the SCN and the light cycle (Stokkan et al. 2001). Mammals demonstrate feeding rhythms in behavior. Periodic availability of food and periodic feeding dictate adaptive behavioral and appropriate metabolic responses. Recently defined a nutrient anticipation metabolic oscillator (NAMO) is thought to arrange metabolic processes in visceral organs. NAMO is similar with the food anticipatory oscillator in the central nervous system (Khapre et al. 2014). Actually the mammalian target of rapamycin (mTOR) signaling pathway controls many processes that generate or use large amounts of energy and nutrients (Laplante and Sabatini 2012). It was shown that mTOR/the eukaryotic translational initiation factor 4E binding protein 1 (4E-BP1)-mediated translational control regulates entrainment and synchrony of the master clock (Cao et al. 2013). mTORC1 couples nutrient abundance to cell growth and proliferation by sensing and integrating a variety of inputs arising from nutritional status (Kim et al. 2013). These evidences suggest that NAMO may signal to the circadian clock through mTORC1 (Khapre et al. 2014).

Functional clock genes of gastrointestinal tract are molecular core components of the circadian clock. Synchronization of gastrointestinal clock genes during the daytime feeding in nocturnal animals is independent to the central clock and is not mediated through the vagal nerve (Hoogerwerf et al. 2007). Food-entrained oscillator consists of an unidentified network between the central and peripheral structures. However, clock genes and their metabolic oscillations are not essential for the persistence of food-anticipatory activity (Escobar et al. 2009). In mammals, peripheral and brain oscillators are synchronized indirectly and the SCN output pathways serve as input pathways for peripheral tissues (Dibner et al. 2010).

4 Food-Entrained Oscillator and Clock Genes

Light- and food-entrainable circadian rhythms share many properties, including limits of entrainment in the circadian range, free running under constant conditions and transients following phase shifts, but not neural basis (Mistlberger 1994). Functional CLOCK-based oscillators are not necessary for food-entrained circadian locomotor rhythms. However, both food and light temporally control locomotor behavior and that each circadian clock system can operate independently of the other. Although under normal ad

libitum conditions, these two systems are in phase with each other, but daytime food restriction disturbs the synchrony (Pitts et al. 2003).

The mammalian circadian clock is based on a transcription-translation feedback loop in which CLOCK (Circadian Locomotor Output Cycles Kaput) and BMAL1 proteins act as transcriptional activators of Cryptochrome (CRY) and Period (PER) genes. These genes encode proteins that repress CLOCK-BMAL1 with a 24-h periodicity. In the presence of CRY, nuclear entry of PER inhibits its transcription by displacing CLOCK-BMAL1 from the promoter (Ye et al. 2014). CLOCK dimerizes with BMAL1 to activate transcription. BMAL1 can also dimerize with other CLOCK homologs, such as neuronal PAS (PER, ARNT, SIM) domain protein 2 (NPAS2), to activate transcription and sustain rhythmicity (Asher and Schibler 2006; Debruyne et al. 2006). Eventually the regulatory targets of CLOCK:BMAL1, PER1, PER2, and PER3 genes together with the CRY1 and CRY2 genes function as negative regulators by blocking CLOCK:BMAL1-mediated transcriptional activation (Froy et al. 2002). On the other hand, many clock gene products function as transcription factors, which possess PAS and basic helix-loop-helix (bHLH) domains involve in protein-protein and protein-DNA interactions, respectively. These factors ultimately activate or repress their own expression and, thus, constitute self-sustained transcriptional feedback loops (Froy and Miskin 2010).

In homozygous CLOCK/CLOCK mice, the circadian rhythms are severely disrupted in constant darkness, but the locomotor behavior in light-dark cycle is less affected. This indicates that CLOCK protein is a necessary component of the light-entrainable oscillator (Vitaterna et al. 1994), whereas food-anticipatory activity is sustained in homozygous CLOCK/CLOCK mice under both entrained and constant conditions. This would suggest that the food-entrainable oscillator is not a CLOCK-based oscillator and therefore utilizes a different circadian molecular mechanism than that of the SCN (Pitts et al. 2003). The transcriptional regulation of CRY1 and CRY2 is under CLOCK control in both the SCN and in peripheral clocks. Direct inhibitory effect of the CRY1 and CRY2 proteins on the CLOCK-BMAL1–E box complex inhibits transcription by directly interacting with the PER proteins and translocating them into the nucleus for subsequent transcriptional effects (Kume et al. 1999). When the levels of cytosolic PER and CRY proteins rise, they associate, translocate to the nucleus, and repress their own gene transcription through direct interaction with CLOCK/BMAL1. The molecular clock consists of the three transcriptional regulatory feedback loops. The CLOCK incorporates to CLOCK, BMAL1 and ROR as transcriptional activators, PER, CRY, and REV-ERB as transcriptional repressors, and casein kinase 1 as a posttranslational regulator (Bechtold 2008). In this case, casein kinase I epsilon regulates the circadian clock by periodic phosphorylation of the proteins PER1 and PER2, controlling their stability and localization (Virshup et al. 2007). Nervous system-specific deletion of BMAL1 creates a marked deficit in entrainment of locomotor activity by periodic feeding. This is accompanied by reduced food intake and subsequent loss of body weight. That means SCN-independent food-entrained oscillator in the nervous system requires BMAL1 and plays a critical role in the adaptation of circadian locomotor activity and food intake to periodic feeding (Mieda and Sakurai 2011). While the SCN directly entrains feeding behavior to the light-dark cycle, daytime feeding shifts the timing of PER expression in the liver (Damiola et al. 2000; Hara et al. 2001). Day time restricted feeding forces the food-entrainable oscillator and SCN, which are normally in synchrony, to be out of phase with each other (Pitts et al. 2003).

5 Metabolic Feedback and Clock Genes

CLOCK genes are expressed in both subcutaneous and visceral fat tissues. Visceral obesity-associated cardiovascular risk is an indicator of the potential role of these clock genes in the metabolic disturbances (Gómez-Abellán et al. 2008). Metabolic dysfunction is associated with circadian disturbances at both central and peripheral levels

and, eventual disruption of circadian clock functioning can lead to obesity (Delezie and Challet 2011). Circadian oscillator genes (Npas2, BMAL1, PER1-3, and CRY1-2) and clock-controlled downstream genes (REV-ERB alpha, REV-ERB beta, Dbp, E4bp4, Stra13, and Id2) both are expressed in adipose tissues. Furthermore, temporally restricted feeding causes a coordinated phase-shift in circadian expression of these genes (Zvonic et al. 2006). In particular REV-ERB alpha acts as a major circadian regulator of BMAL1 expression in the SCN and in the liver. REV-ERB alpha also participates in the regulation of circadian CLOCK expression (Preitner et al. 2002). The expression levels of BMAL1 and REV-ERB alpha are attenuated in high fat diet-induced obesity as well as in genetically obese animals. While CLOCK expression levels are increased with high fat diet-induced obesity, CRY1 expression levels are decreased. In addition, peroxisome proliferator-activated receptor (PPAR) alpha increases the transcriptional level of BMAL1 (Kaneko et al. 2009).

Thus the diurnal effect dominates the transcriptome of the human adipose tissues, with more than 25% of the transcribed genes being diurnally regulated. The genes linked to PER1-led oscillations are defined as a novel point of obesity (Loboda et al. 2009). All transcript levels of the CLOCK genes and adipocytokines show 24 h rhythms. However, the rhythmic expression of these genes is attenuated in obesity (Ando et al. 2005) (Fig. 2.1).

Loss of BMAL1 expression leads to a significant decrease in adipogenesis and gene expression of several key adipogenic/lipogenic factors. Contrarily over-expression of BMAL1 in adipocytes increases lipid synthesis (Shimba et al. 2005). Several master lipid metabolism regulators and enzymes involved in triglyceride metabolism sustain their circadian expression in clock-disrupted animals (Adamovich et al. 2014). Lipid biosynthesis is regulated by sterol regulatory element-binding proteins (SREBP). The orphan receptor REV-ERB alpha participates in the circadian modulation of SREBP activity and expression, as well as SREBP targets, fatty acid synthase and acetyl-CoA carboxylase alpha, independently of feeding regimen. REV-ERB alpha also controls the timing of cyclic accumulation of SREBP in the nucleus (Le Martelot et al. 2009). Disruption of clock genes results in dyslipidemia, insulin resistance and obesity. The nuclear receptor REV-ERB alpha plays an important role in keeping proper timing of the clock by cross-talking with several other nuclear receptors involved in energy homeostasis (Duez and Staels 2008). Elevated levels of palmitate, a predominant saturated fatty acid in diet and fatty acid biosynthesis, alter cellular function. It is likely that palmitate-induced signal transduction cascades lead to changes in circadian transcript expression such as an increase in BMAL1 and CLOCK and a decrease in PER2 and REV-ERB alpha through AMPK-mediated regulation (Lee and Kim 2013).

REV-ERB alpha is a target gene of PPAR-gamma in adipose tissue. Expression of REV-ERB alpha promotes the effect of PPAR-gamma on adipocyte differentiation and insulin sensitivity (Fontaine et al. 2003). PPAR-gamma coactivator-1alpha (PGC-1alpha) is necessary for appropriate adaptation to the metabolic and physiologic stressors and plays a central role in the maintenance of glucose, lipid and energy homeostasis (Leone et al. 2005). Additionally, PGC-1alpha stimulates the expression of CLOCK genes, notably BMAL1 and REV-ERB alpha (Nr1d1), through co-activation of the ROR family of orphan nuclear receptors. Furthermore, PGC-1alpha deficiency causes both metabolic and circadian abnormalities (Liu et al. 2007).

In addition to PGC-1alpha, the circadian expression of the PPAR genes are regulated by peripheral oscillators in a CLOCK-dependent manner. CLOCK and BMAL1 play an important role in lipid homeostasis by regulating the transcription of a key protein, PPAR alpha (Oishi et al. 2005). The promoter activities of PPAR target genes acyl-CoA oxidase (AOX), 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) synthase and cellular retinol binding protein II (CRBPII) are increased by the expression of CLOCK/BMAL1 via the peroxisome PPAR response element (PPRE) (Inoue et al. 2005). Increased body mass, higher levels of plasmatic and hepatic triglycerides, higher levels of pro-

inflammatory and lower levels of anti-inflammatory adipokines, impairment of glucose metabolism, abnormal fat pad mass distribution, higher number of larger adipocytes, hepatic steatosis, higher expression of lipogenic proteins in high-fat diet are associated with the decreased expression of PPAR alpha and carnitine palmitoyltransferase I (CPT-1) in liver, and diminished expression of PPAR gamma and adiponectin in white adipose tissue (Magliano et al. 2013). Increase in the BMAL1 transcriptional level of obese subjects and insulin resistance by PPAR alpha indicates the involvement of PPAR alpha in the attenuation of circadian rhythms in the nucleus of the solitary tract in obesity (Kaneko et al. 2009).

When Clock-mutant mice fed with a high-fat diet, CLOCK mutation causes less triglyceride accumulation in the liver through the suppression of Acsl4 and Fabp1 gene expression compared to wild-type (Kudo et al. 2007). Thus high-fat diet-induced obesity alters the circadian-clock system. Obesity with or without metabolic syndrome are highly correlated with the expressions of circadian-clock genes including PER1-3, CRY1-2, BMAL1, Dbp, E4BP4, CK1, PEPCK, PDK4 and NHE3 (Hsieh et al. 2010).

The circadian clock synchronizes mitochondrial ATP production to meet daily alterations in cellular energy demands. AMP-activated protein kinase (AMPK) is activated by liver kinase B1 when the AMP/ATP ratio increases. This information is translated into SIRT1-dependent deacetylation of the transcriptional regulators PGC-1alpha and FOXO1 (Cantó et al. 2010). AMPK activation is linked to regulation of the circadian clock, which couples daily light and dark cycles to the control of energy demand in a wide variety of tissues and the hypothalamus (Bass and Takahashi 2010). The activities of AMPK display circadian rhythms in peripheral tissues as a crucial cellular energy sensor. AMPK transmits energy-dependent signals to the mammalian clock by driving the phosphorylation and destabilization of CRY and PER proteins. In addition, AMPK subunit composition, subcellular localization, and substrate phosphorylation are dependent on clock time (Jordan and Lamia 2013). The loss of AMPK signaling in vivo stabilizes CRYs and disrupts circadian rhythms. AMPK-mediated phosphorylation of CRY and Casein kinases I regulates the negative feedback control of circadian clock by proteolytic degradation of PER2. AMPK can also modulate the circadian rhythms through another metabolic sensor the NAD^+-dependent type III deacetylase SIRT1 (Lee and Kim 2013). SIRT1 controls DNA repair, apoptosis, circadian clocks, inflammatory pathways, insulin secretion and mitochondrial biogenesis (Chalkiadaki and Guarente 2012), whereas AMPK controls the expression of genes involved in energy metabolism in muscle by acting in coordination with SIRT1. AMPK enhances SIRT1 activity by increasing cellular NAD^+ levels, resulting in the deacetylation and modulation of the activity of downstream SIRT1 targets that include the PGC-1alpha and the FOXO1 and FOXO3a transcription factors (Cantó et al. 2009). AMPK is upstream of extracellular-signal-regulated kinase (ERK) and mTOR complex 1 (mTORC1) but downstream of adenylyl cyclase in regulating the circadian rhythm of photoreceptor L-type voltage-gated calcium channels (L-VGCCs) (Huang et al. 2015).

AMPK also increases the NAMPT expression and intracellular NAD^+ levels, which induces deacetylation of SIRT1 (Cantó et al. 2009). The circadian regulation of the NAMPT-SIRT1-PGC-1alpha pathway is achieved by AMPK. Conversely the circadian rhythm is disrupted in AMPK-deficient animals (Um et al. 2011). Hypothalamic metabolic sensors play an important role in the control of feeding and energy homeostasis. In this regard PAS kinase (PASK), AMPK and mTOR are important nutrient sensors of glucose metabolism and cellular energy requirement. Hypothalamic AMPK and S6K1 are highly activated under fasted/re-fed conditions. PASK function has critical importance for preserving the nutrient effect on AMPK and mTOR/S6K1 pathways (Hurtado-Carneiro et al. 2014). Alteration of hypothalamic AMPK activity is sufficient to change food intake and body weight. Acetyl-coenzyme A carboxylase/malonyl-coenzyme A/carnitine palmitoyltransferase-1/

fatty acid oxidation and mTOR signaling are putative downstream pathways for food intake regulation in response to hypothalamic AMPK (Minokoshi et al. 2008). On the other hand, defects in the genes encoding leptin or its receptor lead to hyperphagia and severe obesity. Diet-induced obesity alters alpha2-AMPK and signal transducer and activator of transcription 3 (STAT3) signaling in hypothalamus and subsequently impairs the effects of leptin on these signaling pathways. Defective responses of AMPK to leptin may contribute to resistance to leptin action on food intake and energy expenditure (Martin et al. 2006a). Eventually AMPK is a principal mediator of the effects of leptin on fatty-acid metabolism (Minokoshi et al. 2002). Furthermore, a high-fat diet modulates carbohydrate metabolism by amplifying circadian variation in glucose tolerance and insulin sensitivity (Rudic et al. 2004). In fact, adipose tissue is most vulnerable to clock gene disruption secondary to obesity, which is associated with marked disruption of downstream clock-regulated genes in cellular metabolic homeostasis including AMPK and of AMPK protein. Despite the diversity between rhythm loss and impairment of tissue insulin signaling, adipose tissue is most sensitive to rhythm loss. This discrepancy suggested that insulin resistance and clock gene dysfunction in obesity may arise by different mechanisms (Prasai et al. 2013).

The DNA-binding activity of the Clock:BMAL1 and NPAS2:BMAL1 heterodimers is regulated by the redox state of NAD cofactors. The reduced forms of the redox cofactors, NAD(H) and NADP(H), strongly enhance DNA binding of the Clock:BMAL1 and NPAS2:BMAL1 heterodimers, whereas the oxidized forms inhibit (Rutter et al. 2001).

Expression of NAMPT protein and NAMPT-RNA oscillations are circadian in nature with a reduction in NAMPT protein levels prior to the onset of the dark period. The rhythmic oscillation in RNA and protein levels of NAMPT leads to a circadian oscillation of NAD^+ levels (Ramsey et al. 2009). NAD^+ dependent deacetylase SIRT1 is a regulator responsible for various biological effects, depending on its localization in organism. Hence NAD^+ dependent histone deacetylases are very important for the mammalian metabolic clock (Rehan et al. 2014). SIRT1 and NAMPT constitute essential parts of the mammalian circadian clock feedback cycle. NAMPT is under the transcriptional regulation of a CLOCK-BMAL-SIRT1 complex, which increases the conversion of NAM to NAD^+. This in turn activates SIRT1, which reactivates NAMPT expression (Nakahata et al. 2009; Ramsey et al. 2009). NAD displays circadian oscillation and modulates CLOCK:BMAL1-mediated circadian transcriptional regulation through SIRT1. Actually this cycle exhibits a new function of NAD as a "metabolic oscillator" (Imai 2010). SIRT1 levels are responsive to environmental stimuli such as daylight, cell stress, and calorie restriction. SIRT1 binds to and deacetylates a number of important transcription factors, such as PPAR-gamma, PPAR-alpha, PGC-1alpha, and FOXO1. These transcription factors manage metabolic responses such as insulin secretion, gluconeogenesis, and fatty acid oxidation (Haigis and Sinclair 2010). SIRT1 functions as a molecular regulator of CLOCK-mediated histone acetyltransferases (HAT). Thereby SIRT1 transduces signals originated by cellular metabolites to the circadian machinery. Furthermore, SIRT1 also modulates the circadian machinery by controlling the acetylation levels of BMAL1. The histone deacetylases (HDAC) activity of SIRT1 is regulated in a circadian manner (Nakahata et al. 2008). Thus, the cross talk between the biological clock and the NAMPT/NAD^+/SIRT1 pathway provides a connection between the circadian system and nutrient-sensing pathways (Marcheva et al. 2013). CLOCK/BMAL1 binds to the SIRT1 promoter to enhance its expression and regulates hepatic insulin sensitivity by SIRT1. In addition, constant darkness-induced circadian misalignment in mice decreases hepatic BMAL1 and SIRT1 levels and induces insulin resistance. Actually CLOCK and BMAL1, two core circadian transcription factors, are correlated with hepatic insulin sensitivity (Zhou et al. 2014).

Poly(ADP-ribose) polymerase 1 (PARP-1) modulates the activities of several transcriptional

regulatory proteins either by direct protein-protein interaction or by NAD+-dependent poly ADP ribosylation (Hassa et al. 2006). PARP-1 poly ADP-ribosylation activity is circadian and regulated by feeding. PARP-1 binds to CLOCK-BMAL1 in a daytime-dependent manner and poly ADP-ribosylates CLOCK in a circadian manner. Poly ADP-ribosylation of CLOCK reduces the DNA-binding activity of CLOCK-BMAL1 and its interaction with the PER and CRY proteins (Asher et al. 2010).

The ratio between oxidized and reduced forms of NAD and NADP cofactors or increased levels of oxidized cofactors, NAD^+ or $NADP^+$ decrease the ability of CLOCK/BMAL1 and NPAS2 (neuronal PAS domain-containing protein)/BMAL1 to bind to DNA, suggesting that cellular redox changes may be sufficient to entrain clocks. NPAS2 promote transcription of PER (Rutter et al. 2001). Therefore, the nuclear redox state is similarly fundamental for the activation of several redox-regulated transcription factors including CLOCK, NPAS2 (Rutter et al. 2001) and REV-ERB beta (Gupta and Ragsdale 2011). Sensing and responding to oxidative cycles in cellular environments could have driven the evolution of circadian rhythms, and maintained the intrinsic link between clocks and metabolism (Edgar et al. 2012).

CLOCK-mediated acetylation together with the SIRT1 deacetylation cycles are central mechanisms in the CLOCK-directed rhythmic expression of clock-responsive genes (Bechtold 2008). As mentioned above, alterations in NAD levels could change activities of important enzymes in metabolic pathways. Thereby NAD would also affect NAD-dependent deacetylase SIRT1 or PARP (Revollo et al. 2007). While the reduced forms, NADH and NADPH increase binding of the clock heterodimers, the oxidized forms, NAD^+ and $NADP^+$ decrease their binding. Although NAD^+ is involved in cellular redox reactions within the mitochondria, it also serves as a substrate for the nutrient-responsive SIRT. In this case the activity of SIRT1 is directly coupled to the redox status, as well as it negatively regulates the activity of CLOCK/BMAL1 (Ramsey and Bass 2011).

Genotype and haplotype analysis in 537 individuals associated with metabolic risk of insulin resistance revealed that genetic variation in the CLOCK genes play a significant role in the development of obesity (Scott et al. 2008). Indeed, CLOCK polymorphism and related haplotypes increase the risk of overweight or obesity by 1.8-fold via altering circadian rhythmicity (Sookoian et al. 2008). Actually the photic regulation of the circadian system can be altered by eating a diet enriched in saturated fatty acids and leads to abdominal adiposity. In this case photic induction of two regulatory proteins, c-FOS and P-ERK, in the suprachiasmatic CLOCK are also markedly reduced during the high fat feeding (Mendoza et al. 2008). Although genetic variation at the CLOCK gene is associated with the metabolic syndrome features, no association is found between CLOCK gene polymorphism and fasting state lipid profiles. However, CLOCK polymorphisms interact with fatty acids to modulate metabolic syndrome traits. Therefore, genetic effects on insulin resistance and obesity phenotypes could be modulated by the dietary intake of the monounsaturated fatty acid (MUFA) or saturated fatty acids (Garaulet et al. 2009). In a total of 1100 individual participants who have CLOCK single-nucleotide polymorphisms, the energy intake with total fat, protein and carbohydrate consumptions are found to be significantly higher in minor allele carriers than in non-carriers. Subjects with the minor allele are 1.33 times more likely to have high energy intake than non-carriers (Garaulet et al. 2010b). Carriers of the minor allele C are also less successful in losing weight due to shorter sleep duration, higher plasma ghrelin concentrations. Moreover, they have shown less compliance with a Mediterranean diet pattern (Garaulet et al. 2011). On the other hand, PER2 polymorphisms have been linked with abdominal obesity, and unhealthy feeding behavior phenotypes (Garaulet et al. 2010a). Despite the CLOCK and PER2 gene polymorphism, NAMPT1 gene polymorphism is a rare single-nucleotide type, which is associated with protection from obesity (Blakemore et al. 2009).

Timing of food intake is associated with genetic variance in CLOCK. In 420 overweight/

obese patients undergoing a 20-week weight-loss diet, those who ate their main meal late lost significantly less weight than early eaters. This difference in weight loss success could not be explained by differences in caloric intake only (Garaulet et al. 2013a). Actually unusual feeding time can produce a disruption of the circadian system which might produce metabolic consequences for the development of obesity and for unsuccessful weight loss in humans (Garaulet and Gómez-Abellán 2014). Indeed, unusual feeding time induces internal desynchronisation trough decoupling of peripheral oscillators from the central clock (Lowrey and Takahashi 2004). Association between food timing and obesity has been also verified in shift workers. The majority of evidences indicates that shifts workers are more prone to obesity than day workers (Lowden et al. 2010). Additionally, there are considerable epidemiological evidences indicating that shift work is associated with increased risk for obesity, diabetes and cardio-vascular diseases as a result of unusual eating time and disruption of circadian rhythm (Antunes et al. 2010). In a retrospective cohort study that was conducted involving 21,469 healthy individuals who slept less than 6 h at night were more likely to experience weight gain and to become obese (Kobayashi et al. 2012). The pooled odds ratio that linked short-duration sleep to obesity is 1.89 in children and 1.55 in adults in a total of 634,511 participants (Cappuccio et al. 2008). Furthermore, the prevalence of short sleep duration associated-metabolic syndrome is 8.7% (Kobayashi et al. 2011). Virtually sleep deprivation during only a single night induces insulin resistance via multiple metabolic pathways in healthy subjects (Donga et al. 2010). Sleep restriction with less than 6-h at night for 7 days is associated with serious insulin resistance without significant alterations in the insulin secretory response (Buxton et al. 2010). Shorter REM sleep during the second part of the night is also associated with dysregulation of the HPA-axis and reduced insulin sensitivity (Gonnissen et al. 2013). Briefly, a sleep duration less than 6 h or more than 9 h is associated with increased prevalence of diabetes mellitus and impaired glucose tolerance (Gottlieb et al. 2005). Additionally, sleep deprivation may alter the ability of leptin and ghrelin to accurately signal caloric need and produce a misperception for accurate energy availability (Knutson and Van Cauter 2008). Actually feeding requires the maintenance of wakefulness and the orexin system, which has a key role in the interaction between feeding and arousal. Deficiencies in the orexin system are associated with sleep disorders (Spiegel et al. 2009).

Individuals who have an excessive night time light exposure at home are associated with increased body mass, waist circumference and triglyceride levels, and poor cholesterol balance (Obayashi et al. 2013). The effects of dim light at night and high-fat diet appear additive. Thus, animals exposed to dim light at night that are fed high-fat diet display the greatest increase in body mass and exaggerated peripheral inflammation (Fonken et al. 2013b). Furthermore, continuous exposure to light induces the complete loss of circadian rhythm in energy metabolism and insulin sensitivity decreases due to reduced amplitude of the central clock (Coomans et al. 2013). Constant light desynchronizes clock neurons but does not compromise their ability to regenerate circadian rhythms (Ohta et al. 2005). Indeed, constant light has both acute and long-term disruptive effects on developing biological clocks of infants. Thereby cyclic light conditions have been recommended in neonatal intensive care units (Ohta et al. 2006).

Otherwise, peripheral circadian de-synchrony is an early indicator of metabolic disruption in shift workers due to sleep deprivation-mediated disruption of circadian rhythms. Imposing a strict dark phase feeding rhythm can be employed to reset peripheral clocks and alleviate metabolic perturbations. Strengthening the peripheral circadian rhythm by imposing metabolic rhythms via limiting food intake during the night may counteract comorbidities seen in human shift workers (Barclay et al. 2012). Nevertheless, circadian rhythms in clock expression persist during light at night; however, the amplitude of PER1 and PER2 rhythms is attenuated in the hypothalamus. Changes in the circadian clock are associated with temporal alterations in feeding behavior and

increased weight gain (Fonken et al. 2013a). Thus reduced total daily energy expenditure in humans during nightshift schedules and reduced energy expenditure in response to dinner represent contributing mechanisms in the risk of weight gain. During the biological night, when the circadian clock is promoting sleep, working and eating may increase the risk of weight gain and obesity (McHill et al. 2014). In this manner the increase in exposure to light at night parallels the global increase in the prevalence of obesity and metabolic disorders (Fonken and Nelson 2014). Caloric intake during typical sleep time leads to greater weight gain than the same caloric intake during typical wake time (Arble et al. 2009). Eventually disruption of the timing of food intake and other metabolic signals at dim light at night leads to excess weight gain (Fonken et al. 2010).

Additionally, appetite-regulating hormones are altered during circadian misalignment. Thus, insufficient sleep reduces leptin and increases ghrelin (Bayon et al. 2014). In fact, the term of "circadian misalignment" describes either inappropriately timed sleep-wake or misalignment of sleep/wake with feeding rhythms, or misaligned central and peripheral rhythms (Baron and Reid 2014). In any case, circadian misalignment during nightshift schedules disturbs the metabolic physiology and contributes to the adverse metabolic health outcomes by reducing total daily energy expenditure (McHill et al. 2014). Later relative timing of meals, particularly eating close to sleep, could lead to weight gain due to a greater number of eating occasions and higher total daily caloric intake (Reid et al. 2014). Actually three major hormones, leptin, ghrelin and NPY, have been shown to exhibit circadian oscillation in metabolism. The progressive derangements in temporal communication imposed by environmental shifts in energy intake may force a positive energy balance culminating in excessive weight gain and obesity (Kalra et al. 2003). Thus, disruption in the rhythmic communication in the leptin-ghrelin-NPY feedback loop results in the loss of hypothalamic control, leading to abnormal weight gain and obesity (Kalra et al. 2005). The central circadian clock regulates leptin expression. Even so feeding time cannot be affected by the rhythmicity of leptin release, ablation of the SCN and the regular feeding has been shown to eliminate leptin circadian rhythmicity (Kalsbeek et al. 2001). Leptin is systematically lower when the behavioral cycle is misaligned with the circadian cycle. Leptin suppression is maximal when the behavioral cycle is misaligned by 12 h with the circadian cycle (Scheer et al. 2009).

The expression of receptors for metabolic hormones, such as leptin and ghrelin, allows the ARC to sense the information from the periphery and signal it to the central nervous system. Anatomical and functional pathway for peripheral hormonal feedback to the hypothalamus may serve to modulate the activity of the SCN (Yi et al. 2006). In particular, the ghrelin receptors play an important role in modulating the activity of the circadian system by exerting a direct action on the SCN both under normal conditions and under restricted feeding schedules (Lamont et al. 2014; Yannielli et al. 2007). The SCN also contains leptin receptors. Thus, leptin can directly modulate the electrical properties of SCN neurons and, in this way, may contribute to the mechanism by which metabolic processes influence the circadian clock (Inyushkin et al. 2009). The circadian system importantly contributes to the reduced glucose tolerance observed in the evening compared with the morning. Circadian misalignment reduces glucose tolerance (Morris et al. 2015). Daily blood glucose homeostasis is also controlled by the hypothalamic clock in the SCN as well as by peripheral clocks. Thereby both CLOCK mutant and BMAL1 deficient animals exhibit delayed recovery from insulin-induced hypoglycemia, impaired glucose tolerance and blunted insulin sensitivity (Kalsbeek et al. 2014). Actually BMAL1 regulates mitochondrial energy metabolism to maintain normal glucose-stimulated insulin secretion. Its circadian disruption leads to diabetes due to a loss of glucose-stimulated insulin secretion (Lee et al. 2011). Loss of glucose-stimulated insulin secretion is frequently depended on the accumulation of reactive oxygen species (ROS) as well as a consequence of mitochondrial uncoupling.

Hence it is fully rescued by scavenging of the ROS or by inhibition of uncoupling protein 2 (Lee et al. 2013).

An endogenous ligand for the G protein-coupled receptors FM-3/GPR66 and FM-4/TGR-1, neuromedin S (NMS) is expressed in the SCN of the hypothalamus. NMS increases proopiomelanocortin (POMC) mRNA expression in the ARC and corticotropin-releasing hormone mRNA in the paraventricular nucleus, and induces c-Fos expression in the POMC neurons of the ARC. Consequently, NMS is implicated in the regulation of circadian rhythm and feeding behavior (Miyazato et al. 2008; Mori et al. 2005). However, Neuromedin U (NMU) isoform has potent actions on appetite and energy expenditure. NMS or NMU dose-dependently decreases food intake, increases metabolic rate, and leads to significant weight loss in animals. The two NMU-binding receptors (NMU-R1 and NMU-R2) are also expressed in the SCN, but their phase angles are different. Furthermore, the expression of NMS mRNA fluctuated within the SCN under light/dark cycling, but not under conditions of constant darkness. NMU mRNA expression shows a circadian rhythm in the SCN shell of rats maintained under constant darkness (Nakahara et al. 2004). Amino acid variants in NMU associate with overweight and obesity, suggesting that NMU is involved in energy regulation in humans (Hainerová et al. 2006). Although NMU mRNA is significantly down-regulated in fasting, contrarily NMU overloading markedly suppresses food intake (Howard et al. 2000).

Another chronobiotic hormone which acts via high affinity G protein-coupled membrane receptors is melatonin. The abilities of this hormone for re-synchronization of sleep and circadian rhythm disturbances have been demonstrated in clinical trials (Barrenetxe et al. 2004). The nocturnal synthesis and release of melatonin by the pineal gland are tightly controlled by the SCN clock and inhibited by light exposure. Thus melatonin signals are used for the synchronization of peripheral oscillators. Moreover, melatonin receptors are also expressed by the SCN, hence endogenous melatonin is able to feedback onto the master clock (Pevet and Challet 2011). Melatonin is necessary for the proper synthesis, secretion, and action of insulin. Therefore, the activity/feeding phase of the day is associated with high insulin sensitivity, and the rest/fasting is synchronized to the insulin-resistant metabolic phase of the day. The reduction in plasma melatonin levels during shift-work at night induces insulin resistance, glucose intolerance, sleep disturbance, and metabolic circadian disorganization leading to obesity (Cipolla-Neto et al. 2014). Ghrelin and serotonin are biochemically and functionally linked to the melatonin, which is an internal transducer of photic environmental changes (Kirsz and Zieba 2012). The neuroendocrine circadian patterns in the night eating syndrome (NES) have been distinguished by an attenuated nocturnal rise in the plasma concentrations of melatonin and leptin, despite a greater increase in the concentrations of cortisol (Birketvedt et al. 2012). Patients with NES characteristically demonstrate a significant change in the timing and amplitude of various behavioral and physiological circadian markers such as reduced amplitudes in the circadian rhythms of food intake, cortisol, ghrelin, and insulin, but increased thyroid-stimulating hormone (TSH) amplitude. In this respect a delayed circadian pattern of food intake with a normal sleep-wake cycle occurs in NES. Thus NES may result from dissociations between central timing mechanisms and putative oscillators elsewhere in the central nervous system or periphery (Goel et al. 2009).

6 Effect of Feeding Regimens on Circadian Rhythms

6.1 Restricted Feeding

Daytime restricted feeding involves food intake for 2–4 h in the middle of the light period. After few days, anticipatory activity may occur (Mistlberger 2009). In this case the expression of alternative circadian oscillators can be strongly affected by daily feeding cycles, which are independent to the SCN (Mendoza 2007). Daily food is consumed in a limited time. When foods are received everyday at the same time for only a few

hours, organism adjusts to the feeding period within a few days (Froy et al. 2008). However, there is an association between food-entrainable oscillations and the expression of mPER1 and mPER2 in the cerebral cortex and hippocampus (Wakamatsu et al. 2001). Food-restricted animals are able to predict meal time, whereas lack of food anticipation is associated with a mutation of PER2. Mutations of CLOCK or PER1 do not impair expression of food anticipatory components, suggesting that these clock genes are not essential for food-entrainable oscillations. By contrast, NPAS2 mutation or CRY1 and CRY2 deficiencies show more or less altered responses to restricted feeding conditions (Feillet et al. 2006). SCN circadian pacemaker is entrainable to restricted feeding under continuous darkness. This suggests that circadian clock system can be reset by a signal associated with feeding time (Abe et al. 2007). Functional CLOCK is not required for an entrainment of peripheral clocks to restricted feeding. The rhythmic expression of REV-ERB alpha is not involved in the restricted feeding-induced circadian expression of BMAL1 mRNA, although REV-ERBalpha has been identified as a major regulator of BMAL1 transcription (Oishi et al. 2002). Food-entrained activity rhythms are likely mediated by a circadian oscillators system, which is sensitive to multiple feeding related inputs (Patton and Mistlberger 2013). Indeed, to restrict the food-availability to a specific period of the day causes profound changes on the behavior and physiology of animals. Increase in gastro-intestinal motility, and activity of digestive enzymes 2–3-h prior to the next scheduled feeding is called food anticipatory activity. Metabolic activities of the anterior piriform cortex, the olfactory tubercle and olfactory bulb increase during food anticipatory activity independent of the geographical time and the metabolic activity in SCN (Olivo et al. 2014). In this case although the mechanism is not known exactly, food intake associated stimuli sensitivity of circadian oscillators outside of the SCN enables animals to uncouple rhythms of behavior and physiology from light-dark cycles and align these with predictable daily mealtimes (Mistlberger and Antle 2011). Uncoupling of the stimulus associated with food intake from the central pacemaker suggests that nutritional regulation of clock oscillators in peripheral tissues may play a direct role in coordination of metabolic oscillations (Lin et al. 2008). Restricting the timing of meals to light time in contrast to restricted feeding during the night causes internal desynchronization with the loss of phase relationship between central-light entrained and peripheral clocks (Sunderram et al. 2014). In this manner peripheral oscillators become uncoupled from the central pacemaker when food availability becomes restricted, a long-lasting temporal conflict occurs with the central pacemaker. As soon as food availability returns to normal, the SCN clock, whose phase remains unaffected, resets the peripheral oscillators (Damiola et al. 2000). Time-restricted feeding entails the delivery of a certain amount of calories with the standard nutritional intake at specific time intervals of specific duration. Calorie restriction entails an overall reduction in caloric intake, albeit without malnutrition (Redman and Ravussin 2011).

Inter-individual analyses showed that subjects with relatively less REM sleep, particularly during the second part of the night, associated with dysregulation of the hypothalamic–pituitary–adrenal (HPA)-axis, higher cortisol concentrations, reduced insulin sensitivity and a higher homeostatic model assessment of insulin resistance (HOMA-IR) index. There is a negative correlation between total sleeping time and fasting insulin concentrations or between total sleeping time and the HOMA-IR index (Gonnissen et al. 2013). The time-restricted feeding regimen entrained circadian clock and metabolic regulators fix feeding times and prevent high fat diet-induced disruption of the normal cellular metabolic program (Hatori et al. 2012). High-energy food intake in the evening and fasting in the morning have both been associated with the development of obesity. In this regard skipping breakfast impairs postprandial insulin sensitivity and fasting lipid levels in humans (Farshchi et al. 2005). Eventually the synchrony between circadian and metabolic processes plays an important role in the regulation of energy balance and body weight control. Modifying the time of feeding

alone can greatly affect body weight (Arble et al. 2009). Temporal restricted feeding is a potent synchronizer of peripheral oscillators (Mendoza et al. 2005). Thus temporal feeding restriction under light-dark or dark-dark conditions can change the phase of circadian gene expression in peripheral cell types by up to 12 h. Sudden large changes in feeding time, similar to abrupt changes in the photoperiod, reset the phase of rhythmic gene expression (Damiola et al. 2000).

6.2 Calorie Restriction

Calorie restriction limits the amount of daily calorie intake to 60–70% of ad libitum feeding. In mammals, calorie restriction prevents or delays the onset of age-related diseases. In humans, long-termed calorie restriction results in sustained beneficial effects on major risk factors for atherosclerosis, Type 2 diabetes, and inflammation (Fontana 2009). A comprehensive review of 372 recorded comparisons revealed that dietary restriction has little effect on mitochondrial ROS production or antioxidant capacity. However more than half of the observations indicated that oxidative damage is reduced with dietary restriction by increasing antioxidant enzyme activity or the turnover of oxidized macromolecules (Walsh et al. 2014).

Calorie restriction upregulates NAMPT mRNA and protein levels in rat skeletal muscle and white adipose tissue. Inhibition of NAMPT activity attenuates the calorie restriction-induced SIRT3 activity, the calorie restriction-induced decrease of oxidative stress and the calorie restriction-induced improvements of antioxidant activity. Thereby, calorie restriction-induced beneficial effects on oxidative stress, mitochondrial biogenesis, and metabolic adaptation require NAMPT (Song et al. 2014). Analysis of the subjects who are included into different levels of chronic caloric restriction programs revealed that severe calorie restriction and acute fasting increase oxidative damage and decrease antioxidant capacity whereas moderate calorie restriction increases antioxidant capacity due to increase in manganese superoxide dismutase (Mn-SOD) activity and glutathione (GSH) concentration (Stankovic et al. 2013).

The mTOR pathway integrates nutrient, energy, and mitogen signals to regulate cell growth and cell division (Hay and Sonenberg 2004; Wullschleger et al. 2006). Actually calorie restriction can lead to activation of SIRT1 and suppression of mTOR and S6K1 activation (Ma et al. 2015). Dietary energy restriction reduces levels of phospho mTOR. This signaling pathway acts as a sensor of the nutritional and energetic state in the cell. Its principal upstream regulators are AMPK and Akt, and its downstream targets are the mTOR translation effectors p70 S6K, ribosomal S6 protein (S6) and 4E-BP1 S6K (Jiang et al. 2008). While light stimulates the co-localized activation of p70 S6K and ERK, pharmacological disruption of ERK signaling abolishes light-induced mTOR activity. This means that light-activated signaling coordinates activation of CREB and mTOR-mediated signals in the central pacemaker (Cao et al. 2008). Calorie restricted-fed animals resemble restricted feeding-treated animals, as they usually consume all or most of their food within a short period of time. Thus, due to the temporal component of food intake, calorie restriction similarly to restricted feeding synchronizes peripheral clocks and influences clock-controlled output systems (Froy and Miskin 2010). Calorie restriction entrains the SCN clock, whereas timed meals entrain peripheral oscillators (Froy 2007). When restricted feeding is coupled with caloric restriction, timing of clock gene expression is altered within the SCN, indicating that calorie restriction resets circadian rhythms by changing SCN clock gene expression and effects photic responses of the circadian system (Challet et al. 2003).

The normal alignment of feeding and activity with the environmental light cycle is critical for the maintenance of energy homeostasis (Marcheva et al. 2013).

Contrary to temporal restricted feeding, timed calorie restriction modifies clock gene expression in the SCN. Moreover, both temporal gating of light induced phase shifts and light induction of PER1 are strongly modified with daily calorie restriction. Diurnal hypoca-

loric feeding affects not only the transient regulation of the SCN clockwork and circadian outputs under light/dark cycle but also photic responses of the circadian system. Thus the changes in energy metabolism modulates circadian rhythmicity and gating of photic inputs in mammals (Mendoza et al. 2005). The circadian changes in clock and clock-controlled proteins and their acute responses to light in the SCN demonstrate that metabolic cues induced by a calorie restriction modulate the translational regulation of the SCN clock (Mendoza et al. 2007). In addition to timing of food availability affecting the circadian outputs of the clock, caloric restriction induces phase advances behavioral and physiological circadian rhythms and alters expression of clock genes and neuropeptides in SCN (Challet 2010). Under low-fat diet, adiponectin signaling pathway exhibits circadian rhythmicity. However, fasting and high fat diet alters this circadian expression. High fat diet leads to obesity by changing daily rhythm of clock genes and components of adiponectin signaling pathway (Barnea et al. 2009). When compared high fat diet ad libitum, the timed high fat diet restores the expression phase of the clock genes CLOCK and CRY1 and phase-advanced PER1, PER2, CRY2, BMAL1, ROR-alpha, and REV-ERB alpha. High fat diet provides 18% reduction in body weight and improves insulin sensitivity by 3.7-fold. Timing can prevent the harmful effects of high fat diet (Sherman et al. 2012). Actually the serum resistin levels are associated positively with saturated fat intake and inversely with monounsaturated fat intake. In a cross-sectional study of 6637 randomly recruited adults, the resistin level is also found to be inversely associated with adiposity and with adherence to the Mediterranean diet (Cabrera de León et al. 2014). The diurnal pattern of resistin expression is negatively correlated with the gastric contents and serum insulin. Insulin stimulates resistin expression and that circulating resistin follows a contrary circadian pattern in comparison to insulin (Oliver et al. 2006).

6.3 Intermittent Fasting

Intermittent fasting or reduced meal frequency and caloric restriction extend lifespan and improve the health of overweight humans. These feeding regimens enhance cardiovascular and brain functions and improve several risk factors for coronary artery disease and stroke including reduction in blood pressure and increased insulin sensitivity. The beneficial effects of intermittent fasting and caloric restriction are primarily due to reduced oxidative damage and reinforced cellular resistance mechanisms against stress (Mattson and Wan 2005). Additionally, intermittent fasting results in increased production of brain-derived neurotrophic factor (BDNF), which increases the resistance of neurons to degenerative processes. BDNF signaling may also mediate beneficial effects of intermittent fasting on glucose regulation and cardiovascular functions (Mattson 2005).

Furthermore, caloric restriction, intermittent fasting or every other day feeding regimens provide increased resistance to oxidative, metabolic and excitotoxic insults. In this respect PGC-1 is regulated by several signaling pathways via the connection between intermittent fasting and caloric restriction. These include FoxO transcription factors (through an insulin/insulin-like growth factor-I -dependent pathway), glucagon-stimulated CREB, stress-activated protein kinases (p38 and c-jun N-terminal kinase) and SIRT1 (Martin et al. 2006b). These pathways stimulate the production of protein chaperones, neurotrophic factors and antioxidant enzymes, all of which help cells fight with stress and resist disease (Martin et al. 2006b). Chausse et al. showed that intermittent fasting promotes tissue-specific changes in mitochondrial bioenergetics and tissue redox state. However intermittent fasting surprisingly increases protein oxidative damage without measurable changes in mitochondrial function of the brain unlike the other studies. This restrictive dietary intervention may also be detrimental toward liver oxidative balance, as reflected by the enhanced levels of protein carbonylation and induction of glutathione synthesis

(Chausse et al. 2015). Proteomic analysis of hepatic lipid droplets isolated from animals exposed to intermittent fasting and caloric restriction showed significantly higher levels of proteasome 26S subunit, non-ATPase 9 (PSMD9) (co-activator Bridge-1), macrophage migration inhibitor factor (MIF), transcription elongation factor B (SIII), polypeptide 2 (TCEB2)), aminoacylase 1 (ACY1) and fatty acid binding protein 5 (FABP5), and a marked reduction of glutathione S-transferase alpha 3 (GSTA3). In addition, accumulation of diacylglycerols (DAGs) is significantly reduced in hepatocytes of intermittent fasting and caloric restriction animals (Baumeier et al. 2015). If intermittent fasting combined with caloric restriction, liquid meals is an effective strategy to help obese women for decreasing weight and lower coronary heart disease risk (Klempel et al. 2012). Intermittent fasting can affect circadian rhythms differently depending on the timing of food availability and light conditions. This suggests that this regimen affects the SCN clock, similarly to caloric restriction (Froy and Miskin 2010). Actually intermittent fasting is not as dominant as restricted feeding in dictating peripheral rhythms. Nevertheless, intermittent fasting exhibits some similarities with restricted feeding, as reflected by the anticipatory feeding behavior. This precedes food availability or restoration of circadian rhythms under disruptive light conditions, due to the effect on the food entrainable oscillator. Co-activation of both the food entrainable oscillator and the SCN would yield rhythms at two opposite phases leading to the overall arrhythmicity (Froy et al. 2009).

7 Circadian Rhythms and Obesity

The daily variations between sleep/fasting/catabolism and wakefulness/feeding/anabolism are coordinated by a master hypothalamic clock, mainly reseted by ambient light. Peripheral clocks are normally synchronized by the master clock, but they are also sensitive to feeding time, especially when meals take place during the usual resting period (Challet 2013). Circadian desynchronization of hormonal rhythms may participate in internal desynchronization and is associated with increase in metabolic risks favoring obesity (Challet 2015). Homozygous CLOCK mutation shows an attenuated diurnal rhythm of feeding behavior, as well as profound changes in body weight regulation and fuel metabolism, which leads to obesity. In addition to the severely altered diurnal rhythm in food intake, CLOCK mutant animals show a significant increase in energy intake and body weight. PER2, orexin and ghrelin are dramatically reduced in CLOCK mutant animals at all time points of the light-dark cycle (Turek et al. 2005). BMAL1/CLOCK generates circadian rhythm of C/EBP alpha-mediated leptin transcription in adipose tissue. PER and CRY mutant animals show a similar disruption of peripheral clock and deregulation of leptin. Actually coupling of the central and peripheral CLOCK controls leptin homeostasis. Hence leptin resistance has an important role in circadian dysfunction-induced obesity and metabolic syndromes (Kettner et al. 2015). In diet induced obesity, expression of CLOCK in the ARC is down-regulated. In this case inhibition of CLOCK may be involved in leptin resistance and regulation of suppressor of cytokine signaling-3 in ARC (Xie et al. 2013). Leptin and adiponectin are strongly associated with glucose and lipid metabolism. Their synthesis and secretion display circadian rhythms that are disturbed in the obese state, while hyperleptinemia resulting in leptin resistance, adiponectin deficiency has been linked to the pathophysiology of the obesity-related disorders (Szewczyk-Golec et al. 2015).

On the other hand, impaired brown adipose tissue activity is an important mediator in the association between disturbed circadian rhythm and adiposity. Increasing the daily hours of light exposure decreases the uptake of fatty acids from triglyceride-rich lipoproteins, as well as of glucose from plasma selectively by brown adipose tissue (Kooijman et al. 2015). Adipose tissue clock genes regulate the hydrolysis of adipose tissue triglycerides and provide a rhythmic release of free fatty acids and glycerol from adipocytes. Disruption of circadian function decreases overall daily lipolytic activity and

blunts the lipolytic response to fasting (Yoshino and Klein 2013). Saturated fatty acid intake interacts with a high obesity genetic risk score in increasing BMI (Casas-Agustench et al. 2014). Loss of clock gene function or misalignment of circadian rhythms with feeding cycles results in impaired lipid homeostasis (Gooley and Chua 2014). About 13% of 263 lipid metabolites shows circadian variation in humans. Surprisingly inter-individual agreement for lipid metabolites identified as rhythmic is only about 20%. Eventually it is stated that there are different circadian metabolic phenotypes and an extensive diversity in circadian regulation of different lipid species in humans (Chua et al. 2013). In 1465 overweight/obese subjects carrying minor alleles at variants of SIRT1 and CLOCK were found to have a higher resistance to weight loss. This could be related to the chronotype of the subject, their higher plasma levels of ghrelin and less adherence to Mediterranean diet patterns (Garaulet et al. 2012).

8 The Risk of Obesity in Adulthood

Human genetic studies report associations between polymorphisms of CLOCK and obesity (Scott et al. 2008). Exposure to maternal obesity from pre-conception to birth as well as high fat diets influences the risk of obesity in the offspring. Hepatic mRNA expression of circadian (CLOCK, BMAL1, REV-ERB alpha, CRY, PER) and metabolic (PPAR alpha, SIRT1) genes were strongly suppressed in offspring exposed to both maternal obesity and high fat diet (Borengasser et al. 2014). Maternal obesity and diabetes associated with high birth weight, excessive nutrition in neonates, and rapid catchup growth also increase the risk of adult-onset obesity. Conversely, maternal undernutrition results in low birth weight with increased risk for long-lasting energy balance disorders with the impaired glucose uptake, insulin and leptin resistance, low-grade inflammation, modified sympathetic activity with reduced noradrenergic innervations, and thermogenesis (Lukaszewski et al. 2013). The hypothalamus-adipose axis plays a pivotal role in the maintenance of energy homoeostasis by controlling the nutritional status and energy storage level. The perinatal period largely corresponds to the period of brain maturation, neuronal differentiation and active adipogenesis. Impaired neurogenesis, neuronal functionality, nuclei structural organization and misalignment of circadian rhythms with feeding cycles led to a persistent reprogrammed appetite system that favors the orexigenic pathways, leptin/insulin resistance and hyperphagia (Breton 2013). Offspring from high-fat/high-sucrose-fed dams are heavier and have increased hepatic triglycerides, hepatic glycogen, blood glucose and plasma insulin compared with the offspring from chow-fed dams. In a similar manner supplementation of chocolate and soft drink during gestation and lactation contributes to early onset of hepatic steatosis (Kjaergaard et al. 2014).

The mRNA of PER1, PER2, BMAL1, and CRY1 clock genes are expressed in human hearts. Although PER1, PER2, and BMAL1 mRNAs reveal clear circadian rhythms in the human heart, no circadian rhythm is detected in CRY1 mRNA (Leibetseder et al. 2009). Furthermore, maternal obesity has a profound influence in the protein expression genes in heart and liver of offspring. The circadian clock undergoes nutritional programing, which may contribute to the alternations in energy metabolism associated with the development of metabolic disorders in early life and adulthood. Metabolic and inflammatory genes, CPT1b, PPAR alpha, PER2 show antiphase oscillations, whereas BMAL1 has greater oscillation amplitudes (Wang et al. 2015). Offspring exposed to maternal obesity and high fat consumption during development are more susceptible to developing behavioral disorders such as anxiety, depression, attention deficit, hyperactivity and autism spectrum disorders (Sullivan et al. 2014). Additionally, hepatic mRNA expression of circadian; CLOCK, BMAL1, REV-ERB alpha, CRY, PER genes and metabolic; PPAR alpha, SIRT1 genes are strongly suppressed in offspring exposed to both maternal obesity and high fat diet. Offspring from obese animals may exhibit impaired liver metabolism

in response to high fat diet via reduced PPAR alpha expression prior to the development of obesity (Borengasser et al. 2014). In offspring of obese dams, hepatic fatty acid oxidation decreases due to twofold reduction of hepatic long chain acyl-CoA dehydrogenase and three-fold reduction of SIRT3 protein levels. In this manner mitochondrial dysfunction precedes the development of detrimental obesity associated co-morbidities such as insulin resistance and nonalcoholic fatty liver disease (Borengasser et al. 2011).

The incidence of overweight or obese is increasing all over the world. Weight loss surgery for obesity seems to be the most effective option when other treatment modalities have failed. Surgery results in a greater improvement in weight loss related outcomes and weight associated comorbidities compared with the non-surgical interventions. Very few data have been obtained related to the effect of bariatric surgery on the circadian desynchronization (Colquitt et al. 2014). Severely obese individuals show a decrease in circulating free fatty acid 6 months after biliopancreatic diversion. Malabsorptive bariatric operations are associated with a drastic reduction in 24-h circulating free fatty acid concentrations, improvement of insulin resistance and changes in the pattern of leptin peaks independent of weight loss (Raffaelli et al. 2015). Actually all forms of weight loss surgery lead to caloric restriction, weight loss and decrease in fat mass (Gumbs et al. 2005). While duodenal-jejunal bypass induces marked expressions of glucose transporter-2 (GLUT2) in the liver, gluconeogenic enzymes, phosphoenolpyruvate carboxykinase-1 and glucose-6-phosphatase decrease. In these animals, circadian transcription factors CRY1 and PER2 increase in the liver and decrease in the intestine (Kim et al. 2015).

9 Conclusion

In addition to the influence of dietary nutrients on circadian rhythms, the daily timing of food intake has itself been shown to affect body weight regulation in mammals (Summa and Turek 2014). Altered timing of food intake can lead to uncoupling of peripheral clocks from the central pacemaker (Oosterman et al. 2015). In other word, unusual feeding time can cause the disruption of the circadian system which might reveal unhealthy consequences in humans (Garaulet and Gómez-Abellán 2014). Late lunch eaters loose less weight and display a slower weight-loss rate than early eaters (Garaulet et al. 2013b). Calorie restriction entrains the SCN clock, whereas timed meals entrain peripheral oscillators (Froy 2007).

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Eat and Death: Chronic Over-Eating

3

Atilla Engin

Abstract

Obesity-related co-morbidities decrease life quality, reduce working ability and lead to early death. The total amount of dietary fat consumption may be the most potent food-related risk factor for weight gain. In this respect, dietary intake of high-caloric, high-fat diets due to chronic over-eating and sedentary lifestyle lead to increased storage of triglycerides not only in adipose tissue but also ectopically in other tissues. Increased plasma concentrations of non-esterified free fatty acids and lipid-overloaded hypertrophic adipocytes may cause insulin resistance in an inflammation-independent manner. Even in the absence of metabolic disorders, mismatch between fatty acid uptake and utilization leads to the accumulation of toxic lipid species resulting in organ dysfunction. Lipid-induced apoptosis, ceramide accumulation, reactive oxygen species overproduction, endoplasmic reticulum stress, and mitochondrial dysfunction may play role in the pathogenesis of lipotoxicity. The hypothalamus senses availability of circulating levels of glucose, lipids and amino acids, thereby modifies feeding according to the levels of those molecules. However, the hypothalamus is also similarly vulnerable to lipotoxicity as the other ectopic lipid accumulated tissues. Chronic overnutrition most likely provides repetitive and persistent signals that up-regulate inhibitor of nuclear factor kappa B kinase beta subunit/nuclear factor kappa B (IKKβ/NF-κB) in the hypothalamus before the onset of obesity. However, the mechanisms by which high-fat diet induced peripheral signals affect the hypothalamic arcuate nucleus remain largely unknown. In this chapter, besides lipids and leptin, the role of glucose and insulin on specialized fuel-sensing neurons of hypothalamic neuronal circuits has been debated.

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Keywords

Obesity • Lipotoxicity • Free fatty acid • Mitochondria • Obesity paradox • Chronic overnutrition • Overfeeding • Heart failure • Uncoupling protein (UCP) • Mammalian target of rapamycin (mTOR) • Adenosine monophosphate-activated protein kinase (AMPK) activity • Ceramide

1 Introduction

It is possible that the lifespan will increase up to hundred years or more if persons stop destroying themselves with negative emotions and bad habits, including unhealthy food and overeating (Rzheshevsky 2013). Excess lipid utilization or activation of signaling pathways by lipid metabolites may disrupt cellular homeostasis and contribute to cell death which defines the concept of lipotoxicity. Lipotoxicity occurs in multiple organs, including cardiac and vascular tissues (Wende et al. 2012). Elevated free fatty acid concentrations lead to necrotic cell death, which depends on functional mitochondria. The accumulation of reactive oxygen species (ROS) by loss of membrane integrity may cause the release of nuclear high mobility group box 1 (HMGB1), which is a highly conserved DNA-binding protein (Rockenfeller et al. 2010). Chronic exposure of the heart to high plasma levels of free fatty acids may promote the accumulation of toxic lipid intermediates within cardiomyocytes. Furthermore, suppression of glucose oxidation by increased fatty acid uptake shunts glucose into the oxidative pentose phosphate and hexosamine biosynthetic pathways, both of which yield potentially harmful products (Chess and Stanley 2008).

Actually mitochondria sense the status of metabolism and change their functions to regulate energy production, cell death, and thermogenesis. Thereby abnormalities in mitochondrial division and fusion are linked to the obesity (Roy et al. 2015). Mitochondria are highly dynamic organelles and the balance in fusion/fission is strictly associated with their bioenergetics. Fusion processes are associated with the optimization of mitochondrial function, whereas fission processes are associated with the removal of damaged mitochondria. In contrast to saturated fatty acids, omega 3 polyunsaturated fatty acids induce fusion processes and improve mitochondrial function (Putti et al. 2015). Thus palmitate-induced endoplasmic reticulum (ER) stress also induce adiponectin resistance, assessed by the adenosine monophosphate-activated protein kinase (AMPK) phosphorylation, via reducing an adaptor protein containing pleckstrin homology (PH) domain, phosphotyrosine binding (PTB) domain, and adaptor protein, phosphotyrosine interacting with PH domain and leucine zipper 1motif (APPL1) expression (Park et al. 2015). APPL1 transmits signals from adiponectin receptors to downstream targets by directly interacting with the NH2-terminal intracellular region of adiponectin receptor protein 1 (AdipoR1) and AdipoR2 (Mao et al. 2006).

Overfeeding with different types of fat results in the different cardiometabolic effects. If the excess energy is provided from polyunsaturated fatty acids (PUFA) versus saturated fatty acids (SFA), atherogenic lipoproteins reduces. Furthermore, modest weight gain in young individuals induces hyperproinsulinemia and increases biomarkers of endothelial dysfunction (Iggman et al. 2014). The evaluation of 607 randomized controlled trials and prospective cohort studies revealed that the partial replacement of SFA with PUFA decreases the risk of cardiovascular diseases. Furthermore, PUFA or monounsaturated fat (MUFA) lowers fasting serum/plasma total and LDL cholesterol concentrations. Nevertheless highly controlled randomized trials and prospective cohort studies with the sufficient number of subjects and long enough duration are required in order to clarify the cardiometabolic

effects of the quality of dietary fat (Schwab et al. 2014). Adipose tissue is able to determine the functions of excess dietary lipids whether the body homeostasis will be maintained or a state of inflammation and insulin resistance will be produced with deleterious cardiovascular consequences (Bastien et al. 2014). Actually, lipid accumulation in the heart, skeletal muscle, pancreas, liver, and kidney due to excessive plasma non esterified fatty acids (NEFA) levels results in myocardial triglyceride accumulation and obesity-associated heart failure (Schaffer 2003). During over-nutrition, lipids over-accumulate in nonadipose tissues and subsequently fatty acids are utilized in ceramide production pathway, which is through increased nitric oxide formation, causes apoptosis of lipid-laden beta-cells and cardiomyocytes (Unger 2002). Thus Zhou et al. found two to three times higher levels of ceramide, a mediator of apoptosis, and four times greater inducible nitric oxide synthase levels in obese subjects than controls. Furthermore, myocardial DNA laddering, an index of apoptosis, reaches 20 times of normal levels. In this case obesity-related cardiac dysfunction is caused by lipoapoptosis and is prevented by reducing cardiac lipids (Zhou et al. 2000). Left ventricular structure, diastolic and systolic functions and the other risk factors for heart failure, including hypertension and coronary heart disease are markedly increased in obese patients (Lavie et al. 2013). However, combining the right heart hemodynamic variables with a functional evaluation of the right ventricle is more accurate to define the risk of heart failure (Ghio et al. 2001). Obesity may result in heart failure and death by inducing haemodynamic and myocardial changes that lead to cardiac dysfunction (Ebong et al. 2014). Progressive caloric restriction also induces a dose-dependent increase in myocardial triglyceride content and a dose-dependent decrease in diastolic function in lean healthy men (Hammer et al. 2008b). Recently it has been shown that the risk for overall mortality, in particular cardiovascular mortality and length of hospitalization is highest in patients with chronic heart failure who are underweight, when compared with obese subjects (Sharma et al. 2015). Despite the obesity-related coronary heart disease and heart failure, high body mass index (BMI) and body fat is found to be paradoxically associated with improved survival in secondary care of coronary heart disease and heart failure (De Schutter et al. 2013). At once the heart failure has developed; it is thought that the already existing obesity confers a beneficial influence on prognosis. This event is defined as obesity paradox (Rayner et al. 2015). Indeed, cardiorespiratory fitness modifies the inverse association between obesity and mortality in patients with known or suspected cardiovascular disease. Among the 30,104 overweight patients who suffer from cardiovascular diseases, in case of high cardiorespiratory fitness the obesity paradox persists and risk of all-cause mortality is the lowest. (McAuley and Beavers 2014). In fact, cardiorespiratory fitness is a measurement of metabolic equivalent achieved during a maximal exercise test. Highly fit overweight men have the lowest mortality risk (McAuley et al. 2010). Resolving the mechanism of the "obesity paradox" in patients with heart failure is necessary before recommendations are made concerning the weight control (Curtis et al. 2005).

2 Chronic Overnutrition

Obesity-related comorbidities decrease life quality, reduce working ability and lead to early death. Environmental factors as well as habitual diet play an important role in the obesity epidemic. Genetic determinants of habitual dietary intake may cause an interplay between diet, genes and obesity (Hasselbalch 2010). Obesity may also develop by the way of behavioral risk factors in addition to genetic mechanisms. Although a high-fat diet does not guarantee that an individual will be obese, considerable evidences indicate that the total amount of dietary fat consumption may be the most potent food-related risk factor for weight gain (Blundell and Cooling 1999). Chronic overnutrition is associated with increased plasma concentrations of NEFA. Fasting NEFA levels are higher in obese individuals compared to the lean participants. Additionally, metabolically healthy obese individuals have higher fasting NEFA levels

versus their insulin-resistant counterparts (Il'yasova et al. 2010; Normand-Lauzière et al. 2010). Accelerated fat deposition with perinatal over-nutrition may alter the sensitivity of adipose tissue to obesity-induced inflammation. Consequently, accelerated fat gain during development could induce pathological remodeling of the extracellular matrix. In this case early postnatal over-nutrition may be a critical determinant of ectopic fat accumulation and insulin resistance in obese adults by programming the inflammatory capacity of adipose tissue (Kayser et al. 2015). However, lipid-overloaded hypertrophic adipocytes alone may cause insulin resistance in inflammation-independent manner (Kim et al. 2015). A high correlation occurs between the eating behaviour pattern and the metabolic syndrome. Thus obese individuals show the hyperphagic type of eating behavior pattern when compared with the healthy participants. With the emergence of insulin resistance, hypertrophy of visceral adipose tissue develops gradually. Moreover, significant alterations in cortisol and adrenocorticotropic hormone (ACTH) secretions may occur in obesity (Benbaibeche et al. 2015). In high-fat diet induced obesity, the genes related to lipolysis, fatty acid metabolism, mitochondrial energy transduction, oxidation-reduction and insulin sensitivity are down-regulated, whereas extracellular matrix components- and inflammation-associated genes are up-regulated (Choi et al. 2015). Actually, lipogenic proteins, inflammatory molecules, peroxisome proliferator-activated receptor-gamma (PPAR-gamma) coactivator-1alpha (PGC-1alpha), and protein arginine methyl transferase-1 (PRMT1) are increased in the livers of high-fat diet fed animals. In this instance thioredoxin-interacting protein mediates hepatic lipogenesis and inflammation via PRMT1 and PGC-1alpha regulation (Park et al. 2014). The methyl donor deficiency abolishes fatty acid oxidation and energy metabolism of myocardium through imbalanced methylation/acetylation of PGC-1alpha and decreased expression of PPAR alpha (Garcia et al. 2011). Impaired mitochondrial fatty acid oxidation due to hypomethylation of PGC1-alpha indicates the presence of a link between methyl donor deficiency and epigenomic deregulations of energy metabolism (Pooya et al. 2012). Overfeeding in addition to deficit in methyl donors increases central fat mass and leads to a dramatic increase of plasma free fatty acids in offspring. High-fat diet and overfeeding impair AMPK-dependent phosphorylation of PGC-1alpha, while methyl donor deficiency decreases PGC-1alpha methylation through decreased expression of PRMT1 and cellular level of S-adenosyl methionine (Guéant et al. 2014). In parallel with the progression of obesity, the incidence of its associated endocrine, metabolic and other medical disorders increase. Meanwhile, lipid accumulation in the body augments the synthesis of various inflammatory mediators (Bellentani and Marino 2009; Golden et al. 2009; Hevener et al. 2010). Inherently, a chronic nutrient overload leads to an increase in adipose tissue. Subsequent to adipocyte hypertrophy, the ER stress may activate the metabolic pathways that trigger insulin resistance, release of macrophage chemoattractant proteins, and chronic inflammation. Insulin-resistant adipocytes enhance the release of free fatty acids. Increased circulating free fatty acid levels lead to decreased lipid oxidation in non-adipose tissues. As a result of this vicious cycle, ectopic lipid accumulation causes lipotoxicity and insulin resistance in non-adipose tissues (Lionetti et al. 2009). In a similar manner the increase in plasma free fatty acid has been associated with an increased cardiac fatty acid uptake. While the increase in plasma free fatty acid is two-fold after partial starvation, this increase reaches to three-fold following complete starvation. Simultaneous rise in myocardial lipid contents are two and 3.6-fold after partial and complete starvation, respectively (Bilet et al. 2011; Hammer et al. 2008b). Human homologue of fatty acid transporter protein (FAT)/CD36, plasma membrane fraction of fatty acid-binding protein (FABP-pm) and fatty acid transport protein (FATP)1, 4 and 6 may be effective in carrying the myocardial lipid (Glatz et al. 2010). However, the first two appears to be key transporters for myocardial tissue (Luiken et al. 1999). Increased involvement of FAT/CD36 is causally linked to the increase in fatty acid uptake by the cardiac

myocytes in the presence of AMPK activation. Furthermore, the stimulatory effect of activation on fatty acid uptake is independent of insulin (Luiken et al. 2003). AMPK activation simultaneously stimulates the protein expression of both fatty acid transporters, FAT/CD36 and FABPpm in dose-dependent manner via the AMPK signaling pathway (Chabowski et al. 2006). Long-chain fatty acids (LCFAs) transporters and rates of LCFA transport are also altered in obesity. By contrast with the findings of Luiken et al., insulin rapidly up-regulates FAT/CD36 protein expression by cardiac myocytes via the phosphatidylinositol 3-kinase (PI3K)/Akt insulin-signaling pathway, whereas the increase in synchronous expression of FABPpm cannot achieved by this hormone (Chabowski et al. 2004). Even in the absence of metabolic disorders, mismatch between myocardial fatty acid uptake and utilization leads to the accumulation of cardiotoxic lipid species that results in cardiac dysfunction (Chiu et al. 2005). Thereby computing of lipid over-accumulation provides better results than the weight gain for identifying cardiovascular risk (Kahn 2005). In particular lipid accumulation products predict mortality in nondiabetic obese patients with high risk for cardiovascular diseases (Ioachimescu et al. 2010). Nevertheless in adult fasting mammals, most of the cardiac energy demands are provided by the fatty acids oxidation (Siess 1980). However, in fed-state higher concentration of circulating insulin ensures a partial switch to glucose oxidation, while the cardiac fatty acid loading is reduced. For this reason, energy production pattern in the heart is regulated by insulin signaling as well as the availability of fatty acids (Liepinsh et al. 2014).

Sterol-regulatory element binding protein (SREBP)-1c/PPAR gamma pathway also contributes to heart lipotoxicity by promoting lipid accumulation within myocytes of patients with metabolic syndrome (Marfella et al. 2009). Excess fatty acids are converted to myocardial triacylglycerol (TAG) and stored in lipid droplets (LDs) for subsequent energy production via mitochondrial beta-oxidation (Kienesberger et al. 2013). Actually excess TAG accumulation contributes to obesity-induced cardiomyopathy. Cardiomyocyte-specific adipose triglyceride lipase (ATGL) overexpression prevents cardiac steatosis and decreases fatty acid utilization (Pulinilkunnil et al. 2014). Perilipin 5 (Plin5)-coated lipid droplets are resistant toward ATGL-mediated triglycerides catabolism. Cardiac muscle expressed Plin5 protects the cardiac triglyceride pool from uncontrolled hydrolysis and the excessive release of free fatty acids and glycerol (Pollak et al. 2013). In this respect Plin5 protects heart lipid droplets from attack by ATGL under physiological conditions (Kuramoto et al. 2012). Distribution of ATGL occurs from the cytosol and from large lipid droplets to small lipid droplets. Hormone-sensitive lipase or ATGL overexpression indicates the increased triglyceride-specific hydrolase capacity, but only ATGL overexpression increases whole cell lipolysis (Bezaire et al. 2009). Moreover, lipolysis increases in ectopic perilipin 5 and ATGL expressing cells following activation of protein kinase A (Wang et al. 2011b). Activation of protein kinase A by cyclic adenosine monophosphate leads to polyphosphorylation of perilipin 5 and stimulation of triglyceride lipase activity (Brasaemle 2007). ATGL activity is inhibited by long-chain acyl-CoAs in a non-competitive manner. In this case inhibition of the major lipolytic enzymes ATGL and hormone-sensitive lipase by long-chain acyl-CoAs protects the cells from lipotoxic concentrations of fatty acids and fatty acid-derived lipid metabolites (Nagy et al. 2014).

On the other hand, stored triglycerides are synthesized by the enzyme diacylglycerol acyl transferase (DGAT). Forced expression of DGAT1 in the heart may cause the development of a significant cardiomyopathy due to triglyceride accumulation (Glenn et al. 2011). By contrast with the previous findings, Liu et al. showed that overexpression of DGAT1 is not toxic to the heart but reduces levels of toxic lipids and improves lipotoxic cardiomyopathy (Liu et al. 2012). At first DGAT1 reduces diacylglycerol and ceramide accumulation by enhancing triglyceride synthesis. Most probably DGAT plays a dual role in the development of lipotoxic cardiomyopathy. (Liu et al. 2012). However, with the progression of obesity, excess fatty acid uptake of cardiomyocyte exceeds mitochondrial oxidative

capacity and cardiac steatosis arises. Lipotoxic intermediates, ceramide and acylcarnitine synthesis increases. Insulin-resistant heart in obesity is obliged to utilize fatty acids for energy demand. Increased fatty acid oxidation and triglyceride accumulation is the feature of lipotoxic cardiomyopathy (Zhang and Ren 2011). Although some studies indicate a correlation between cardiac function and triglyceride content, it is not proven that changes in triglyceride content directly cause cardiac dysfunction. Nevertheless, lipid-induced apoptosis, ceramide accumulation, ROS overproduction, ER stress, and mitochondrial dysfunction may play role in the pathogenesis of lipotoxic cardiomyopathy (Wende and Abel 2010). The induction of PPAR-gamma by SREBP-1c is extremely important in the regulation of intracellular lipid stores in human heart. In this respect overfeeding leads to the non-oxidative or oxidative cardiomyocyte death and heart dysfunction following a series of sequential steps. Initially, increased expression of the lipogenic transcription factor SREBP-1c and of PPAR gamma enhances lipogenesis, later on ectopic lipid deposition occurs in myocardium (Marfella et al. 2009). Since lipotoxic cardiomyopathy develops in states of obesity, high rates of fatty acid oxidation are associated with the accumulation of neutral lipid in the form of triglyceride within the myocyte. The PGC-1 coactivators are key regulators of mitochondrial function and biogenesis. Cardiac failure relates to mitochondrial dysfunction which depends on the loss of the PGC-1 coactivators-mediated control (Scarpulla et al. 2012).

Furthermore, cardiac metabolism impairment related to western diet-induced obesity is probably due to damaged myocardial oxidative capacity, reduced mitochondrial biogenesis and mitochondrial uncoupling (Neves et al. 2014). With the rise of fatty acids, cardiac mitochondrial uncoupling proteins (UCPs) increase and myocardial glucose transporter protein decreases. In this manner actual heart failure caused by energy deficiency depends on increased mitochondrial UCPs (Murray et al. 2004). In brief, fatty acid-induced mitochondrial uncoupling reduces cardiac efficiency by limiting ATP production and increasing myocardial oxygen consumption in obesity (Boudina et al. 2005). If the fatty acid delivery to the mitochondria exceeds oxidative capacity, human UCP3 is up-regulated, whereas the oxidative capacity of mitochondria is enhanced, UCP3 is down-regulated. In first mentioned case, NEFAs cannot pass through the inner mitochondrial membrane and accumulate inside the mitochondrial matrix. Thus mitochondria are protected by up-regulation of UCP3 against the accumulation of fatty acids (Schrauwen et al. 2001). The main function of UCP3 is to export fatty acid anions away from the mitochondrial matrix. In this process fatty acids are exchanged with protons through the uncoupling activity of UCP3 (Hesselink et al. 2003). UCPs 2 and 3 are not only activated by ROS or ROS by-products to induce proton leak, but also are controlled by covalent modification by glutathione to decrease ROS emission (Mailloux and Harper 2011). Increased oxidative stress and exacerbated inflammatory outcomes are key features of obesity. Thus, protons may be shifted to the mitochondrial matrix via different uncoupling proteins (Bondia-Pons et al. 2012). Mitochondrial uncoupling alleviates the production of ROS by the release of excessive antioxidant capacities (Jezek and Hlavatá 2005). Nevertheless, accumulation of visceral fat promotes synthesis of proinflammatory adipokines and the ROS formation. In the cardiometabolic syndrome visceral adipose tissue-specific increase in ROS are derived substantially from NADPH oxidase reaction (DeMarco et al. 2010). In the absence of exogenous substrates, cardiac mitochondria have a surprisingly large capacity to oxidize endogenous substrates in response to a decrease in membrane potential. Extramitochondrial environment can remove mitochondrial H_2O_2 emission which is formed during oxidation of lipid-based substrates. Thereby, a reduced environment is maintained in the matrix. This mechanism may be important for maintaining-oxidation flux and ATP production if mitochondrial uptake of fatty acids is acutely inadequate (Korge and Weiss 2006).

Dietary intake of high-caloric high-fat diets and sedentary lifestyle lead to increased storage

of triglycerides not only in adipose tissue but also ectopically in other tissues (Szendroedi and Roden 2009). Given all the evidence, myocardial triglyceride may be related to the specific cause of disease rather than the severity of cardiac dysfunction. In dilated cardiomyopathy, myocardial triglyceride may be affected by an overweight state rather than cardiac sympathetic nerve dysfunction (Nakae et al. 2010). Prolonged caloric restriction in obese type 2 diabetes mellitus patients decreases BMI and improves blood glucose regulation associated with decreased myocardial triglyceride content and improved cardiac functions (Hammer et al. 2008a), whereas short-term caloric restriction induces accumulation of myocardial triglycerides (van der Meer et al. 2007). Upon high-fat feeding, impaired calcium handling due to a lower cardiac ATP supply is the main cause of cardiac dysfunction in the absence of apoptosis (Relling et al. 2006). Increased myocardial fat uptake leads to reduced energy efficiency by inducing mitochondrial damage and uncoupling, increasing ROS production and impairing mitochondrial calcium handling. In advanced stages of obesity, palmitate toxicity, ceramide and DAG formation, ER stress, membrane destabilization and inflammation, all at once may also contribute to the lipoapoptosis and subsequent decreased function of the fatty heart (van de Weijer et al. 2011).

When compared with the lean subjects, the markers for apoptosis are higher in the myocardium of obese patients. Furthermore, mRNA levels of enzymes involved in synthesis and degradation of ceramides are markedly increased in obese subjects (Baranowski et al. 2010). ER stress is a critical intracellular event that induces metabolic diseases under conditions of overnutrition (Ozcan et al. 2004, 2006).

In obesity, increased lipid delivery can stimulate an increased influx of unfolded proteins in the ER, which can lead to a mismatch between the unfolded protein response and protein translation, inducing stress of the ER (Ron and Walter 2007). If the ER stress is prolonged, or the adaptive response fails, apoptotic cell death ensues (Gorman et al. 2012). On aggregation of unfolded proteins, GRP78 dissociates from the three ER stress receptors, pancreatic ER kinase (PKR)-like ER kinase (PERK), activating transcription factor 6 (ATF6) and inositol-requiring enzyme 1 (IRE1), allowing their activation. Signaling through PERK, ATF6 and IRE1 can trigger proapoptotic signals during prolonged ER stress. Upon activation of the transcription factor C/EBP homologous protein (CHOP) and the c-Jun N-terminal kinase (JNK) pathway, at first the anti-apoptotic effect of B-cell lymphoma 2 (BCL2) is eliminated, later ER stress-induced apoptosis is initiated (Szegezdi et al. 2006). Furthermore, over-nutrition causes ER stress and hypothalamic IKKβ/NF-κB activation, which is reflected by increased levels of both PERK and eukaryotic initiation factor 2 alpha (eIF2alpha) phosphorylation. Chronic overnutrition provides repetitive and persistent signals that up-regulate IKKβ/NF-κB in the hypothalamus. Consequently, IKKβ/NF-κB activation results in both central insulin and leptin resistance (Zhang et al. 2008). Recent research shows that suppressor of cytokine signaling 3 (SOCS3) is a critical inhibitor for both insulin and leptin signaling in the hypothalamus (Howard et al. 2004; Mori et al. 2004; Ueki et al. 2004). Activation of IKKβ/NF-κB at the intracellular level in the hypothalamic neurons by chronic overnutrition discriminates the chronic overnutrition from obesity (Zhang et al. 2008). Recent research has revealed that SOCS3 is a common inhibitor for leptin and insulin signaling (Howard and Flier 2006). The pathogenic induction of hypothalamic SOCS3 during over-nutrition also requires NF-κB (Zhang et al. 2008) (Fig. 3.1).

Epicardial adipose tissue is a source of several inflammatory mediators in high-risk cardiac patients. (Mazurek et al. 2003). Main physiological functions of human epicardial adipose tissue are lipid storage for the energy needs of the myocardium and regulation of luminal size of the coronary arteries. In contrast, epicardial adipose tissue plays an adverse paracrine role in lipotoxic cardiomyopathy (Sacks and Fain 2011). Epicardial adipose tissue serves as a buffer for the storage of free fatty acids for the heart. However, the increased epicardial thickness as seen in obesity has been associated with pro-inflammatory cytokine production (Iacobellis

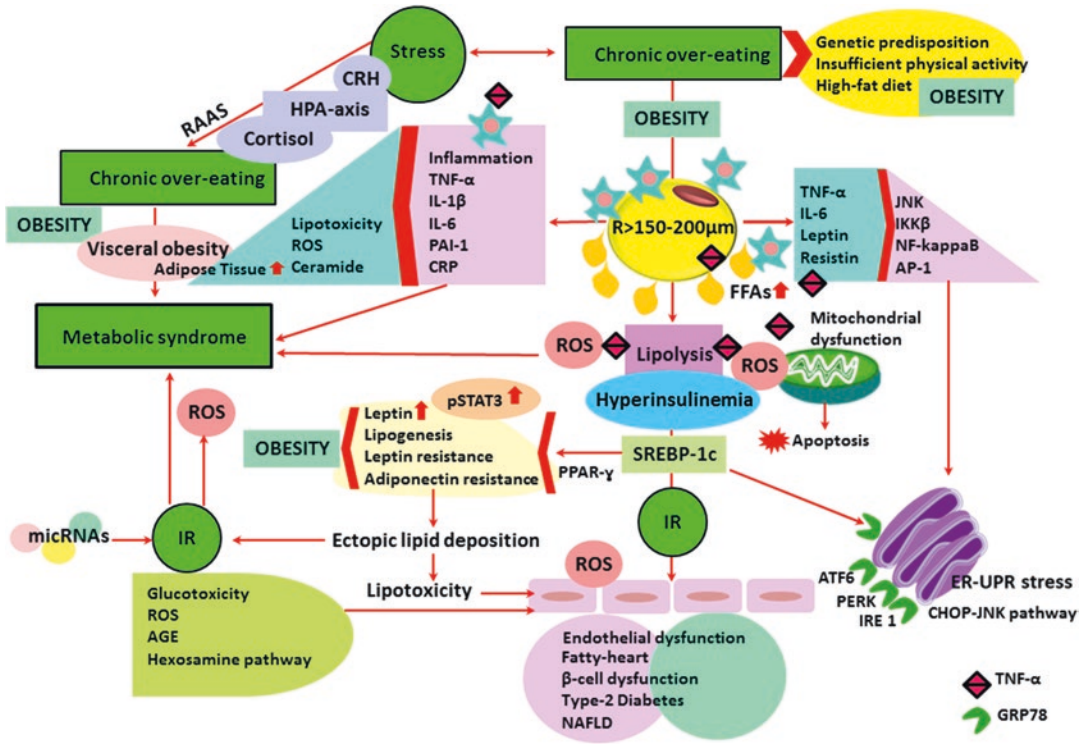

Fig. 3.1 Chronic nutrient overload leads to an increase in adipose tissue. Mismatch between fatty acid uptake and utilization leads to the accumulation of toxic lipid species that results in organ dysfunction. Subsequent to adipocyte hypertrophy, the endoplasmic reticulum stress activates the metabolic pathways that trigger insulin resistance, release of macrophage chemoattractant proteins. Lipid-induced apoptosis, ceramide accumulation, reactive oxygen radical overproduction, endoplasmic reticulum stress, and mitochondrial dysfunction play role in the pathogenesis of lipotoxicity (*CRH* Corticotropin Releasing Hormone; *HPA axis* Hypothalamic-Pituitary-Adrenal Axis; *RAAS* Renin-angiotensin-aldosterone system; *ROS*Reactive oxygen radicals; *TNF-α* Tumor necrosis factor-alpha; *IL-1β* Interleukin-1 beta; *IL-6* Interleukin-6; *PAI-1* plasminogen activator inhibitor-1; *CRP* C-reactive protein; *JNK* c-Jun N-terminal kinase; *IKKβ* Inhibitor kappa B kinase-beta; *NF-kappaB* Nuclear factor-kappa B; *AP-1* activating protein-1; *AGE* advanced glycation end product; *FFA* Free fatty acid; *SREBP-1c* Sterol regulatory element-binding protein-1c; *PPAR-ɣ* Peroxisome proliferator-activated receptor-gamma; *IR* Insulin resistance; *micRNAs* MicroRNA; *pSTAT3* Phosphorylated signal transducer and activator transcription 3; *ATF6* Activating transcription factor 6; *CHOP* CCAAT/enhancer-binding protein homologous protein; *IRE1* Inositol requiring enzyme 1; *PERK* PRKR-like endoplasmic reticulum kinase; *GRP78* 78-kD glucose-regulated/binding immunoglobulin protein; *NAFLD* Nonalcoholic fatty liver disease; *ER* Endoplasmic reticulum; *UPR* Unfolded protein response)

et al. 2005). Thirteen cytokines are identified in obesity-associated epicardial adipose tissue. Among these, interleukin 6 (IL-6), IL-8, monocyte chemoattractant protein 1 (MCP-1), plasminogen activator inhibitor 1 (PAI-1), growth-related oncogene-alpha, and macrophage migration inhibitory factor (MIF) are the most abundant ones in epicardial adipose stores of patients with obesity and coronary artery disease, whereas adiponectin release is suppressed (Karastergiou et al. 2010). As mentioned above epicardial adipose tissue has been identified as an active source of inflammatory mediators (Mazurek et al. 2003). Due to the close relationship between this fat depot and the myocardial tissue, coupled with the lack of fascial boundaries, epicardial adipose tissue may locally interact and modulate the myocardium through secretion of pro- and anti-inflammatory cytokines (Iacobellis et al. 2005).

On the other hand, obesity-induced inflammation may be a causal pathway for depression. In

this case inflammatory cytokines interact with the neurotransmitter metabolism, neuroendocrine functions, and synaptic plasticity which are all relevant to risk for depression. In some cases, prior depression may increase risk for the subsequent development of adiposity (Shelton and Miller 2010). Obese individuals are at an increased risk of suicide. This risk seems to persist despite treatment of obesity with bariatric surgery (Heneghan et al. 2012). Bariatric surgery is one of the most effective treatments for morbid obesity and decreases the overall mortality due to obesity in patients who received bariatric surgery. However bariatric surgery patients show higher suicide rates than the general population (Peterhänsel et al. 2013). Thus compared with age and sex-matched suicide rates in the United States of America, there was a substantial excess of suicides among all patients who had bariatric surgery. The overall rate is 6.6/10,000, of these 13.7 per 10,000 are among men and 5.2 per 10,000 are among women. While about 30% of suicides occur within the first 2 years following surgery, almost 70% occurs within 3 years (Tindle et al. 2010).

3 AMPK–Acetyl CoA Carboxylase–Malonyl CoA Pathway in the Regulation of Food Intake and Body Weight

Obesity results from an imbalance between energy intake and energy expenditure. The hypothalamus, in particular the arcuate nucleus has an important impact in the regulation of energy balance by modulating the hormonal signals as well as nutritional signals (Wisse et al. 2007). In the hypothalamus, malonyl CoA controls food intake and energy expenditure as a critical intermediate of fatty acid metabolism (Loftus et al. 2000). Actually, hypothalamic malonyl-CoA levels depend on the activities of acetyl-CoA carboxylase and fatty acid synthase. It also regulates the feeding behavior by monitoring global energy status (Hu et al. 2003). While fasting hypothalamic malonyl-CoA decreases, refeeding increases hypothalamic malonyl-CoA. However, malonyl-CoA level is under the control of AMPK which phosphorylates/inactivates acetyl CoA carboxylase. On the other hand, malonyl-CoA is an inhibitor of carnitine palmitoyl-CoA transferase-1 (CPT1), which is an outer mitochondrial membrane enzyme that regulates the entry of fatty acids into mitochondria and subsequent mitochondrial oxidation of fatty acids. Paradoxically, CPT1c protects against the effects of a high-fat diet (Lane et al. 2008). The increase in fatty acid oxidation and the phosphorylation/inactivation of acetyl-CoA carboxylase leads to reduced malonyl-CoA concentration. In this case, decrease in malonyl-CoA releases carnitine/CPT1 from inhibitory restriction and facilitates the entry of fatty acids into mitochondria for beta oxidation. Thereby, the hypothalamic malonyl-CoA signal regulates fatty acid oxidation and energy expenditure by communicating with the expression of PPARalpha, a transcriptional activator of fatty acid oxidizing enzymes, and UCP3, a thermogenic mitochondrial uncoupling protein (Cha et al. 2005).

The cellular level of malonyl-CoA depends on its rate of synthesis catalyzed by acetyl-CoA carboxylase, relative to its rate of utilization and degradation catalyzed by fatty acid synthase and malonyl-CoA decarboxylase, respectively. Fatty acid synthase inhibitors increase the hypothalamic malonyl-CoA levels and decrease food intake (Hu et al. 2005). The enhanced expression of malonyl-coenzyme A decarboxylase within hypothalamic region increases food intake and provokes progressive weight gain. The increase in hypothalamic malonyl-coenzyme A appears to be the primary disorder in the central nutrient-sensing pathway, which disrupts energy homeostasis and induces obesity (He et al. 2006).

4 Hormonal Regulation of Hypothalamic Appetite Control

Over-nutrition-induced metabolic dysfunction and excess lipid accumulation in non-adipose tissues may cause severe health disorders. Lipid

overload results from mismatch between free fatty acid uptake and utilization. Lipotoxicity due to lipid accumulation in the heart, skeletal muscle, pancreas, liver, and kidney play an important role in the pathogenesis of obesity-related cellular stress and inflammation (Schaffer 2003; van Herpen and Schrauwen-Hinderling 2008). The hypothalamus senses availability of circulating levels of glucose, lipids and amino acids, thereby modifies feeding according to the levels of those molecules. However, the hypothalamus is also similarly vulnerable to lipotoxicity as the other ectopic lipid accumulated tissues (Lam et al. 2005; López et al. 2007). Furthermore, hypothalamic neurons themselves are capable of an inflammatory response to fatty acid excess, and that inducing fatty acid oxidation in the neurons themselves can reduce this. Therefore, the increase in hypothalamic fatty acid oxidation may reverse the response to high-fat diet (McFadden et al. 2014). In this respect long-chain saturated fatty acids exert an inflammatory stimulus in hypothalamus by triggering the intracellular signaling network via toll-like receptor 4 (TLR4). Actually TLR4 acts as a predominant molecular target for saturated fatty acids in the hypothalamus (Milanski et al. 2009). On the other hand, insulin controls fatty acid release from white adipose tissue through direct effects on adipocytes and indirectly through hypothalamic signaling. Overfeeding induces insulin resistance and lipolysis in white adipose tissue, as well as simultaneously abolishes hypothalamic insulin action (Scherer et al. 2012). Indeed, IKKβ/NF-κB in the hypothalamic neurons responds to the metabolic signals which are produced by over-nutrition. Chronic overnutrition most likely provides repetitive and persistent signals that up-regulate IKKβ/NF-κB in the hypothalamus before the onset of obesity. In fact, activation of IKKβ/NF-κB is not physiologically required. Activating IKKβ/NF-κB elevates ER stress in the hypothalamus, which is reflected by increased levels of both PERK and eIF2alpha phosphorylation. Thus IKKβ/NF-κB in the hypothalamus mediates both hypothalamic insulin and leptin resistance (Zhang et al. 2008).

As an intermediate in fatty acid synthesis, hypothalamic malonyl-CoA appears to be an anorectic mediator in the central control of feeding. Malonyl-CoA inhibits the acyltransferase activity of CPT-1. Thereby, CPT-1 is considered as a downstream effector in hypothalamic malonyl-CoA action on feeding. However, the inhibition of CPT-1 acyltransferase activity does not play an important role in the feeding effect of either leptin or fatty acid synthase inhibitor cerulenin. Furthermore, CPT-1c may link malonyl-CoA to ceramide metabolism to affect food intake in the arcuate nucleus (Gao et al. 2013).

Energy restriction up-regulates expression of the orexigenic neuropeptide Y (NPY) and agouti related peptide (AgRP) and down-regulates that of the anorexigenic alpha-melanocyte stimulating hormone or its precursor pro-opiomelanocortin and the co-expressed cocaine and amphetamine-regulated transcript in the arcuate nucleus of the hypothalamus (Sainsbury and Zhang 2010). In this manner, the arcuate nucleus consists a neuronal cycle together with the neurons which are located in the other hypothalamic nuclei (Lopaschuk et al. 2010). Moreover, nuclei within the hypothalamus integrate peripheral signals such as adiposity and caloric intake to regulate important pathways within the central nervous system controlling food intake and energy expenditure. In this regard, firmly established pathways involve the orexigenic NPY/AgRP and the anorexigenic pro-opiomelanocortin/cocaine-and amphetamine-related transcript (POMC/CART) neurons in the arcuate nucleus of the hypothalamus (Simpson et al. 2009).

Fatty acid availability in the hypothalamus is important for the regulation of energy balance. The major pools of circulating fatty acids are either albumin-bound free fatty acids released by lipolysis from adipose tissue triglyceride storage pools or free fatty acids contained within triglyceride-rich lipoproteins (Wang et al. 2011a). However, the blood-brain barrier acts as an interface between the central nervous system and peripheral tissues. This communication network is an important tool in the control of feeding-related behaviors and regulates the transport of the eating-related peptides and regulatory pro-

teins produced by peripheral tissues (Banks 2010). In this respect, triglycerides inhibit the transport of leptin across the blood-brain barrier and provide a mechanism for peripheral leptin resistance by attenuating the leptin signal across the blood-brain barrier. Since triglycerides are elevated in both starvation and obesity, the blood-brain barrier plays important roles in the progression of obesity (Banks 2008). Additionally, defects in hypothalamic leptin receptor signal transduction involving reduced leptin receptor expression or the induction of feedback inhibitors have been found in leptin resistance (Munzberg 2010). However, the hypothalamus, and in particular the arcuate nucleus is characterized by discontinuation of blood–brain barrier, which allows direct access of circulating hormones and nutrients to the central nervous system (Banks 2006).

Tanycytes are bipolar cells bridging the cerebrospinal fluid to the portal capillaries and may link the cerebrospinal fluid to neuroendocrine events (Rodríguez et al. 2005). Interestingly, arcuate tanycytes possess microvilli at their luminal surfaces (Brawer 1972; Bruni et al. 1972). Actually cerebrospinal fluid-contacting neurons may play an important role in sensing molecules in the cerebrospinal fluid. The axons of these neurons transmit information taken up by dendrites and perikarya to synaptic zones of various brain areas (Vígh et al. 2004). This neuron population may also be actively involved in promoting exchanges between the cerebrospinal fluid and the brain compartments at the arcuate nucleus of the hypothalamus. Thereby, the arcuate nucleus of the hypothalamus is a critical component of the neural pathways that regulate energy balance, and therefore plays a key role in conveying blood-borne molecular signals (Mullier et al. 2010). In fact, leptin signaling in the hypothalamic arcuate nucleus prevents the full-obesity syndrome seen in leptin receptor-deficiency. Melanocortin receptor- and NPY receptor-expressing neurons are downstream targets of leptin-responsive hypothalamic arcuate neurons in the leptin-mediated control of glucose homeostasis (Coppari et al. 2005). However, the mechanisms by which peripheral signals reach the hypothalamic arcuate nucleus to mediate their central effects remain largely unknown. The neuropeptides neurokinin B axons target the barrier of tanycytes around fenestrated capillary vessels at the hypothalamic arcuate nucleus. This indicates a control of regional bidirectional permeability (Ciofi et al. 2006). An absence of an intact blood-brain barrier may represent a route of entry for circulating substances to a subpopulation of arcuate nucleus neurons (Norsted et al. 2008). This means that exchanges between the cerebrospinal fluid and the arcuate nucleus neurons can be facilitated (Mullier et al. 2010).

Besides lipids and leptin, glucose and insulin are also detected by specialized fuel-sensing neurons of hypothalamic neuronal circuits (Jordan et al. 2010). The arcuate nucleus neurons have the ability to sense higher glucose concentrations than 5 mmol/l through a K (ATP) channel-independent mechanism (Fioramonti et al. 2004). Two types of glucosensing neurons have been described; either activities are proportional with the changes in glucose concentration or activities are inversely proportional with these changes (Pénicaud et al. 2006).

Actually the central regulation of energy homeostasis is achieved by two functionally opposing neuron populations in the arcuate nucleus of the hypothalamus; the orexigenic NPY/AgRP-expressing neurons and the anorexigenic POMC/CART-expressing neurons (Gropp et al. 2005; Könner et al. 2009). These two distinct neuronal populations have opposite effects on the feeding behavior as well as in energy balance (Sohn 2015). The increase of AgRP gene expression is greater in pre-obese subjects. The initial higher levels of AgRP mRNA in the hypothalamus predict the subsequent increase in food intake, after the period of hyperphagia, AgRP mRNA expression subsides and obesity develops. In this phase AgRP gene expression is relatively reduced but still higher. In this case neuron-specific reduction in lipoprotein lipase gene expression results in progression of severe obesity (Wang et al. 2011a). Indeed, lipoprotein lipase is a key enzyme that controls the partitioning of triglyceride-rich lipoprotein derived fatty acids in peripheral tissues. Lipoprotein lipase

deletion in skeletal muscle ultimately leads to increased lipid partitioning to other tissues, insulin resistance, and obesity, whereas lipoprotein lipase deletion in the heart develops hypertriglyceridemia and cardiac dysfunction (Wang and Eckel 2009). Lipoprotein lipase mRNA is also expressed throughout the nervous system. Actually, the primary role of lipoprotein lipase in the brain may be similar to that played in extracranial tissues. While the corticotropin stimulation results in an increase in adipose lipoprotein lipase activity, a simultaneous decrease may occur in heart lipoprotein lipase activity, but have no discernible effect on lipoprotein lipase in the brain (Ben-Zeev et al. 1990).

The uptake of fatty acids is an integral part of normal lipid metabolism of the central nervous system and may be important in regulating feeding behavior (Bessesen et al. 1993). Progressive changes in energy balance may be due to the lipoprotein lipase-dependent decrease in polyunsaturated fatty acid levels, and increased NPY/AgRP gene expression in the hypothalamus (Wang et al. 2011a).

On the other hand, the promotion of cannabinoid receptor-1 activity while increasing feeding, also enhances satiety by promoting neuronal activity of hypothalamic pro-opiomelanocortin cells (Koch et al. 2015). The melanocortin-4 receptor (MC4R) is crucial for regulating both food intake and energy expenditure. Inactivation of MC4R is associated with hyperphagia, hyperinsulinemia, hyperglycemia and finally results in obesity (Huszar et al. 1997). The tonic satiety signal provided by the constitutive activity of MC4R may be required for maintaining long-term energy homeostasis in humans. Defects in basal signaling is a potential cause of genetic obesity caused by MC4R mutations (Srinivasan et al. 2004). Mutations in the MC4R can also constitutively activate the mitogen-activated protein kinase (MAPK) pathway and cause energy imbalance by significantly enhancing basal extracellular signal-regulated kinase (ERK)1/2 phosphorylation (Huang and Tao 2012).

Alpha-melanocyte-stimulating hormone (alpha-MSH)-induced activation of the melanocortin-4 receptor in hypothalamic neurons increases energy expenditure and inhibits food intake. Alpha-MSH dephosphorylates AMPK at Thr(172) and consequently decreases phosphorylation of the AMPK substrate, acetyl-coenzyme A-carboxylase at Ser(79). Eventually alpha-MSH inhibits AMPK activity via protein kinase A, ERK-1/2, and liver kinase B-1 pathway (Damm et al. 2012). Hypothalamic AMPK regulates energy metabolism by integrating the effects of multiple hormones, peptides, neurotransmitters, and nutrients. Alteration of hypothalamic AMPK activity changes food intake and body weight by regulating acetyl-coenzyme A carboxylase/malonyl-coenzyme A/carnitine palmitoyltransferase-1/fatty acid oxidation and mammalian target of rapamycin (mTOR) signaling (Minokoshi et al. 2008).

Although alpha-MSH is the predominant POMC-derived neuropeptide in the central regulation of human energy balance, in 538 patients with severe, early-onset obesity, five unrelated probands who are heterozygous variant in the region encoding beta-MSH are identified. Compared to wild-type beta-MSH, the variant peptide is impaired in its ability to bind to and activate signaling from the MC4R (Lee et al. 2006). Indeed, the POMC-derived neuropeptide beta-MSH plays a critical role in the hypothalamic control of body weight in humans (Biebermann et al. 2006).

The central 5-hydroxytryptamine (5-HT) system, including the 5-HT2C receptors (5-HT2CRs) plays critical roles in the regulation of energy homeostasis. Activation of 5-HT2CRs enhances satiety signals to terminate food intake (Xu et al. 2008). Particularly, POMC neurons in the arcuate nucleus of hypothalamus co-express 5-HT2CRs (Heisler et al. 2002). Central 5-HT2CRs expression by POMC neurons is important for the progression of the satiation process and therefore, suppress feeding for maintaining normal feeding behavior and body weight homeostasis (Xu et al. 2008).

Similar to serotonin, the adipose-derived leptin exerts some of its effects by directly activating POMC neurons (Hill et al. 2010). PI3K signaling in POMC neurons is essential for leptin-induced activation and insulin-induced

inhibition of POMC cells and for the acute suppression of food intake elicited by leptin (Hill et al. 2010). However, lacking leptin signaling in POMC neurons causes mildly obesity, hyperleptinemia, and altered expression of hypothalamic neuropeptides (Balthasar et al. 2004). Thus, in addition to the arcuate nucleus, functionally relevant leptin receptors in the ventromedial hypothalamic nucleus (VMH) neurons are necessary for appropriate energy homeostasis. Leptin receptor deficiency in VMH is associated with metabolic syndrome including hepatic steatosis, dyslipidemia, and hyperleptinemia (Bingham et al. 2008). In this case, leptin signaling in the nucleus of solitary tract neuron is required for the regulation of food intake and consumption (Scott et al. 2011). Leptin receptor-expressing populations of neurons outside of the hypothalamus also seems to be necessary in the regulation of feeding behavior and hypothalamic energy balance.

In addition to the known numerous physiological functions the transient receptor potential C (TRPC) channels (Freichel et al. 2005) may mediate the acute effects of the two potent anorexigenic signals within the same neuron, leptin via a Jak2-PI3 K-Phospholipase C(PLC)-gamma pathways (Qiu et al. 2010) and serotonin via PLC-PKC-IP3-dependent signaling pathways (Sohn et al. 2011). Indeed, serotonin and leptin both inhibit food intake and regulate energy balance and both activate TRPC channels to excite POMC neurons. Serotonin and leptin share common signaling TRPC channels in order to modify POMC neuronal activity. About 25% of POMC neurons are depolarized by the 5-HT2CR agonists via activation of TRPC channels. Serotonin and leptin, key anorexigenic signals, activate distinct subpopulations of POMC neurons via activation of TRPC channels (Sohn et al. 2011).

Another potential cellular target of 5-HT2C receptors is the M-current, a subthreshold, noninactivating, voltage-dependent K^+ current. The M-current is ubiquitous in most neuronal cell types (Robbins 2001). 5-HT2C receptors are coupled with G alpha-protein and highly expressed in the arcuate nucleus and in POMC neurons. However, serotonin is known to inhibit the M-current in human cortical neurons (McCormick and Williamson 1989). The inhibition of the M-current by serotonin via the 5-HT2C receptor would increase POMC neuronal activity and activate the melanocortin neuron circuitry to reduce feeding behavior. The M-current as a critical component in control of NPY and POMC neuronal activity by central and peripheral signals regulates energy homeostasis (Roepke et al. 2012). The NPY/AgRP neurons within the arcuate nucleus of hypothalamus are probably the most established orexigenic population in the central nervous system. The NPY/AgRP neurons release NPY (agonist of the Y receptors), AgRP (inverse agonist at the MC4Rs), and the inhibitory neurotransmitter gamma-aminobutyric acid (GABA). The orexigenic pathways involving the GABAergic neurotransmission from the NPY/AgRP neurons increase food intake by the inhibition of the anorexigenic centers in the central nervous system (Sohn 2015).

NPY hormones are the most potent orexigenic molecules, which consist of three native peptide ligands named NPY, pancreatic polypeptide, and peptide YY. This family consists of four G-protein-coupled Y receptors subtypes in humans, as hY(1), hY(2), hY(4), and hY(5) (Lindner et al. 2008).

Similar to orexigenic NPY/AgRP neurons, anorexigenic POMC/CART neurons are also responsive to circulating satiety factors, and the activation of these neurons promotes negative energy balance/increased energy expenditure and/or decreased food intake (Lopaschuk et al. 2010). Leptin action on the hypothalamic regulation of food intake and body weight is mediated by orexigenic as well as anorectic signals (Sahu 2003). While the orexigenic neuropeptides are downregulated by leptin, the anorexigenic neuropeptides are upregulated (Jéquier 2002). Although obese humans have higher plasma leptin concentrations proportional with the size of adipose tissue, these individuals are resistant to the effects of endogenous leptin (Jéquier 2002). Leptin resistance in diet-induced obesity may depend on the defective nutritional regulation of leptin receptor gene expression and reduced signal transducer and activator of transcription-3 (STAT3) signaling. Even so leptin

resistance may occur in the hypothalamic neurons despite an intact Janus kinase-2 (JAK2)-STAT3 pathway of leptin signaling (Sahu 2003). However, the PI3K-phosphodiesterase-3B (PDE3B)-cAMP pathway appears to be a major mechanism of leptin signaling in regulating energy balance. SOCS3 negatively regulates the PI3K pathway of leptin signaling in the hypothalamus. This mechanism plays a significant role in diet-induced obesity (Sahu 2011). Actually PI3K is an upstream regulator of the PDE3B pathway of leptin signaling in hypothalamus (Sahu et al. 2013). SOCS-3 is a leptin-inducible inhibitor of leptin signaling, and a potential mediator of leptin resistance in obesity (Bjørbaek et al. 1998). As mentioned above the arcuate nucleus of the hypothalamus contains two populations of leptin-responsive neurons (Cone 2005). The first expresses two potent appetite-stimulating (orexigenic) peptides, the melanocortin antagonist AgRP and NPY. The second population expresses the peptide CART and the large precursor peptide POMC. Both sets of neurons project to second-order, MC4R expressing neurons within the hypothalamus and elsewhere in the brain (Coll et al. 2007). MC4R deficiency has been found in severely obese humans (Farooqi et al. 2003). Higher circulating non-esterified fatty acids (NEFA) and insulin concentrations with the increased UCP1, MC4R and CART gene expression may occur as an immediate consequence of consuming high-energy diet. However, increased circulating leptin in these high-energy-fed animals reflects the size of adiposity (Archer et al. 2005). Fat distribution is influenced by the genetic variation of the CART locus (Challis et al. 2000). Polymorphisms in linkage disequilibrium with the 5′-flanking region of the CART gene site may be associated with genetic predisposition to obesity (Yamada et al. 2002).

Amino acid transmitters in hypothalamic circuits have significant contribution to the regulation of food intake. The release of GABA from NPY/AgRP neurons in the arcuate nucleus is also required for the normal regulation of energy balance (Tong et al. 2008). Both AgRP and POMC neurons send axons to many of the same target areas. Ablation of inhibitory AgRP neurons leads to anorexia by two mechanisms; first, the loss of inhibition into POMC neurons and their postsynaptic target cells stimulates the melanocortin system, which suppresses feeding. Second, the loss of GABAergic inhibition into neurons in the parabrachial nucleus mimics the activation of these neurons and results in severe anorexia. Contrarily GABA release from NPY/AgRP neurons into the parabrachial nucleus prevents starvation (Wu et al. 2009). GABA released from POMC neurons may act at presynaptic receptors on POMC terminals to limit continued transmission. POMC neurons can rapidly affect the activity of downstream neurons via GABA and glutamate release (Dicken et al. 2012). GABA from NPY neurons participates in the inhibition of POMC neurons. This neuronal interaction seems to favor the tonic inhibition of satiety signal, and also promotes feeding or overfeeding when food is available in excess (Drougard et al. 2015).

Activation of medial arcuate neurons expressing the orexigenic NPY and inhibition of dorsomedial and paraventricular neurons may contribute to hyperphagia and overweight. Histamine H(1)-receptors and GABA(A)-receptors are involved in mediation of these effects (Davidowa 2007). The neuronal responses to leptin, insulin and amylin are reduced in the presence of a GABA(A) receptor antagonist (Davidowa et al. 2006). Furthermore, in the presence of a GABA(A)-receptor antagonists, amylin induces a significant inhibition of medial arcuate neurons. Since the histaminergic system mediates the effects of amylin, the histamine H1-receptor antagonists also reduce the responses to amylin (Davidowa 2007). Amylin is a pancreatic beta-cell hormone that plays an important role in the control of nutrient fluxes (Young 2005). The satiating effect of peripheral amylin is mediated by direct action on area postrema neurons. Amylin sensitivity may be reduced in obesity (Lutz 2010). Amylin is completely transported across the blood-brain barrier (Banks and Kastin 1998). Because of the primary site of peripheral amylin action, area postrema, is free of a blood-brain barrier, amylin can easily access

to its receptive neurons (Lutz 2010). Amylin reduces food intake, slows gastric emptying, and reduces postprandial glucagon secretion, in addition to preventing the expression of orexigenic neuropeptides in the lateral hypothalamic area (Lutz 2009). Plasma levels of amylin are higher in obese individuals. Chronic infusion of amylin into the brain reduces body weight gain and adiposity, whereas infusion of amylin antagonists increases adiposity (Lutz 2012). Concurrent peripheral administration of amylin and leptin elicits synergistic, fat-specific weight loss in leptin-resistant, diet-induced obese rats. Weight loss synergy is specific to amylin treatment, compared with other anorexigenic peptides. These findings indicate that amylin agonism restores leptin responsiveness in diet-induced obesity (Roth et al. 2008). In this respect, amylin and leptin have synergistic effects on body weight and body adiposity. Amylin/leptin similarly increases leptin binding in the ventromedial hypothalamus by 40% and the arcuate nucleus by 70%. In amylin-deficient mice, hypothalamic leptin receptor mRNA expression and leptin-stimulated phosphorylated STAT-3 is reduced by 50% and 40%, respectively within arcuate nucleus and Ventromedial nucleus (Turek et al. 2010).

AMPK is a fuel-sensing enzyme activated by physiological and pathological stresses that deplete cellular ATP. Activation of AMPK represses ATP-consuming anabolic pathways and induces ATP-producing catabolic pathways (Xue and Kahn 2006). AMPK is an intracellular energy sensor maintaining the energy balance within the cell. AMPK is activated subsequent to an increase in the AMP:ATP ratio within the cell (Hardie et al. 2003). Hormonal induced changes in AMPK activity leading to changes in acetyl CoA carboxylase phosphorylation activity, which in turn would alter malonyl-CoA levels to regulate food intake (Andersson et al. 2004). Malonyl-CoA as a downstream mediator of acetyl CoA carboxylase in leptin's signaling pathway in the arcuate nucleus and imply that palmitoyl-CoA, instead of malonyl-CoA, could be an effector in relaying acetyl CoA carboxylase signaling in the paraventricular nucleus (Gao et al. 2007).

Malonyl-CoA functions as a mediator in the hypothalamic sensing of energy balance. Lowering muscle malonyl-CoA, a potent inhibitor of carnitine/CPT1, releases CPT1 from inhibitory constraint, facilitating the entry of fatty acids into mitochondria for beta oxidation (Cha et al. 2005). The overexpression of CPT-1c also blocks leptin-induced down-regulations of orexigenic NPY and brain-specific homeobox transcription factor (Bsx), a transcription factor of NPY. Elevated levels of hypothalamic arcuate nucleus malonyl-CoA, a fatty acid-metabolism intermediate controls the hypothalamic food intake. CPT-1c is implicated in malonyl-CoA action in leptin's hypothalamic anorectic signaling pathways. Leptin also impacts ceramide metabolism through malonyl-CoA and CPT-1c, and de novo biosynthesis of ceramide acts downstream of both malonyl-CoA and CPT-1c (Gao et al. 2011b). Long-chain fatty acyl-CoAs, substrates of CPT-1a, dissociate from malonyl-CoA's actions in the hypothalamic arcuate nucleus under different feeding states. The hypothalamic arcuate nucleus intracellular mechanisms of malonyl-CoA's anorectic actions induced by leptin are independent of CPT-1a. The data suggest that target(s), rather than CPT-1a, mediates malonyl-CoA action on feeding (Gao et al. 2011a). Leptin and adiponectin activate AMPK to alter metabolic pathways in muscle and liver. Counter-regulatory hormones involved in appetite control regulate AMPK activity. Activation of AMPK in the hypothalamus increases food intake. Leptin decreases hypothalamic AMPK activity by reducing food intake (Spiegelman and Flier 2001). Contrarily circulating ghrelin at fasting concentrations may stimulate food intake (Wren et al. 2001).

The adipose-derived hormone leptin acts via a specific receptor in the brain to regulate energy balance and body weight. Even though obese individuals have high levels of circulating leptin, the failure of these high levels to control body weight is dependent on resistance to this hormone (Sáinz et al. 2015). Fat redistribution from the subcutaneous to the visceral depot and increased inflammation in adipose tissue seems to be associated with leptin resistance (Carter

et al. 2013). Actually leptin resistance is defined as decreased sensitivity to the anorexigenic or weight loss effects of leptin. A hallmark of leptin-resistant states is hyperleptinemia. Leptin binds to its cell surface receptor and signals through the JAK/STAT pathway. Leptin activates JAK2 and STAT3, as well as the MAPK (Erk 42/44) and the PI3K pathways. Intact STAT3 signaling is necessary for the effects of leptin on food intake (Martin et al. 2006). On the other hand, the protein-tyrosine phosphatase (PTP)-1B lacking subjects are hypersensitive to insulin and resistant to obesity. PTP1B is expressed in hypothalamic regions harboring leptin-responsive neurons and regulates leptin signaling by targeting JAK2 (Zabolotny et al. 2002). Recent evidence based on the resistance to diet-induced obesity has suggested that PTP1B might be a key regulator of leptin signaling. Upon leptin binding, the leptin receptor activation leads to stimulation of the JAK/STAT signal transduction cascade. The negative regulatory role of PTP1B on leptin signaling is mediated through a direct and selective dephosphorylation of JAK2 and STAT3 (Lund et al. 2005). Furthermore, the substrate-trapping experiments demonstrate that leptin-activated JAK2, but not STAT3 or the leptin receptor, is a substrate of PTP1B. Thus by utilization of a selective PTP1B inhibitor, the leptin-induced STAT3 activation was enhanced in cells. These results suggest that PTP1B negatively regulates leptin signaling, and provide one mechanism by which it regulates obesity (Cheng et al. 2002; Lund et al. 2005).

Leptin influences body weight not only by suppressing food intake but also by increasing energy expenditure reflected in metabolic rate and oxygen consumption. One of the major actions of leptin on fuel utilization is to increase fatty acid oxidation and decrease fat storage in muscle via activation of AMPK. The lower respiratory exchange ratio in diet-induced obesity is consistent with increased AMPK activity and decreased acetyl CoA carboxylase activity in muscle (Martin et al. 2006). AMPK activity is inhibited in arcuate and paraventricular hypothalamus (PVH) by the anorexigenic hormone leptin, and in multiple hypothalamic regions by insulin, high glucose and refeeding. A melanocortin receptor agonist, a potent anorexigen, decreases AMPK activity in PVH, whereas AgRP, increases AMPK activity. Melanocortin receptor signaling is required for leptin and refeeding effects on AMPK in PVH. Dominant negative AMPK expression in the hypothalamus reduces food intake and body weight, whereas constitutively active AMPK increases both. Alterations of hypothalamic AMPK activity augment changes in arcuate neuropeptide expression induced by fasting and feeding. Furthermore, inhibition of hypothalamic AMPK is necessary for leptin's effects on food intake and body weight, as constitutively active AMPK blocks these effects. Thus, hypothalamic AMPK plays a critical role in hormonal and nutrient-derived anorexigenic and orexigenic signals and in energy balance (Minokoshi et al. 2004). Inhibition of fatty acid synthase activity and stimulation of CPT-1 activity in the hypothalamus can alter energy perception via AMPK. AMPK-fatty acid synthase pathway may act in a leptin-independent manner (Kim et al. 2004).

On the other hand, adiponectin has also been shown to increase food intake by activating AMPK in the arcuate hypothalamus. Recent data have shown that acetyl-coenzyme A carboxylase/malonyl-coenzyme A/carnitine palmitoyltransferase-1/fatty acid oxidation and mTOR signaling are putative downstream pathways for food intake regulation in response to hypothalamic AMPK. Thus, these results suggest that food intake and nutrient metabolism are coordinately regulated by the common signaling pathway of AMPK in the hypothalamus (Minokoshi et al. 2008).

Systemic hypoglycaemia causes hypothalamic AMPK activation, which is important for counter-regulatory hormonal responses (Han et al. 2005). AMPK in the ventromedial hypothalamus plays a key role in the detection of acute hypoglycemia and initiation of the glucose counter-regulatory response. AMPK downregulation in the ventromedial hypothalamus leads to a marked suppression of the glucose counter-regulatory response to hypoglycemia (McCrimmon et al. 2008). Both cannabinoids

and ghrelin while stimulating AMPK activity in the hypothalamus and the heart, inhibit AMPK activity in the liver and adipose tissue. Peripheral inhibition of AMPK by cannabinoids and ghrelin may lead to fat storage (Kola et al. 2005).

Counter-regulatory hormones involved in appetite control regulate AMPK activity and that pharmacological activation of AMPK in the hypothalamus increases food intake. In vivo administration of leptin, which leads to a reduction in food intake, decreases hypothalamic AMPK activity. By contrast, injection of ghrelin in vivo, which increases food intake, stimulates AMPK activity in the hypothalamus (Andersson et al. 2004). Pharmacological and genetic activation or inhibition of hypothalamic AMPK lead to increased or reduced food intake, respectively (Kola 2008). Furthermore, inhibition of fatty acid synthase and stimulation of CPT1 may increase ATP levels in hypothalamic neurons and signal a positive energy balance, leading to a decrease in AMPK activity and resulting in a decrease in NPY expression. In fasting, when energy is depleted, AMPK is stimulated, thereby the cAMP response element binding protein-NPY pathway is activated and food intake is increased (Kim et al. 2004).

CPT1 regulates LCFA entry into mitochondria, where the LCFAs undergo beta-oxidation. Either genetic or biochemical inhibition of hypothalamic CPT1 activity diminishes food intake and endogenous glucose production. Changes in the rate of lipid oxidation in hypothalamic neurons adjust nutrient availability to the hypothalamus, which in turn modulates the exogenous and endogenous inputs of nutrients into the circulation (Obici et al. 2003). Central inhibition of lipid oxidation restores hypothalamic levels of LCFA-CoAs and markedly inhibits feeding behavior and hepatic glucose fluxes in overfed rats (Pocai et al. 2006).

Hypothalamic AMPK activity regulates hypoglycaemia-induced counter-regulatory hormone responses (Xue and Kahn 2006). Hypothalamic neurons may sense the changes of glucose levels and participate in the counter-regulatory response by altering AMPK activity. Insulin induced hypoglycaemia activates AMPK in the arcuate nucleus/ventromedial hypothalamus and paraventricular hypothalamus. Hypothalamic AMPK senses the whole-body energy state and regulates not only energy homeostasis but also neuroendocrine functions (Han et al. 2005). One of the other factors which is mTOR pathway, senses nutrient, energy and oxygen availability. mTOR signaling regulates cell energy homeostasis by coordinating anabolic and catabolic processes for survival. mTOR is a serine/threonine kinase that exists in two different complexes, mTORC1 and mTORC2 in mammals. The role of hypothalamic mTOR complex in cellular energy sensing indicates the importance of this metabolic sensor in cellular and whole body energy management (Martínez de Morentin et al. 2014).

mTOR co-localizes with NPY and pro-opiomelanocortin neurons in the arcuate nucleus and its signaling is controlled by energy status of the hypothalamus (Cota et al. 2006). High-protein diet decreases AMPK and increases mTOR activity in the hypothalamus, leading to inhibition of NPY and stimulation of pro-opiomelanocortin expression. A cross-regulation between AMPK and mTOR controls food intake (Ropelle et al. 2008). Thus, mTOR senses extracellular amino acids, glucose, growth factors, and neurotransmitters, and regulates anabolic reactions in response to these signals. Thereby activated mTOR reduces food intake by increasing protein and lipid synthesis (Takei et al. 2014). AMPK is thought to be the molecular background to this specific nutrient dependency. Expression of constitutive AMPK-alpha subunit counteracts brain-derived neurotrophic factors-induced phosphorylation of p70S6K and enhances protein synthesis in cortical neurons. These results indicate that AMPK inhibits the effects of brain-derived neurotrophic factors on mTORC1-mediated translation in neurons (Ishizuka et al. 2013). Brain-derived neurotrophic factors exert anorexigenic function through a neural circuit in the hypothalamus and induces phosphorylation of its target, p70S6 kinase, at Thr389 in neurons by activating mTORC1 (Ishizuka et al. 2013; Takei et al. 2014). In addition to AMPK and mTOR, Per-Arnt-Sim kinase

(PASK) is also a nutrient sensor which is proposed as a regulator of glucose metabolism and cellular energy. AMPK and mTOR/S6K1 pathways are regulated in the ventromedial and lateral hypothalamus in response to nutritional states, whereas PASK-deficient mice have an impaired activation response of AMPK and mTOR/S6K1 pathways and resist diet-induced obesity. Eventually the PASK function is critical for preserving the nutrient effect on AMPK and mTOR/S6K1 pathways (Hurtado-Carneiro et al. 2014, p. 1). In fact, the interaction between AMPK with hypothalamic lipid metabolism and other metabolic sensors, such as the UCP2, the mTOR and the nicotinamide adenine dinucleotide-dependentdeacetylase sirtuin 1 (SIRT1), may play a role in the hypothalamic control of feeding and energy expenditure (Blanco Martínez de Morentin et al. 2011). Ghrelin exerts its orexigenic action through specific modulation of Sirtuin1 (SIRT1)/p53 and AMPK pathways, simultaneously it elicits a marked upregulation of the hypothalamic mTOR signaling pathway. Central inhibition of mTOR signaling with rapamycin decreases ghrelin's orexigenic action and the mRNA expression of AgRP and NPY in the arcuate nucleus of the hypothalamus in obesity (Martins et al. 2012).

5 Conclusion

In addition to the primary induction of hypothalamic IKKbeta/NF-kappaB by chronic overnutrition and increased plasma concentrations of NEFAs, pathophysiological changes during the progress of obesity, such as hyperlipidemia, hyperglycemia, hyperinsulinemia, hyperleptinemia, and elevated inflammatory substances in the peripheral tissues and circulation, might secondarily, but significantly, promote ER stress and mitochondrial dysfunction that accelerate the obesity-related diseases.

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Obesity, Persistent Organic Pollutants and Related Health Problems

4

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Abstract

The present review aims to delve into persistent organic pollutants (POPs), as xenobiotics, in correlation to human health. POPs exhibit a group of common characteristics, including lipophilicity, persistence to decomposition and bioaccumulation in tissues. POPs have been thoroughly studied by former researchers, as they offer a particular interest in the elucidation of metabolic, endocrine and immune perturbation caused by their synergy with intracellular mechanisms. Herein particular focus is attributed to the relationship of POPs with obesity provocation. Obesity nowadays receives epidemic dimensions, as its prevalence elevates in an exponential degree. POPs-induced obesity rotates around interfering in metabolic and endocrinal procedures and interacting with peroxisome-proliferator and retinoic receptors. Moreover, polymorphisms in CYP gene families exert a negative result, as they incapacitate detoxification of POPs. Obesity could be deemed as a multidimensional condition, as various factors interact to lead to an obesogenic result. Therefore, concomitant disorders may occur, from mild to lethal, and get intensified due to POPs exposure. POPs exact function mechanisms remain rather enigmatic, thus further investigation should be prospectively performed, for a more lucid picture of this issue, and, consequently for the establishment of alternative solutions.

Keywords

Persistent organic pollutants • Xenobiotics • Bioaccumulation • Toxicokinetics • Obesity • Diabetes • Metabolism • Endocrine signaling • CYP polymorphism

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1 Introduction

Persistent Organic Pollutants are organic compounds, widely used as pesticides and chemicals. They persist in the environment and bioaccumulate lengthwise the food chain, thus dangers lurk to cause adverse effects to the environment and the human health (Colosio et al. 2013).

In the last 50 years, the human population has increased by more than double, and global agricultural production has similarly risen (Almeida-Gonzalez et al. 2011). However, the available arable land has only increased by 10%, fact that made the use of pesticides imperative. Pesticides are used to control, repel or kill certain pests (e.g. animals, plants). They can be discerned in insecticides, herbicides and fungicides. According to their chemical structures, they can also be classified as carbamates, dithiocarbamates, synthetic pyrethroids, organochlorines, organophosphorus, thiocarbamates, phenoxyacetates, coumarins and quaternary ammonium compounds.

Many pesticides are not novel, as some of them are modeled on natural insecticides, if we consider the ability of plants, marine organisms and others to produce chemicals for attack and defense purposes. On the other hand, some are synthetic and in the past they were as well extensively used as pesticides, industrial chemicals and by-products. Also, conception of chemical weapons is a very historically recent event. These are, in a biochemical perspective, foreign compounds, or xenobiotics.

The first systematic observations of the results of organochlorines in the ecosystem were made by Rachel Carson in the USA and were published in her landmark book "Silent Spring" (1962). She raised public cognizance of the effects of pesticide use on human health and environment. This book was rightly considered as the beginning of ecological "spring" of our times.

Therefore, the international community urged upon the impact of POPs on human and environmental health at the Stockholm Convention of POPs, for their use to become eliminated.

POPs display specific chemical properties, which result to accumulation in human tissues, with the primary pathway being the dietary intake. They have been linked to a broad spectrum of human health dangers, varying from short-term impacts to chronic. To the same direction, recent epidemiological data suggest an interrelation between POPs, obesity and related health problems.

2 Persistent Organic Pollutants

2.1 Definition of POPs

As afore-mentioned, many POPs are of anthropogenic source. They are ubiquitous pollutants, found worldwide.

POPs exhibit common properties, including persistence, lipophilicity, toxicity and bioaccumulation Mrema et al. (2013). They tend to be found in soil forming sediments, as they have low aqueous (water) solubility. Moreover, most POPs have reduced vapor pressure, which means that equally diminished backflow of POPs in gas phase will occur, leading to their accumulation. Their half-lives range from several months to decades and some present also semi-volatile properties, as they have the ability to get vaporized towards the atmosphere and return back to the earth via precipitations. Due to their high lipophilic properties, POPs are resistant to biological, proteolytic and chemical degradation in humans and the environment (Letcher et al. 2010, Filatov et al. 2015, Henriquez-Hernandez et al. 2016). With the passing of time, POPs accumulate in tissues of biota, and subsequently get magnified, as trophic levels of food chains ascend. They are, therefore, a significant threat to global health.

For convenience reasons, POPs can be divided into three categories:

- **Pesticides**: aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, hexachlorobenzene, mirex, toxaphene;
- **Industrial chemicals**: hexachlorobenzene, polychlorinated biphenyls (PCBs); and
- **By-products**: hexachlorobenzene; polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans (PCDD/PCDF), and PCBs.

Organochlorines, which are a class of compounds with common chemical structure and belong to POPs, were the first pesticides that contributed successfully in parasite control, albeit had severe consequences of morbidity and mortality in humans. Discovery of DDT provided a significant solution against the reduction of malaria and other parasitic diseases after WWII, as it was broadly used to protect soldiers and civilians. After the war, DDT continued to be used against mosquitoes in several countries to restrain malaria.

The international community discussed the impact of POPs on human and environmental health on May 22, 2001 at the Stockholm Convention on Persistent Organic Pollutants. The main purpose was the elimination or restriction of their production, in order to protect human health and the environment. The convention and its participants have conceded the potential human and environmental toxicity of POPs. They recognized that POPs feature abilities of long range transport, bioaccumulation and biomagnification. As objective, the convention's inquiry comprised the investigation of chemicals through their classification as POPs or not. The first conference in 2001 commenced this attempt, by compiling a list of chemicals identified as POPs ("the dirty dozen") :

- *Aldrin*, used as insecticide. Exposure occurs mainly through dairy and meat consumption.
- *Chlordane*, a broad-spectrum insecticide applied on agricultural crops. Human exposure to chlordane occurs predominantly through the air and has been incriminated of causing abnormalities in the immune system and carcinogenesis.
- *DDT*, which some countries continue to use as a solution to confine malaria. It exhibits stability and persistence properties. It has been emphasized that 50% of its total applied quantity, 10–15 years after, remains in the soil. It is impressive that DDT remnants have also been traced in the Arctic region (Bonefeld-Jorgensen 2010). Though short-term effects are limited, long-term exposure is correlated to chronic health problems; DDT has even been detected in maternal milk, raising considerable doubts about infant health (Freire et al. 2011).
- *Dieldrin,* used mainly to control isoptera, moths and other stored fabric pests. Dietary uptake represents the primary mean of exposure.
- *Endrin,* which is sprayed on the leaves of crops such as cotton and groins. The main way of exposure for the general human population is through food, although its dietary uptake content lies under the limits defined as safe by world health authorities.
- *Heptachlor,* primarily used to kill soil insects, termites, grasshoppers and other crop pests. It is assorted as potential human carcinogen.
- *Hexachlorobenzene (HCB),* is a fungicide. HCB has been accused of causing skin damage, colic pain and fatigue, as it was observed in a percentage of Turkish population that consumed HCB-treated seed grain, during the period 1954–1959. HCB incites adverse effects in many animal species, including reproductive incapacity and even death.
- *Mirex,* used against ants and termites. Studies performed on rodents have proved a carcinogenic action, though immediate exposure to mirex is not presented to cause hazardous effects on humans. It is viewed as one of the most stable substances, as its half-life elevates up to 10 years. Mirex is observed to get transferred through alimentation, especially found in fish and meat.
- *Toxaphene,* used in seeds, cereals, fruit and nuts, to control ectoparasites in farm animals. Exposure to toxaphene in humans occurs mainly through food. Carcinogenic action has been noted in laboratory animals, though in humans low toxic results have been recorded.
- *Polychlorinated biphenyls (PCB),* used on a universal scale for industrial implementation, e.g. in electrical capacitors, transformers and hydraulic systems. Herein, food intake is again the predominant route of exposure (Agudo et al. 2009, Mori et al. 2014). Contaminated rice crops by PCB in 1968 in Japan were responsible for symptoms of nail pigmentation, eyelid tumescence and debilitation. A same case in Taiwan 11 years later, led to birth of children presenting behavioral disorders

and mental disablement. PCB is as well affecting function of the immune system.

- *Polychlorinated dibenzo-p-dioxins (PCDD),* emitted mostly from incineration of waste material, automobile, coal and wood due to incomplete combustion (Eljarrat et al. 2003). Dioxins induce immune disorders and possibly carcinogenic effects. In laboratory animals there was an augment in stillbirth and intrinsic abnormalities. Also a fish population, shortly after the exposure occurred, deceased.
- *Polychlorinated dibenzofurans (PCDF),* deriving from the production of PCBs and dioxins. Food, especially animal products, is the primal source of exposure for human population. Furans have also been found in infants due to maternal milk.

The Stockholm Convention was adopted on May 2001, but entered into force on May 17th, 2004. Since 2001, the foregoing list has been expanded to include other compounds, such as:

- *Chlordecone*, a synthetic chlorinated organic compound, primarily used as an agricultural pesticide, akin to DDT and Mirex. Chlordecone is toxic to aquatic organisms, and categorized as a possible human carcinogen.
- *Lindane (γ-hexachlorocyclohexane)*, a pesticide used as a wide range insecticide for seeds and plants, and against ectoparasites in animals and humans. It can bioaccumulate rapidly and cause immunotoxicity, neurotoxicity, liver and kidney damage. It is also referred as carcinogenic. Tsatsakis et al. (2008) performed an investigation among Greek residents of an agricultural area, and concentrations of organochlorine pesticides were evaluated in hair samples. It was discovered that levels of lindane as well as DDT could be still detected in high concentrations in specimen, despite their restriction.
- *α-Hexachlorocyclohexane (α-HCH)* and *β-Hexachlorocyclohexane (β-HCH)* are insecticides as well as by-products in the production of lindane. They have been associated with Parkinson's and Alzheimer's disease.
- *Hexabromodiphenyl ether* and *heptabromodiphenyl* ether are principal components of octabromodiphenyl ether. Commercial octaBDE is highly persistent in the environment, whose only degradation pathway is through debromination and production of bromodiphenyl ethers, which in turn can exponentially increase toxicity.
- *Pentachlorobenzene (PeCB)*, is a pesticide, fungicide and inadvertent by-product. PeCB has also been used in PCB products, colorants and pigments, as a flame retardant, and a chemical intermediate.
- *Tetrabromodiphenyl ether (tetraBDE)* and *pentabromodiphenyl ether (pentaBDE)* are industrial chemicals and the primary components of trade name pentabromodiphenyl ether (pentaBDE). PentaBDE has been detected in humans globally.
- *Perfluorooctanesulfonic acid (PFOS)* is used in the production of fluoropolymers. PFOS and related compounds are highly persistent, bioaccumulating and biomagnifying (Jones et al. 2003).
- *Endosulfans* are insecticides to control pests on crops and ectoparasites of cattle. They are toxic both to humans and aquatic and terrestrial organisms, linked to innate physical problems, mental retardation, and death. Endosulfans exhibit endocrine-perturbing ability acting as antiandrogens.
- *Hexabromocyclododecane (HBCD)* is a brominated flame retardant principally used in thermal insulation in construction industry. HBCD is persistent therefore toxic, with bioaccumulative and long-range transport properties.

Moreover, organochlorines can be divided into five subcategories Smith (1991):

1. DDT group and about other ten equivalent compounds (e.g. dicofol, chlorfenethol, chlorobenzilate, chloropropylate, methoxychlor, Prolan).
2. HCH and eight isomers (Lindane).
3. Chlorinated cyclodienes (including Aldrin, Isodrin, Dieldrin, Endrin, Telodrin, Heptachlor, Chlordane, Endosulfan).

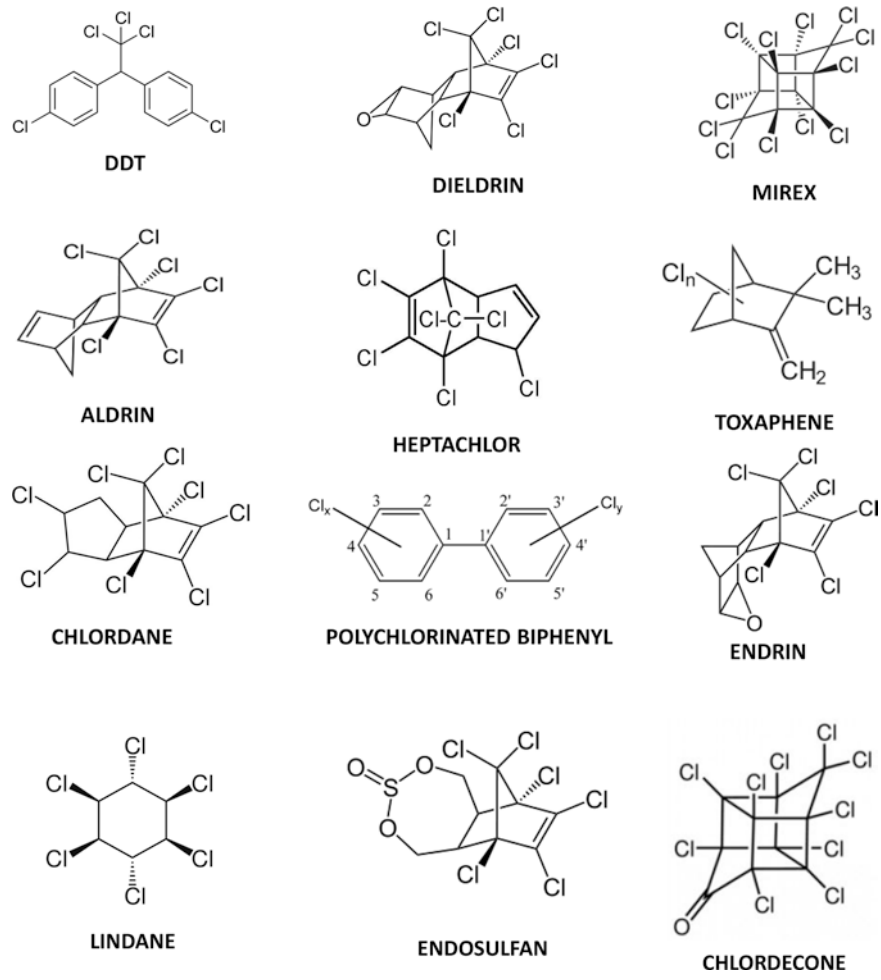

Fig. 4.1 Structures of some organochlorine pesticides

4. Toxaphene.
5. Pesticides with caged structure (Mirex, Chlordecon) (Fig. 4.1).

In the U.S., production and distribution of many POPs have been prohibited under the Safe Chemicals Act of 2011.

However, almost 54 years after Carson accentuated the health and environmental aftereffects of DDT, use of equally perilous pesticides has only been intensified. According to the Environmental Protection Agency, chronic POPs exposure perpetuates due to contamination of the food chain, in artificial as well as natural environments, and their use in developing countries still continues with deficient or non-existent legal regime.

2.2 POPs Action Mechanism

Bioaccumulation is the process of the increase of toxic chemical substances in organisms' tissues as the food chain moves up. By accumulation of non-biodegradable substances human is affected, as he usually comprises the last link in many food chains.

This accumulative trait has been the focal point of the research by Koureas et al. (2016). Peripheral venous samples were collected from 103 volunteers from the city of Larissa, Greece. The most prevalent POP was found to be p′-p DDE and hexachlorobenzane, whereas other substances consisted a minority. The results can be summarized as follow (Table 4.1):

Table 4.1 Serum levels of OCs in relation to demographic data (modified table from: Koureas et al. 2016)

Serum concentrations (ng/ml) of OCs according to demographic characteristics										
			Hexachlorobenzane				p′-p DDE			
		Count	Median	Percentile 25	Percentile 75	p value	Median	Percentile 25	Percentile 75	p value
Sex	Male	81	0.12	<LOQ	0.31	0.440	1.21	0.74	2.96	0.850
	Female	22	0.23	<LOQ	0.39	Reference	1.42	0.65	1.91	Reference
Age	53+	25	0.29	0.12	0.92	<0.001	2.58	1.14	3.97	0.002
	47–52	23	0.15	<LOQ	0.38	0.004	1.66	0.98	2.42	0.015
	40–46	27	0.17	<LOQ	0.33	0.016	1.24	0.77	2.28	0.059
	0–39	28	<LOQ	<LOQ	0.12	Reference	0.85	0.54	1.85	Reference
Smoking	Regular/daily	49	0.12	<LOQ	0.31	0.672	1.16	0.70	2.12	0.210
	Non smoker or less than 1 per day	54	0.14	<LOQ	0.39	Reference	1.62	0.80	3.05	Reference
Alcohol consumption	Regular/daily	12	0.12	<LOQ	0.29	0.700	1.37	0.83	1.99	0.658
	Less than 1 per day	91	0.13	<LOQ	0.35	Reference	1.25	0.70	2.73	Reference
Education	Higher	54	0.15	<LOQ	0.34	0.043	1.85	1.00	2.69	0.902
	Secondary	39	0.12	<LOQ	0.25	0.008	0.96	0.62	1.81	0.472
	Primary or none	10	0.56	0.12	1.55	Reference	1.99	0.33	6.40	Reference

It is evident that age has a strong relation to p-p′ DDE and hexachlorobenzane concentrations, a fact that can be explained if long half biological lives of these xenobiotics are taken into account.

Furthermore, POPs' interactions include interstitial factor interactions, toxicokinetic and toxicodynamic interactions.

In a broad classification, the elements that define toxicity and persistence are:

1. Sites of action; when a chemical interaction occurs, a toxic effect will occur on the organism if the concentration surpasses a certain threshold (the chemical affects the organism).
2. Sites of metabolism, leading to detoxication or activation.
3. Sites of storage. When placed there, the chemical has no observable toxic effect, is not metabolized and is not available for secretion.

Toxicity mechanism of POPs in animal organisms is the reaction result with their receptors, *biochemical lesion,* as a first stage. The toxicity is expressed by the dose causing lethality in the 50% of samples in tests with laboratory animals. In the case of DDT, the biochemical reaction with the receptor occurs in the anoxic membranes of nervous filaments. Organochlorines are mostly neurotoxic, as well as have the ability to penetrate through the pulmonary system and the skin. LC_{50} in food fluctuates between 14–5000 mg/kg in birds, and LC_{50} (aq) for 96 h range from 1 μg/l to 4300 mg/l.

Toxicity can be distinguished in two types, short and long term toxicity. In short term toxicity, intracellular organelles are affected, leading to leakage and formation of surface "blebs" (Fig. 4.2):

To a step further, long-term toxicity provokes apoptosis and/or necrosis. Depending the intensity and duration of exposure, even proliferation and carcinogenesis may be provoked instead of senescence and cellular death (Kim et al. 2015).

Natural mechanisms with which POPs act have been studied in a biochemical level. In laboratory tests, toxicokinetic models are important

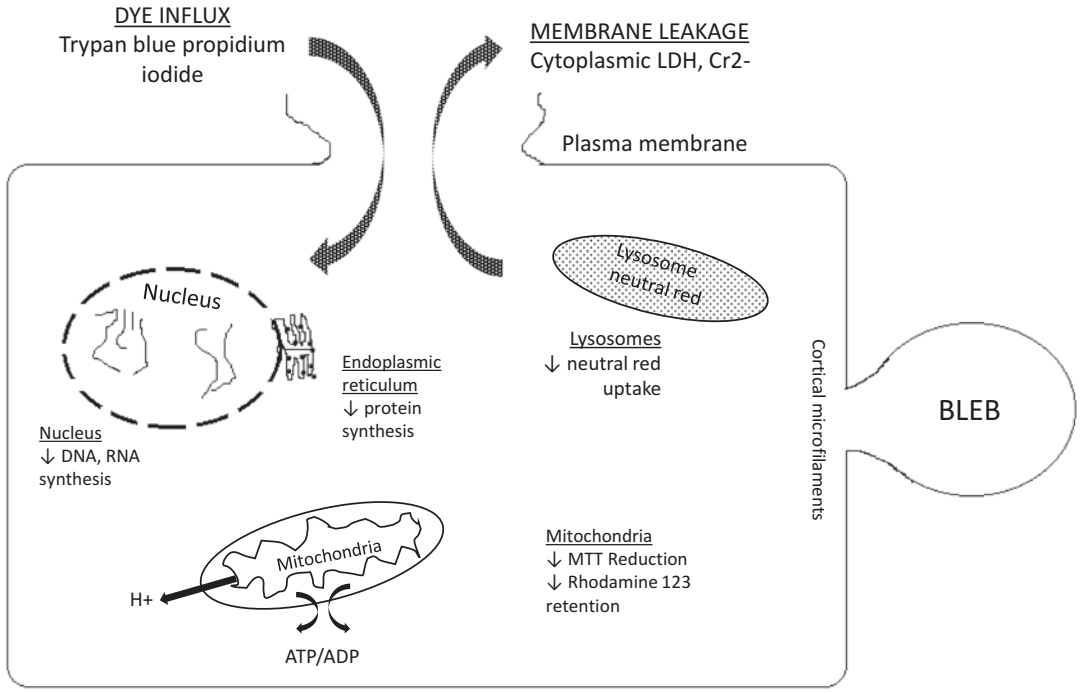

Fig. 4.2 Short-term toxicity outline (Modified image from: A Textbook of Modern Toxicology, 3rd ed., E. Hodgson, New York: Wiley, 2004)

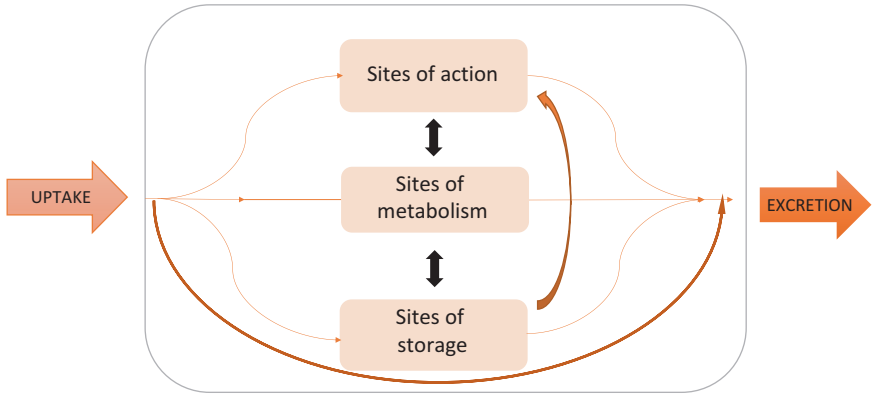

Fig. 4.3 Toxicokinetic model (modified figure from: C.H. Walker (2001), Organic Pollutants: An Ecotoxicological Perspective, Taylor & Francis Ed)

for the determination of possible parameters of components and the clarification of chemical interactions between the pesticides. The term *toxicokinetics* refers to the way a xenobiotic follows to gain entrance in the body. Once the xenobiotic ensures intracellular entrance, a number of genes are involved, giving molecular information about the target and actions of xenobiotic, also known as "reporter" genes. Xenobiotics aim at attacking cellular equipment and metabolism. This fact triggers the expression of chaperone-molecules, which can protect cellular enzymes form the xenobiotic (Mumtaz et al. 2002).

The predominant type of exposure is via dermal (García-García et al. 2016). and respiratory pathways. Intrusion of the skin varies congruently with the type of substance, temperature, relative humidity, type of unprotected exposed area of the body, period of contact and the presence of skin abnormalities (wounds, lesions). Absorption via the gastrointestinal tract has also been referred, albeit in rare cases (Aprea 2012). Of course, velocity of absorption depends upon forms of POPs on each occasion; oily solutions are absorbed faster in intestinal mucosa, whereas those dissolved in organic solvents pass preferably through the skin. Fate of POPs once they enter, involves biomodification in the liver, after a procedure of biological activation. Excretion happens renally, biliary and through feces (Fig. 4.3).

Toxicokinetics of individual compounds may change when exposure to multiple pesticides occurs, which means that the predicted toxicity will get altered. The final outcome of a pesticide modifying the absorption, distribution, metabolism and elimination of others, is the toxicokinetic interaction. It manifests at any dose levels, though the effects might not be measurable at low rates. This interaction leads most likely to alteration of the correlation between the external dose and the corresponding level of a pesticide at its target site, which in turn leads to an alteration in the threshold of effects (The Interdepartmental Group of Health Risks from Chemicals 2009). For the toxicodynamic interaction to be presented, an adequate quantity of pesticide to reach the target tissue is required, so that the normal tissue physiology becomes agitated. Also, a second toxicant is needed to approach the same tissue and cause a second disruption, either of aggravation or reaction result of the first substance. Again the prerequisite for toxicodynamic interactions is that the dose levels are sufficient enough to cause an effect, thus when dose levels are under the threshold of effect, no interaction is expected.

The two basic principles of two pesticides' influence to one another, are the concept of *additivity* and *interaction*. Additivity refers to the ability of two compounds acting independently, whereas interaction emerges in case of the result is divergent to what was expected. The notion of interaction includes the terms of synergism (namely higher total result) and antagonism (Hernández et al. 2013a, b).

To sum up, when pesticides' mechanism action is the same, the combined effect is the aggregate of each individual substance. If each compound differs in the way of action and the dose is below the threshold, the toxicological effect is none. Moreover, if the pesticides affect multiple sites, they may present various toxic results, with the dispensing of some leading to greater toxicity, than each one administered individually (synergism phenomenon).

It is known that toxicity among organisms is affected by age, gender, type of exposure, synergy with other pollutants and chemicals and other (Gasull et al. 2013). Furthermore, various factors, such as nutrition (Kahleova et al. 2016), very low temperature and reproductive activity play an important role as well.

3 Effects of POPs in Human Health

The mechanism of pesticide-mediated toxicity is a combination of various enzyme-inhibitory, metabolic and transcriptional processes occurring at a cellular and molecular level. Thus, POPs are linked with pathogenic conditions in individual organs and systems.

The mechanisms of biochemical changes, gene mutations and oxidative stress after cumulative or repeated exposure to residual doses of pesticides are described and potentially can cause common ailments, incurable, up to deadly diseases, especially in vulnerable population groups and in subsequent generations Kouretas et al. (2013). The cardinal acute toxic action of organochlorine pesticides is on the central nervous system, as they interfere with the Cl^- channels, blocking the neurotransmitter GABA in the postsynaptic termination. As a result, a hyperexcitable state in the brain is induced, leading to myoclonus, paresthesias, tremor, ataxia and hyperreflexia. Convulsions caused by cyclodienes may recur over periods of several days and are also typical of acute organochlorine poisoning. Other symptoms like rhabdomyolysis, hyperthermia, cardiac arrhythmia, decrease of blood pressure and renal failure may also be encountered in acute poisoning (Table 4.2).

Organochlorines are also reported as potential carcinogens. It has been investigated that four OCs (dieldrin, endosulfan, heptachlor, and lindane) affect mitogen-activated protein kinase (MAPK) pathways. Additionally, organochlorines induce disturbance of hepatic biochemistry, including fluctuation of SGOT and SGPT levels, and ALP liver fraction, the presence of which in plasma is pathological.

It is observed that POPs under experimental conditions exhibit a similar potency to estrogens. They activate the production of hepatic microsomal enzymes that leads to hydroxylation of steroids, which in turn burdens the reproductive

Table 4.2 Effects of short-term exposure to POPs

	Systems/organs affected	Mechanism	Clinical manifestations
Acute Toxicity	Central Nervous System (CNS)	Impede Cl- channels/ Block GABA postsynaptically	Myoclonus, paresthesia, tremor, ataxia, hyperreflexia, convulsions, seizures
	Musculoskeletal	Muscular cell hypoxia, lesion of cellular membrane	Rhabdomyolysis
	Kidneys	Myoglobin sediments in renal tubules (due to rhabdomyolysis)	Renal failure
	Cardiovascular	↑ myocardial excitation	Arrhythmia
	Pulmonary	Imbalanced gas exchange, metabolic acidosis	Respiratory depression
	Dermatological	Permeation of stratum corneum, haptenization	Porphyria cutanea tarda
	Gastrointestinal	Irritability of gastric mucosa, activation of intestinal aquatic secretory mechanism	Nausea, regurgitation, secretory diarrhea

ability. Other impacts in organisms pertain to thyroidal secretions Petreas et al. (2011), adrenaline function, biogenic amines and immune system. As POPs accumulate in tissues, they interact with other toxic substances, metabolites and residues. These interactions may be additive, synergetic, augmentative or antagonistic.

POPs function as endocrine disruptors, as they act as a hindrance to hormone signaling and cell communication. Apart from obesity-linked diabetes, POPs may provoke reproductive dysfunction (Lundin et al. 2016) through mutations (e.g. sperm DNA methylation, Consales et al. 2016) and modifications in steroidogenesis (van den Dungen et al. 2015). In female reproductive system, polybrominated biphenyls have been reported to instigate precocious antral follicles luteinization (Gregoraszczuk and Ptak 2013). Estradiol activity was also found to be diminished when exposure to multiple pollutants occurred (Carpenter et al. 2002).

POPs and obesity, as an interface Ibrahim et al. 2011, may get extended to breast cancer incitement (Fredslund et al. 2012, Artacho-Cordon et al. 2015, Reaves et al. 2015). Certain POPs can perform estrogenic action and affect leptin levels, parameters that are intensely linked to activation of HER-2 and ERα receptors, expression of gene proliferators and hormonal signaling Lautenbach et al. (2009), Lemaire et al. (2006). A study on Greenlandic Inuit showed that plasma PFCs were at an elevated level in breast cancer cases (Bonefeld-Jorgensen et al. 2011).

Exposure to POPs may affect cardiac function. POPs of certain chemical structure (e.g. p′,p-DDE), have been reported to increase blood pressure, thus may act as risk factors (Henríquez-Hernández et al. 2014). Goncharov et al. (2011) have also detected a positive association between plasma β-HCH and PCB levels and systolic as well as diastolic blood pressure. However, POPs configuring BP is an issue of conflict, as DDT, β-HCH and oxychlordane were found to not trigger hypertension (Valera et al. 2013). Moreover, Wayman et al. (2012) have stated the aspect that non-dioxin-like PCBs act beneficially to the ryanodine receptor calcium ion channels (Ca^{2+}) of myocardium, leading to impairment of the left ventricle. Similar conclusions have been deduced by Lind et al. (2013), where a correlation of serum POPs and activity of the left ventricle was designated Sjφberg et al. (2013a, b).

In an investigation of Arsenescu et al. (2011), PCB77 was administered to male ApoE−/− mice. Interestingly, action of angiotensin II, a peptide hormone increasing mean BP, was enhanced, resulting in a notable rise in abdominal aortic aneurysm and atherosclerosis incidence.

Cardiovascular diseases (CVDs) can be provoked by oxidative stress and endocrine disruption. These two pathophysiological mechanisms, putative of bringing upon CVDs, have been suggested mainly due to the lipophilic character of POPs, despite partly inadequate evidence of how these mechanisms are involved (Zeliger 2013, Ljunggren et al. 2014).

Multiple Chemical Sensitivity (MCS), also known as Environmental Illness, may occur when the organism is incapable of coping with low doses of chemicals. By the time sensitivity to POPs manifests, the potential of subsequent exposure to POPs deteriorates the symptoms of MCS, which give a broad spectrum, from nausea and musculoskeletal problems, to cardiovascular diseases.

Children form an equally risk group, when in contact with POPs. Even at fetal stage, POPs can permeate the bloodstream and affect fetal growth (Long et al. 2015). Lv et al. (2013) found that polybrominated diphenyl ethers were able to permeate the placenta, despite the barrier it forms. A study by Valvi et al. (2014) examined the prenatal exposure to POPs, comparing data (POP levels in serum) from pregnant women and their infants. They found out that exposure to POPs led to precocity and overweight infants. Particularly for PCB, there was an impressive association between its concentration, age of children and period of lactation.

Since development continues and cellular defense mechanisms are under progress, the impact in children is even more intense. As mentioned before, metabolic syndrome is variously related to POPs absorption and bioaccumulation. Herein, this correlation is enhanced. It is observed that, exposure to low doses of POPs in children

Table 4.3 POPs exposure in utero in relation to cardiometabolic risk factors (modified table from Rhea mother-child cohort study, Crete, Greece)

Outcome	Cases (%)/total	Exposure	Adjusted model
Systolic BP (mmHg) [β (95% Cl)]	427	HCB	4.34 (0.63, 8.05)
		DDE	2.31 (−0.07,4.69)
		PCBs	2.16 (−2.03, 6.34)
Diastolic BP (mmHg) [β (95% Cl)]	427	HCB	2.48 (−0.13,5.09)
		DDE	1.79 (0.13, 3.46)
		PCBs	−0.49 (−3.43, 2.45)
C-reactive protein > 3 mg/L [RR (95% Cl)]	46(11.3)/409	HCB	2.88 (0.86, 9.64)
		DDE	2.23 (0.94, 5.29)
		PCBs	4.50 (0.89, 22.76)
Leptin (ng/mL)[β (95% CI)]	440	HCB	2.15 (0.42, 3.89)
		DDE	1.21 (0.16, 2.27)
		PCBs	1.55 (−0.42, 3.52)

may exert a negative impact in triglyceride levels and increase diastolic pressure (Lee et al. 2016). As inhalation consists one of the main gateways of POPs into the body, childhood asthma has been proved to get aggravated when exposure to organochlorines and PCBs occurs (Meng et al. 2016). Respiratory system can also be affected by the elevation of IL10 triggered by HCB exposure (Gascon et al. 2014), fact that indicates also the existence of adverse effects in the immune system.

DDE and HCB have also been described to progress to higher likelihood of adiposity and to increased BP in early childhood (Table 4.3).

Perfluorinated substances have been identified to possess immunotoxic potential, which has been expressed by reported cases of splenic atrophy and shrinkage of thymus gland. Therefore they disabled proper immune response to influenza virus and antibody functionality, as has been observed by Corsini et al. (2014). To a further extend, as POPs are influential towards the immune system, rheumatoid arthritis has been suggested as a consequence of exposure to PCBs. Study by Lee et al. (2007a, b) noticed a positive relation between plasma PCB levels and autoimmune arthritis, with a presumable explanation being the endocrine-disrupting nature of POPs. Moreover, the same conclusion can be inferred if metabolic changes are taken into account, as metabolic disorders due to obesity are implied to incite osteoarthritis. So, similarly, POPs packed in adipose reservoirs are also plausible to lead to osteoarthritis as well.

An aggregate of the consequences to human health, regarding long-term exposure to POPs, could be depicted as follow (Table 4.4):

Herein, the focal point of the present review consists the induction of obesity, as a result of exposure to POPs. To achieve that, a preview of the various aspects of obesity would be of benefit.

4 Obesity as a Medical Situation

4.1 Definition and Description of Obesity

Obesity is a medical condition that pertains to abnormal accumulation of body fat, usually 20% or more over an individual's normal body weight. In a molecular level, adipocyte lipid droplets consist of a phospholipid monolayer and a lipid ester core. When these lipid droplets excess, obesity occurs. It can also be described as a situation, where an imbalance exists between energy uptake and expenditure. Obesity has been associated with a wide range of clinical conditions, in the cluster term *metabolic syndrome*.

Obesity is a complex condition, as it is caused by both genetic factors as well as environmental

Table 4.4 A general overview of the effects, owing to prolonged exposure

	Systems/organs affected	Mechanism	Clinical manifestations
Chronic Exposure	Liver	↑ SGOT, SGPT and ALP plasma fraction → steroid hydroxylation	Hepatic disease, ↓ reproductive ability
		Inhibition of TH	Under further research
	Endocrinal	Affected leptin	Breast cancer
		Sperm DNA methylation	Reproductive dysfunction
		↓ estradiol, luteinization of precocious antral follicles	Osteoporosis, reproductive dysfunction
		Oxidative stress	↑ BP (under investigation)
	Cardiovascular	↓ action of angiotensin II	Atherosclerosis, abdominal aortic aneurysm
		↑ ryanodine receptor Ca^{2+} myocardial channel	Left ventricle impairment
	Immune	Disabled immune response	Splenic atrophy, thymus shrinkage, RA, osteoarthritis
	Pulmonary (in children)	Induction of inflammation processes (IL-10)	asthma
	Impact on fetuses/infants	Intrusion through the placenta	Precocity, overweight, ↑ risk of metabolic syndrome

stimuli. Therefore, it is linked with increased risk morbidity and mortality. BMI (Body Mass Index) is the most broadly used method to portray obesity, and is regularly used in estimating body fat (adiposity). However, the limitations of BMI as a hazard indicator are avowed, thus abiding interest is expressed in identifying complementary assessment tools that link adiposity and disease.

Overall, the "obesogen hypothesis" states that chemical pollutants (obesogens) foster obesity by disturbing appetite controls and promoting adipocyte hypertrophy and hyperplasia, thus inducing weight gain Pereira-Fernandes et al. (2014), Reaves et al. (2015). Bourez et al. (2012), by performing a murine embryonic adipocyte assay, came to the conclusion that triglyceride levels in the lipid droplets are dependent on PCB intake.

Normally, storage of fat serves the protection of the body from free fatty acids, which provoke oxidative stress. Adipose cells have the ability to store triaglycerols and function as an endocrine tissue, indeed the largest in the body (Bengmark 2015). Working as that, adipocytes can communicate via body-fat regulating hormones lectin, visfatin, adiponectin (adipokines), cytokines, complement proteins and growth factors. Therefore, as molecular obesity markers LEP, ADIPOQ, TNFa and PPARγ (peroxisome proliferator-activated receptor gamma) may be of use when investigating VAT and SAT deposit, while LEP and ADIPOQ can also be measures in the case of serum samples. In obese people compared to lean, plasma leptin, fasting glucose, triglyceride and insulin are augmented, whereas HDL and inflammatory indicator concentrations are lower.

Herein, when abundant adipose tissue is stored, the increased lipolysis, stimulated by the enhanced sympathetic function due to obesity, incites the release of free fatty acids. Free fatty acids then induce lipotoxicity, as oxidant stress occurs Kim et al. 2012. Moreover, free fatty acids may cause dysfunction of the insulin receptors (Berndt et al. 2007) and hyperglycemia (Fig. 4.4).

Adipose tissue is described as an active metabolic endocrine organ, and dysregulation provoked by obesity stimulates an enhanced inflammatory tissue process, including modified secretion of cytokines and adipokines. Obesity-stimulated production of these factors is outright interrelated to conditions such as chronic inflammation Myre et al. (2014), cardiovascular disease

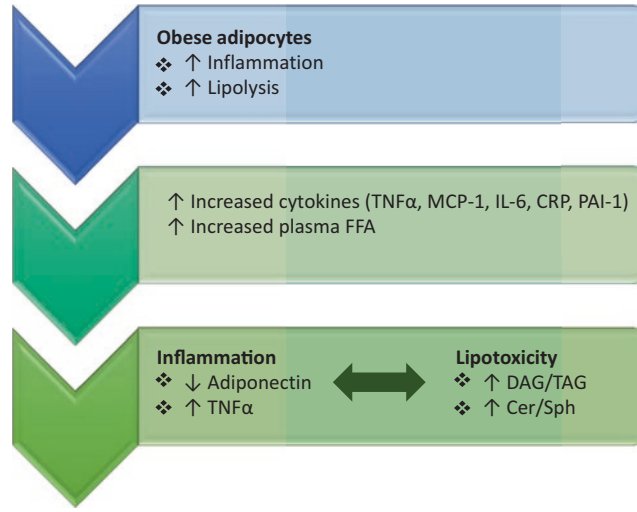

Fig. 4.4 In obesity, adipocytes become enlarged, and trigger systemic inflammation and lipotoxicity, because of the release of cytokines and accumulation of lipids in other tissues (modified graph depicted by Kang et al. 2013)

(Karastergiou et al. 2012), atherosclerosis, cancer Hopperton et al. (2014), Park et al. (2014), and lipotoxicity (Fig. 4.5).

At this point, it may be helpful to briefly adumbrate the different types of adipocytes. As each adipose tissue depot (serum, subcutaneous, visceral and intermuscular) functions differently regarding the endocrine, metabolic and cell-signaling procedures, various cell-subtypes are expressed Yu et al. (2011).

White adipose tissue (WAT), entails a single lipid droplet and has endocrine and immune actions; protecting homeostasis and affecting insulin sensitivity. *Brown adipose tissue (BAT)* contains small lipid droplets, derives from precursor cells and participates in thermogenesis Giralt et al. 2013, ranging from classical brown to beige/"*brite*" *adipocytes*, when a thermogenic stimuli begins. WAT is an important endocrine organ, as its metabolism is controlled by the sympathetic nervous system and by many hormones (insulin, catecholamines, thyroid and steroid hormones). BAT can be primarily found in the cervical-supraclavicular area, it contains many small lipid droplets, a large number of mitochondria, higher oxygen consumption than WAT, high capillary perfusion and sympathetic nervous system innervation. Five main types of POPs have been reported to get stored in WAT; organochlorines, PCBs, dioxins, some BFRs (polybrominated flame retardants) and other pollutants (not categorized yet) (Figs. 4.6 and 4.7).

Nuclear factors with PPAR-γ (peroxisome proliferator-activated receptor gamma) regulate the proliferation of precursor cells and their differentiation into mature cells. This step leads to heterodimerization with RXR (retinoid X receptor), promoting their differentiation. These procedures are going to be analyzed underneath.

In a study (Kim and Lee 2014) about visceral and subcutaneous concentration of POPs congeners in tissue samples, among surgery patients with liver and gallbladder findings of lesions, it was observed that the concentration of PCBs was ten times higher in the visceral than the subcutaneous tissue, which can be translated as a correlation between the adipose tissue features and the attraction to POPs. To the same direction, Bourez et al. (2012), by performing a murine embryonic adipocyte assay, came to the conclusion that the PCB intake is dependent on triglyceride levels in the lipid droplets, which can be interpreted as a preferential reside of POPs in adipose tissue.

Obesity is prevalent mainly in developed countries; from 1980 to 2008, the incidence of obesity has increased up to double in adults and tripled in children (OECD 2013, Health at a Glance) Miller et al. (2012). The worldwide epidemic picture of obesity, nowadays, is a mixture of a diet rich in calories and a rather sedentary

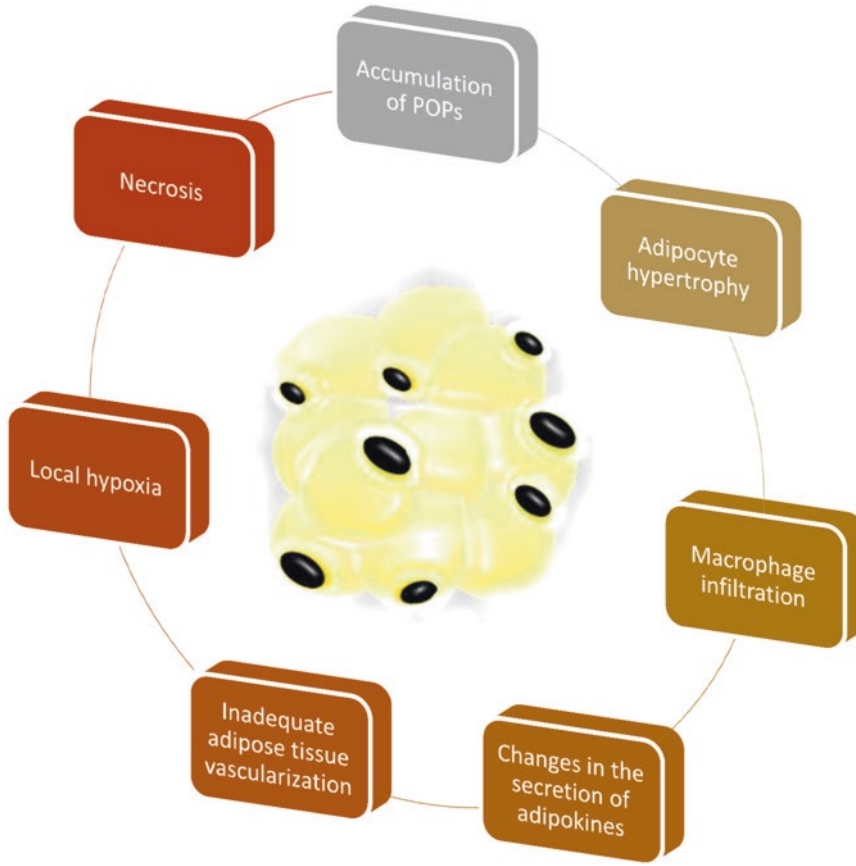

Fig. 4.5 Possible modifications during adipose tissue hypertrophy

lifestyle (Caballero 2007, Caprio et al. 2008). In this scene, a multitude of environmental pollutants, also known as environmental obesogens, come to join in (Decherf et al. 2011, Myrmel et al. 2016). The term is not used arbitrarily, as these pollutants are observed to provoke intrinsic hormonal, obesity-related dysregulation.

4.2 Obesity Linkage to POPs Exposure

POPs are, as mentioned before, hydrophobic compounds that can traverse the phospholipid cell membranes and accumulate in adipose tissues Lyche et al. (2011). Human exposure to POPs occurs mainly through consumption of seafood and livestock, as POPs are water insoluble and not easily metabolized (Baillie-Hamilton 2002).

WAT, as the key-organ for thermoregulation and energy deposit, releases metabolites and adipokines for inter-cell communication. POPs may interfere in this procedure by modifying the action of endogenous ligands of nuclear transcription factors and altering metabolism, differentiation and secretory function of fatty cells.

This can be accomplished by three main mechanisms (Walker et al. 2008):

- Disruption of hormones' function in WAT (androgen, estrogen, thyroid hormone).
- Intervention in retinoic receptors' function in WAT.
- Interaction with transcription of peroxisome proliferator receptors.

POPs may provoke obesity by changing homeostatic metabolic set-points, perturbing

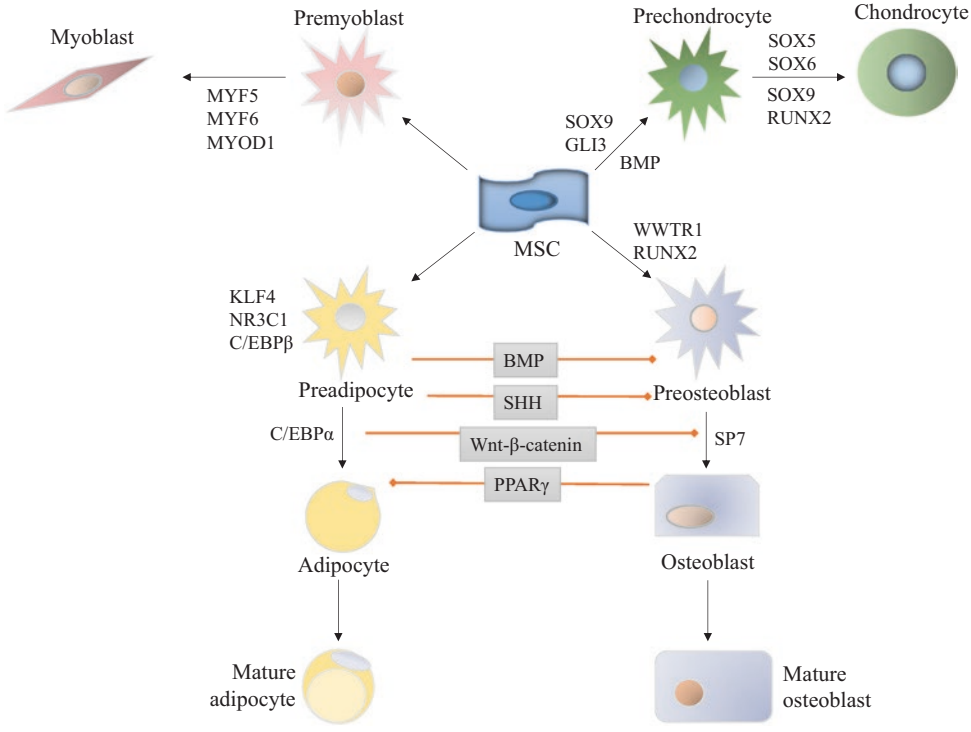

Fig. 4.6 Origin of adipocytes (modified figure, depicted by Takada et al. 2009)

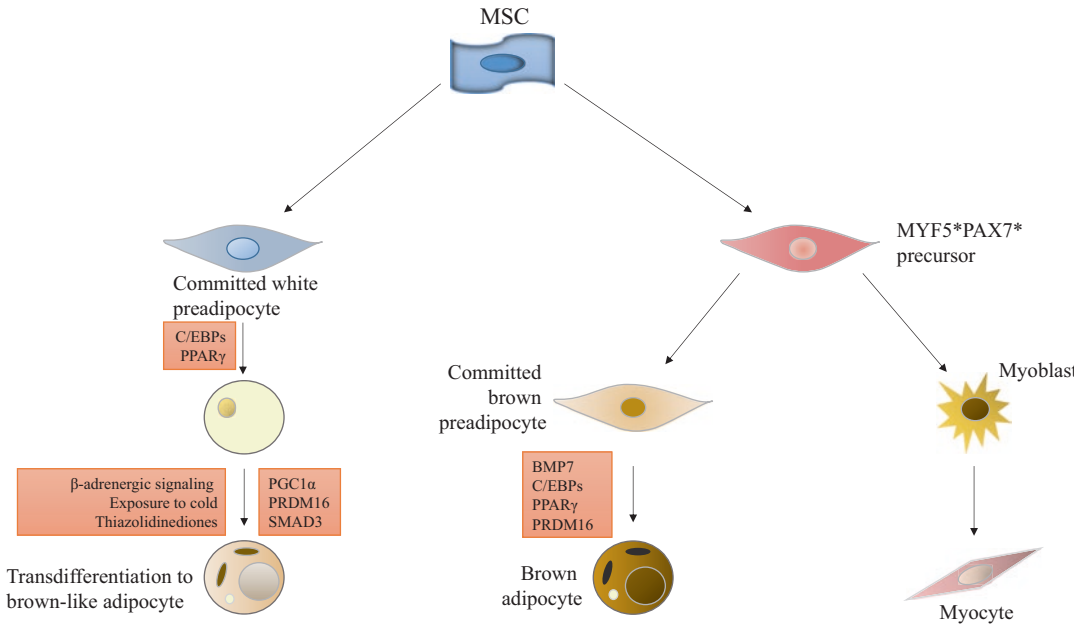

Fig. 4.7 Differentiation of human adipocytes (modified figure, depicted by Cristancho and Lazar 2011)

appetite regulation mechanisms and controls, blocking lipid homeostasis and causing hypertrophy of fat tissue.

4.2.1 POPs as Endocrine Disruptors

POPs, and especially organochlorines and PCBs, act as endocrine disruptors (McKinney et al. 1994), as they can affect physiological development of the embryo and induce, in adulthood, reproductive impairment. A way to achieve that is by mimicking, as they (DDT, endosulfan, lindane) present similarity to steroid and diphenylether natural structural hormone groups, or antagonizing hormones' normal function. Then POPs link with receptors and affect cellular metabolism and fat storage. POPs accumulation is theorized to promote obesity, dysregulating adipose tissue cells. Thus, it can be easily deduced that endocrine abnormalities and disruption of cell signaling derive from the cooperation of POPs existence and obesity-triggering factors, for an even more enhanced result La Merrill et al. (2013).

Intervention of hormone signaling pathways may result to two different situations; either inhibition of the response, or reinforcement at wrong time and place.

Regarding biological systems, dose of hormone and response of receptors exhibit a linear function, when approximately 10% of the receptors are occupied. While hormone dose increases, the rate of occupancy does not display a linear behavior, despite expecting the opposite. Linearity is only present in biological responses to low doses. Therefore, when high dose exposure to hormones occurs, down regulation of receptors is observed, which justifies the downward tendency of the curve. Thus it is concluded that endocrine-disrupting chemicals create inverted U dose-response curves, with specific borders. Moreover, several organochlorine pesticides and PCBs are found to act as predictors of insulin resistance and dyslipidaemia amongst people without evident diabetes. In addition, some POPs have even predicted future obesity (Lee et al. 2011) (Fig. 4.8).

PCB-77 is found to increase adipocyte differentiation, foster the expression of proinflammatory cytokines and elevate the expression of the peroxisome proliferator-activated receptor γ, which regulates cell energy homeostasis (Arsenescu et al. 2008).

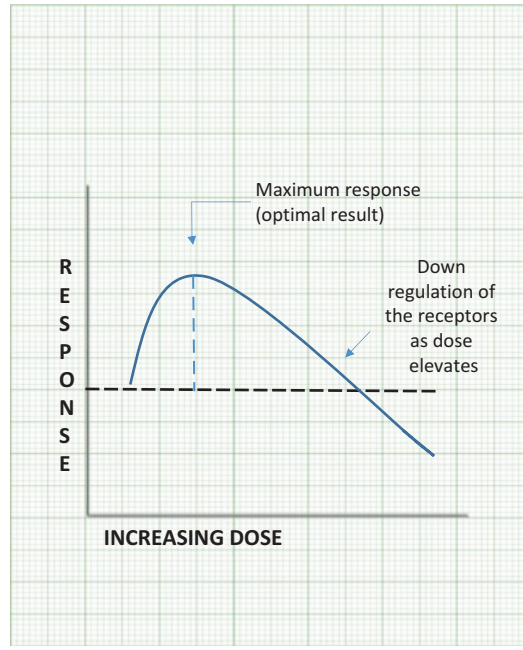

Fig. 4.8 Dose-response inverted U-shaped curve

On the contrary, a survey by Müllerová et al. (2008) underlined a negative relationship between serum adiponectin levels and PCB153, in specimen regarding obese women who were under non-energy-restrictive regime. Although PCB153 was identified as a partial androgen antagonist, herein it exhibits little or no affinity to aryl hydrocarbon receptor Bourdon et al. (2010). At the same wavelength, a negative association between adiponectin and PCB concentration was also identified in the study of Lim and Jee (2015) in Korea. These results lead to the deduction that PCB28, PCB138 and PCB153 may deactivate adiponectin in obese individuals. Diabetes may be fostered through immunotoxic mediation of POPs, by their binding with estrogen receptors. This mechanism would potentially cause a chronic inflammation, reduced functionality of mitochondria, elevated lipolysis rate and oxidation of fatty acids. All the above are notably related to insulin resistance (Arrebola et al. 2013, Lee et al. 2008, Lee 2012).

A cross-sectional study among the U.S. population published in 2006 (Lee et al. 2006), showed a strong association between the serum concentrations of chlorinated POPs (particularly organochlorine pesticides and PCB congeners) and the prevalence of type 2 diabetes. POPs were correlated with insulin resistance and adverse lipid profiles (high triglyceride and low HDL). Even though the concentration of POPs has been reduced worldwide, a possible explanation is that a low yet continuing exposure is more noxious than a high dose (Kim et al. 2010, Lee et al. 2010, Tsatsakis et al. 2012).

4.2.2 POPs as Metabolic Disruptors

Public health concern has been intensified, regarding exposure to POPs and metabolic disorders, as both incidence of obesity and diabetes has worldwide rapidly advanced.

Interference of POPs with endocrine signals leads not only to endocrine disruption, but also metabolic. VAT (visceral adipose tissue) in comparison to SAT (subcutaneous adipose tissue) is known to be involved in metabolic disorders, e.g. cardiovascular diseases, hypertension and diabetes type 2 Roos et al. (2013). WAT also has been proved to constitute a reservoir of POPs and, therefore, provoking modulation of the adipocyte phenotype, inciting metabolic disorders Müllerová et al. (2007).

The relationship between POPs and glucose metabolism comprised the focus of interest of Dirinck et al. (2014). For that purpose, glucose tolerance was defined in obese and lean subjects, with the concurrent comparison of SAT and VAT computed tomography (CT) results. It was found, as expected, that obese individuals had POPs and glucose levels highly correlated. A positive relation was also identified, regarding levels of POPs and waist circumference. Particularly VAT was shown to be related to POPs, as it was apparent in CT scan.

Pestana et al. (2014) displayed by their work the relationship between POPs and metabolic disruption, with the subjects including obese surgery patients. Samples of VAT and SAT were collected and examined for the presence of POPs. Notable was the fact that higher concentrations were detected in VAT over SAT, a possible explanation being the susceptibility of VAT to lipolysis and consequently POPs release. Metabolic disorder suggested the researchers to correlate to elevated BP, risk for evolvement of cardiovascular diseases and dysglycaemia. Dysregulation of fat tissue due to POPs exposure is a key of understanding the following progress of obesity, hence the importance of future research focusing (Fig. 4.9).

Subsequently to exposure to organochlorines comes their storage in adipose tissue. Then dechlorinization, oxidation and conjunction may occur, with finally the excretion through the bile and urine. However, this route is altered, as a multitude of unmetabolized POPs return to the circulation via enterohepatic recycling instead of being emitted. Metabolic residues found in the body emanate mostly from DDT and DDE, dieldrin, mirex, heptachlor epoxide and beta hexachlorocyclohexane isomer, due to their slow breakdown rate. As a result, these POPs are more likely to accumulate in fat deposits, than lindane, toxaphene, endosulfan and endrin, whose metabolic pace is much faster.

The acknowledged bioaccumulative property of POPs was estimated in obese women (n = 20) after diet induced weight loss, in a 5 years follow-up study (Müllerová et al. 2015). Anthropometric data and plasma analysis were compared, for the assessment of seven different serum POPs concentrations. During this 5 years research (from 2006 to 2011), there were increased levels of five of the seven overall examined POPs and the distribution of POPs varied, as obesity progressed. Drastic weight loss is also linked to higher plasma levels in the liver, brain and in a reduced quantity in WAT Kim et al. (2011).

To the same perspective, simultaneous exposure to certain POPs in the general population may contribute to the development of obesity, dyslipidaemia and resistance to insulin, which could lead to diabetes type 2 Rylander et al. (2005), Ruzzin et al. (2010), Taylor et al. (2013), Ngwa et al. (2015), cardiovascular diseases and metabolic syndrome Ruzzin et al. (2012). Obesity results from energy imbalance, which in turn leads to insulin overproduction and finally exhaustion of beta pancreatic cells, resistance

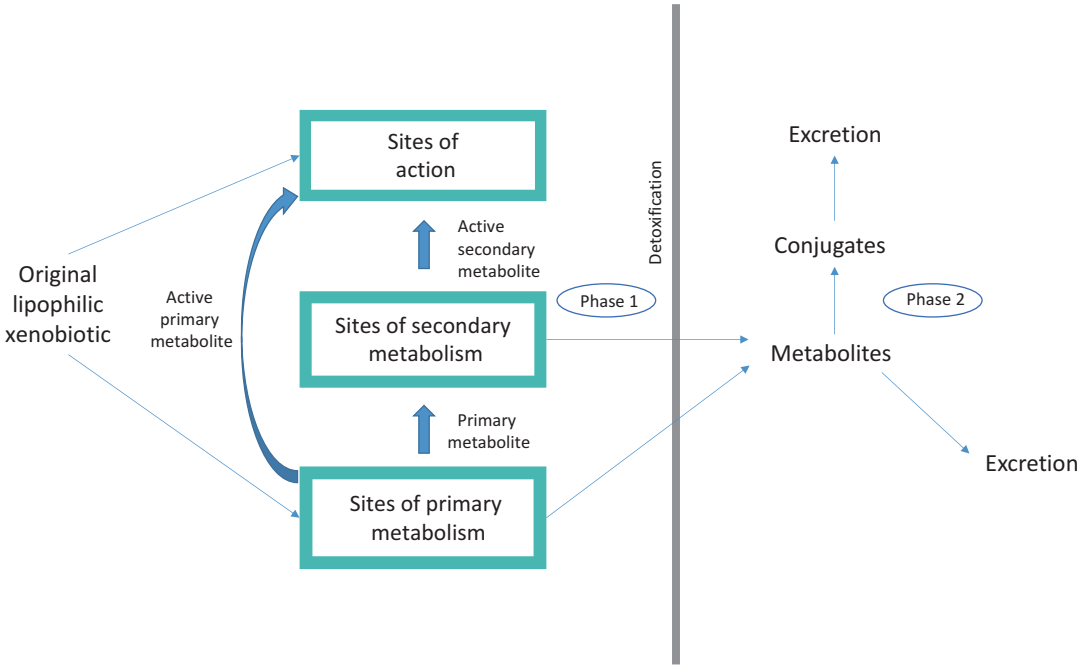

Fig. 4.9 Toxicity and metabolism (modified figure, depicted by: C.H. Walker (2001), Organic Pollutants: An Ecotoxicological Perspective, Taylor & Francis Ed, p. 23)

and diabetes. At first, the pancreatic beta-cells are able to counterbalance this event by increasing insulin secretion, but, as insulin resistance deteriorates, type 2 diabetes occurs when the pancreas is unable to adapt.

Dirinck et al. (2011) performed a cohort study of obese and lean adults, in which serum samples were analyzed for the presence of POPs. The variables assessed were BMI, waist fat mass percentage and total abdominal adipose tissue. A positive relationship between high serum levels of βHCH (beta-hexachlorocyclohexane) and BMI was found, though serum PCB levels were inversely proportional to BMI. In other words, as obese have higher amounts of fat deposits, more PCBs can be captured in adipose tissue and less are in blood circulation. These findings imply that the impact of exposure to POPs is not only obesity-related, but also diabetes-indicative.

Recently, a hypothesis was stated, that environmental chemicals could possibly affect metabolic programming, if the exposure occurs in utero. A very recent cohort study of Péronard et al. (2015), postulated the aspect that prenatal Tang-Peronard et al. 2015 exposure to POPs correlates with increased insulin levels in girls, 5 years of age, as a result of POPs' action as endocrine disruptors. Both leptin and insulin resistance are involved in the pathogenesis of metabolic syndrome. Physiologically, leptin is secreted by the fat tissue and its serum concentration depends on BMI, yet in obese individuals leptin increases independently. In this particular case it was shown that girls with the highest prenatal POP exposure were about 75% more likely to develop higher serum concentration of insulin.

Complementary to that, embryonic exposure to POPs was as well related to postnatal weight gain. Valvi et al. compared serum levels of POPs in pregnant women and their infants. Interestingly, it was evident that exposure to POPs in utero led to precocity and overweight infants, gaining fat mass in a rapid rate.

Rhea Mother-Child cohort study (2015 Crete, Greece) by Vafeiadi and co-workers investigated the relation of POPs exposure in utero to obesity and cardio-metabolic risk factors in 4-year-old

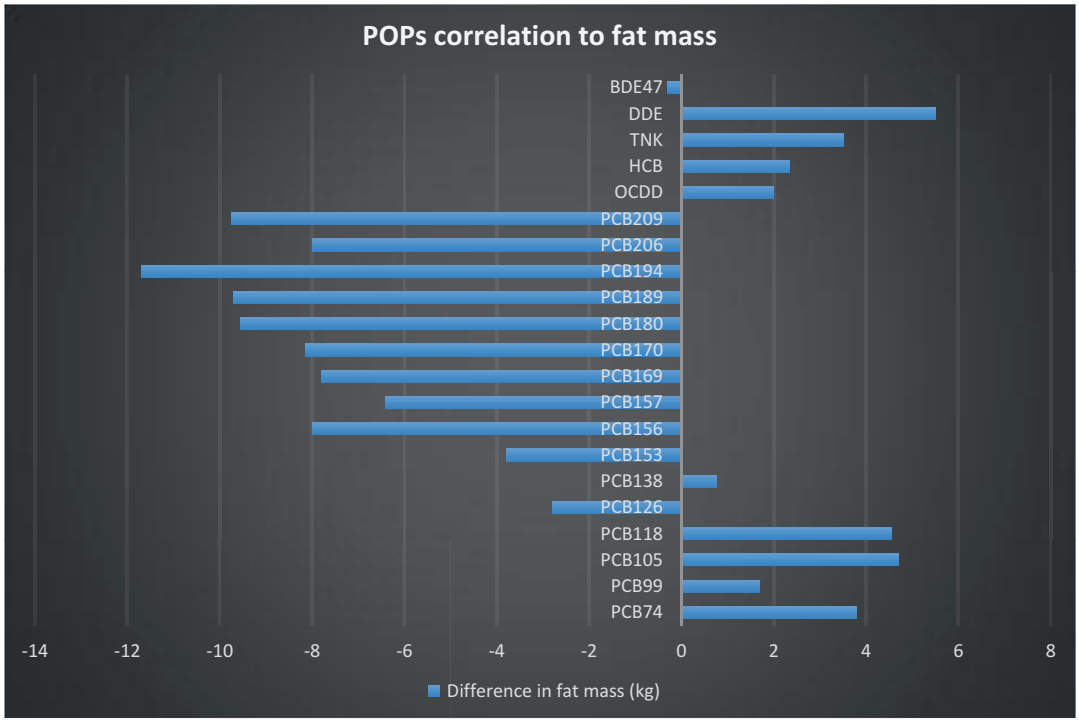

Fig. 4.10 An overall picture of POPs' correlation to fat mass (modified diagram, from Rönn et al. 2011)

children (n = 689). For this purpose, concentrations of PCBs, DDE and HCB were estimated in maternal plasma samples during the first trimester. Obesity in children was determined through anthropometric data and biochemical analysis. It boldly appeared that prenatal exposure to DDE and HCB (and less PCB) was tightly linked with excess adipose tissue and higher blood pressure levels in early childhood. Though the exact mechanism of this occurrence is not very clear, several studies claim that DDE exposure increases proliferation and differentiation of preadipocytes (Chapados et al. 2012; Moreno-Aliaga and Matsumura 2002). The above findings are of particular importance regarding public health implications due to the high prevalence of obesity and exposure to POPs.

Lee et al. (2014) came to the conclusion that especially diabetes type 2 is highly linked with POPs effects, possibly through the interaction with obesity. Even at low doses, type 2 diabetes could be predicted in asymptomatic obese subjects, as metabolic perturbation precedes the manifestation of diabetes (Airaksinen et al. 2011). Serum levels of POPs pertain in multiple ways to body fat mass, measured by dual-energy X-ray absorptiometry (DXA) (Rönn et al. 2011). POPs' concentration was assessed in plasma samples of subjects aged 70 residing in Uppsala, Sweden. Different parameters were estimated, some among of them being total fat mass and fat distribution BMI, gender, alcohol consumption, education level and physical activity. Particular emphasis should be ascribed to the results, as each type of POP affected adipose mass either positively, or an adverse correlation was marked. For instance, PCB118 incited development of fat tissue, whereas PCB180 induced adipocytes' remission. A distinct portrayal of this interaction could be depicted as following (Fig. 4.10):

Nutrigenomics/nutrigenetics consist relatively recent research fields, with the central axis being nutrients as adjusters and genome protectors in relation to harmful pollutant results Marti et al. (2005). They have been presented as a promising solution towards deleterious effects of toxic chemicals, as they may act as biomarkers, boding genome mutations induced by chemicals like

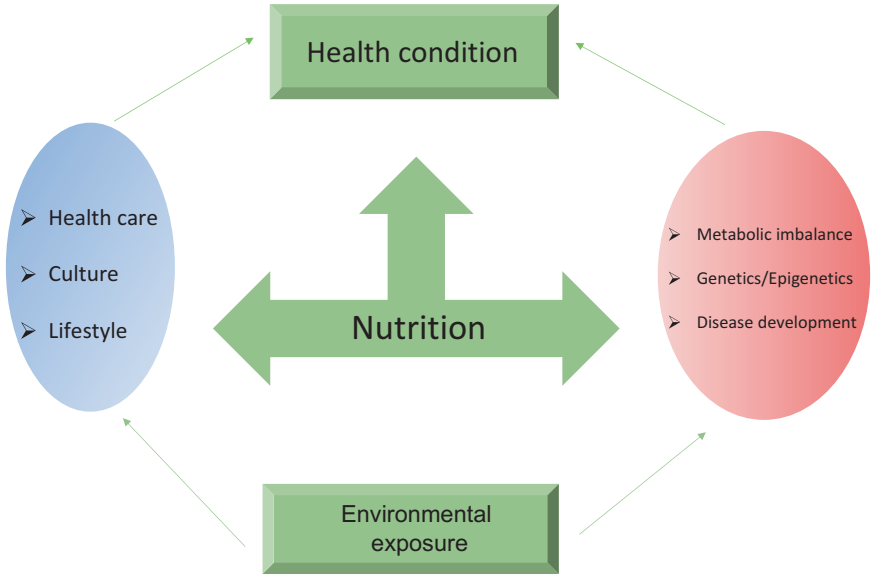

Fig. 4.11 Nutrition as a mediator of environmental exposure to pollutants and health condition (modified diagram from Hennig et al. 2012)

polychlorobiphenyls (Anetor 2010). Healthy dietary uptake encompasses a hopeful objective for decreasing the prevalence of diseases linked with environmental pollutants. Specifically POPs can produce free radicals in the body, inciting inflammatory response and related health problems, such as hypertension, atherosclerosis and diabetes. A survey carried out by Hennig et al. (2005) focused on endothelial functionality in premature atherosclerotic lesions. A connection was established between oxidative stress and activation of inflammatory genes and endothelial impairment provoked by PCBs. The interesting fact of the research was the prominence of certain fats dietary-consumed as positive modulators to dysfunction of endothelial cells, whereas vitamin E, flavonoids, omega-3 fatty acids and other antioxidant nutrients were found to act defensively against cellular destruction brought upon by POPs (Fig. 4.11).

It is also observed that many food nutrients aim at the same molecular targets as POPs, a fact that has been under intense concern. This interaction between them has been confirmed by Cave et al. (2010) study, where PCBs were linked with increased levels of ALT (alanine aminotransferase), an enzyme that consist an indicator of non-alcoholic steatohepatitis. Herein it is worth mentioning that this medical condition is related to obesity.

4.2.3 POPs Interaction with Peroxisome Proliferator Receptors

Radicals, produced during the oxidation or mitochondrial dysfunction, may incite lipid peroxidation and destruction of lipid membranes. Because of the different nature of various cellular membranes (nuclear, mitochondrial, lysosomal, etc.) on different species, different lipid peroxidation can be a crucial event in species' cellular necrosis. Oxidative stress can be effectuated due to exposure to POPs (Androutsopoulos et al. 2013).

Investigation of the relationship between gene expression of obesity indicators (adipokines and PPARγ) and POP plasma levels was performed by Pereira-Fernandes et al. (2014). It is worth giving prominence to the finding that plasma-circulating LEP correlated with several PCBs in women, whereas in men this correlation was not observed. This fact enhances also the notion that gender difference probably exists in obesogenic hypothesis.

A recent survey of Gadupudi et al. (2015) aimed at the clarification of the effects of POPs exposure on adipose tissue, particularly at how

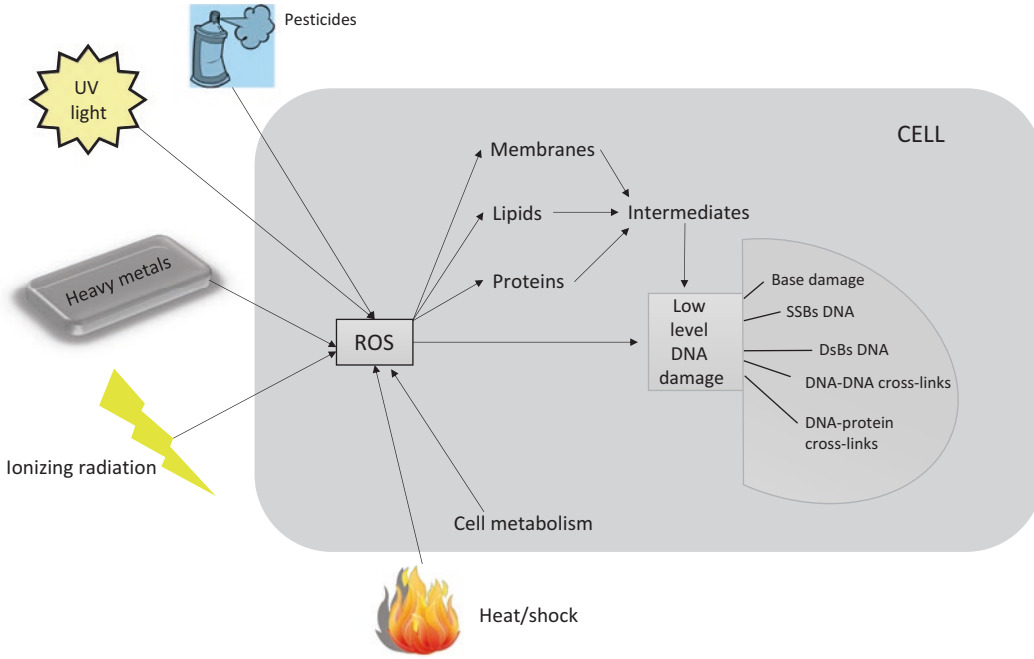

Fig. 4.12 Cell response to ROS (modified figure from: Feinendegen et al. 1999)

human preadipocytes' exposure to PCB126 acts upon their ability to differentiate into mature cells. PCB126, indeed, triggers changes inside the adipocyte, by reducing the transcript rate of PPARγ. PPARγ belongs to a broad family of nuclear receptors, which also consists of PPARα and PPARδ (Casals-Casas et al. 2008). Mainly PPARγ2 and PPARγ3 are expressed in a high rate. Given the fact that the ability of preadipocytes to mature is substantive to development and homeostasis, interference of POPs in the formation of physiological adipose tissue in children and adolescents is the likely outcome. In adults, respectively, chronic exposure may modify the ability of replacing mature adipocytes due to cellular death, again potentially leading to dysfunctional adipose tissue. Consequently, impairment in normal adipose tissue differentiation provokes disease (Fig. 4.12).

Lipid peroxidation, accumulation of reactive oxygen species (ROS) and destruction of DNA structure describe oxidative stress, namely an imbalance between ROS production and body's ability to inactivate these toxics and restore detriment Kouretas et al. (2013), Benedetti et al. (2014). Therefore, ATP molecules are targeted and the cell incurs lysis. Superoxide dismutase initially takes part in antioxidant defense, by catalyzing the superoxide ion conversion into water and O_2, with the contribution of another antioxidant enzyme, catalase. For certain POPs, the exact mechanisms that modify reduction-oxidation reactions within the cells are rather nebulous. Enzymatic conversion to secondary reactive products (e.g. ROS), exhaustion of cellular antioxidant defenses and impediment of antioxidant enzymatic activity, are some suggestions for the perturbation of redox reactions balance.

4.2.4 Role of CYP Gene Families

Of the pesticides in use, the majority are metabolised in the human body by the same P450 family member. In mammals 18 known CYP families exist, but nonetheless only three families play a leading part in xenobiotic metabolism, with some others contributing only to an extend to certain metabolic pathways. For instance, members of the CYP4 family are responsible for hydroxylating long-chained fatty acids. Thus, the remaining mammalian CYP gene families participate in biosynthesis of steroid hormones, each of them at a specific step; CYP7 is involves in cholesterol

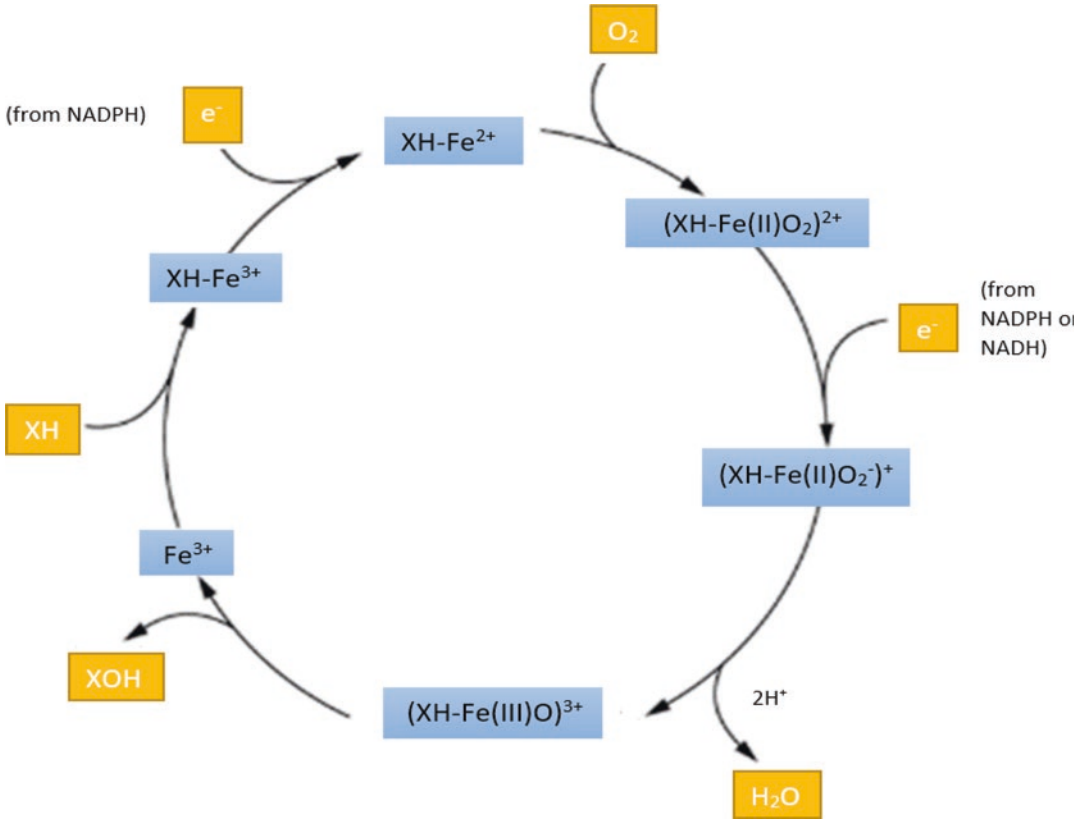

Fig. 4.13 Possible mechanism for monooxugenation by cytochrome P450 (modified figure, depicted by: C.H. Walker (2001), Organic Pollutants: An Ecotoxicological Perspective)

hydroxylation at the 7α-position, while CYP17 and 21 catalyze the 17α and 21-hydroxylations of progesterone, respectively. Another example is CYP19, responsible for the aromatization of androgens to estrogen by the initial step of hydroxylation at the 19-position. Many CYPs active in steroidogenesis can be detected in the adrenal cortex, but those specialized in xenobiotic metabolism mainly reside in liver, kidneys, lungs and olfactory tissues.

Human CYP family members are not the same to CYP families' members belonging to several species. The CYP family members also, differ between people of different races due to CYP polymorphism. Furthermore, overexpression of CYP family members depends on the expression of other family members from different organs and this interaction is not identical in individuals of the same species and the same race. Moreover, CYP family members are not in the same amounts in the same organ of the same species.

Cytochrome P450 genes produce enzymes, which partake in synthesis and metabolism of various cellular molecules, e.g. steroid hormones, cholesterol, fat and bile acids. Exogenous substances can also be metabolized, such as medications, and internal, like toxins. Polymorphisms (variations) in the genes of cytochrome P450 apparently impinge on enzymatic functionality. In the case of POPs, these polymorphisms stipulate the rate of metabolism.

Monooxugenases owe their catalytic ability to cytochrome P450 (Fig. 4.13).

Cytochrome P450 constitutes the active center of microsomal monooxugenase (conducts phase I of biotransformation), hence the participation of CYP gene families 1–4 in the detoxication of most lipophilic xenobiotics. Once lipophilic

compounds get into the endoplasmic reticulum, they become converted by monooxugenase, forming more polar metabolites. This, in turn, leads to the attraction of hydroxyl groups, which get binded with glucuronide or sulfate. From the process of metabolism evade only highly halogenated compounds (high-chlorinated PCBs, dioxin and p,p′-DDE).

During the first stage of metabolism, the system of cytochrome P450 provides biotransformation of the corresponding enzymes. At the following phase, these chemical products form soluble and excretable compounds. Once xenobiotics enter the metabolic mechanism, polar groups are produced, in order to promote the construction of enzymatic substrate, essential for the initiation of phase II. CYP gene family plays a crucial role in the first metabolic phase of xenobiotics. Their activity manifests mainly in the liver, but they can also be detected in the brain, kidney, lungs gastrointestinal tract and the integumentary system.

During metabolic phase I, the alkylations split chemical bonds between phosphorus and carbon atoms, to create the active enzyme centers. More specifically, when phosphorylation processes occur in the esterases, the pollutants form chemical complexes with the enzymes, fact that may result to irreversible situations. Between the enzymes and the xenobiotic, complexes are constructed by phosphorylation processes, with the contribution of esterase enzymes (AChe and BChE). Phosphorylation of hydroxyl groups inhibits the enzymatic action and stability which are exerted in the substrates; as a result retrievable or not complexes are formed, a matter that relies upon the type of xenobiotic and the esterase time reinstatement.

Usually, CYPs are to an extent specific in detoxificating chemical compounds, e.g.: endosulfan and carbosulfan by CYP2B6.

Biosynthesis of estrogen in WAT has reportedly been presented to consist a potential site of interest to POPs, wherein its procedure relies upon androgenic precursors (e.g. testosterone) and aromatase CP450. Aryl hydrocarbon receptor is a ligand-activated transcription factor involved in the regulation of biological responses to planar aromatic hydrocarbons. This receptor has been demonstrated to regulate xenobiotic-metabolizing enzymes such as cytochrome p450.

4.2.5 Disruption of Retinoic Acid Signaling by POPs

Retinoic acid (RA) receptors (RARs) are nuclear receptors that play a crucial role in regulating cellular proliferation, development and differentiation in vertebrates, as a response in endogenous RAs.

Environmental pollution to RAR agonists occurs widely in aquatic environment, and has been detected in several countries (North America, China and Japan). Toxicity of these xenobiotics has been well described by Daisuke et al. (2010).

The existence of POPs exert a negative action to RAR signaling, fact that potently results in possible carcinogenic and teratogenic manifestations. As POPs function as RAR agonists (as xenobiotic compounds), they induce an excess RAR signaling effect and consequently deleterious effects.

4.3 Exposure to POPs and Additional Health Problems Related to Obesity

The predominant diseases associated with obesity pertain to insulin resistance and type 2 diabetes (Lauenborg et al. 2005), ovarian abnormalities, non-alcoholic fatty liver disease Chen et al. (2011), Rantakokko et al. (2015) renal dysfunction (Mathew et al. 2011) and male infertility (Kasturi et al. 2008).

DDT was found by Skinner et al. (2013) to promote obesity-associated testis impairment in a transgenerational level, following the pattern of epigenetic inheritance. Ovarian disease was also studied in relation to DDT exposure in experimental models. Interestingly, DDT was found to promote obesity-associated polycystic ovarian syndrome. The same research team investigated also the manifestation of obesity-linked renal (shrunk glomeruli, cysts formation, thickened Bowman's capsule) and prostate (hyperplasia) disease, deducing the conclusion that DDT

exposure promotes these clinical conditions. Overall, DDT exposure is responsible for the transgenerational transmission of obesity and also related to obesity conditions.

5 Conclusion

Persistent organic pollutants, in a crude outline, own lipophilic properties, are persistent and able to bioaccumulate. These traits transfuse a major toxic potential to organisms exposed to POPs. As afore-mentioned, though attempts for constrained, and ideally ceased, usage of POPs occur for many years, some countries have not complied with the regulations. Plenty of research has been conducted about the effect of POPs in laboratory animals, tissue samples and human populations in communities and countries, the vast majority of them resulting to the confirmation of an initial toxic-positive action hypothesis of various POPs, in conjunction with related health problems. Enactment of an enhanced legal framework in cooperation with scientific force would renew the existent knowledge and refortify restrictive actions. Global strategies should be orientated towards a collective solution, by adapting the possible alternatives to the needs and social as well as economic stamina of different nations.

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Human Protein Kinases and Obesity

5

Atilla Engin

Abstract

The action of protein kinases and protein phosphatases is essential for multiple physiological responses. Each protein kinase displays its own unique substrate specificity, and a regulatory mechanism that may be modulated by association with other proteins. Protein kinases are classified by the target amino acid in their substrates. Some protein kinases can phosphorylate both serine/threonine, as well as tyrosine residues. This group of kinases has been known as dual specificity kinases. Unlike the dual specificity kinases, a heterogeneous group of protein phosphatases are known as dual-specificity phosphatases. These phosphatases remove phosphate groups from tyrosine and serine/threonine residues on their substrate. Dual-specificity phosphatases are important signal transduction enzymes that regulate various cellular processes in coordination with protein kinases. The protein kinase-phosphoproteins interactions play an important role in obesity. In obesity, the pro- and anti-inflammatory effects of adipokines and cytokines through intracellular signaling pathways mainly involve the nuclear factor kappa B (NF-kappaB) and the c-Jun N-terminal kinase (JNK) systems as well as the inhibitor of kappaB-kinase beta (IKK beta). Impairment of insulin signaling in obesity is largely mediated by the activation of the IKKbeta and the JNK. Furthermore, oxidative stress and endoplasmic reticulum (ER) stress activate the JNK pathway which suppresses insulin biosynthesis. Additionally, obesity-activated calcium/calmodulin dependent-protein kinase II/p38 suppresses insulin-induced protein kinase B phosphorylation by activating the ER stress effector, activating transcription factor-4. Obese adults with vascular endothelial dysfunction have greater endothelial cells activation of unfolded protein

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response stress sensors, RNA-dependent protein kinase-like ER eukaryotic initiation factor-2alpha kinase (PERK) and activating transcription factor-6. The transcriptional regulation of adipogenesis in obesity is influenced by AGC (protein kinase A (PKA), PKG, PKC) family signaling kinases. Obesity may induce systemic oxidative stress and increase reactive oxygen species in adipocytes. Increase in intracellular oxidative stress can promote PKC-beta activation. Activated PKC-beta induces growth factor adapter Shc phosphorylation. Shc-generated peroxides reduce mitochondrial oxygen consumption and enhances triglyceride accumulation. Obesity is fundamentally caused by cellular energy imbalance and dysregulation. Like adenosine monophosphate (AMP)-activated protein kinase (AMPK) and mammalian target of rapamycin (mTOR), N-terminal Per-ARNT-Sim (PAS) kinase are nutrient responsive protein kinases and important for proper regulation of glucose metabolism in mammals at both the hormonal and cellular level. Defective responses of AMPK to leptin may contribute to resistance to leptin action on food intake and energy expenditure in obese states.

Keywords

Protein kinases • Protein phosphatases • Dual specificity kinases • Adenosine monophosphate (AMP)-activated protein kinase (AMPK) • Mitogen-activated protein kinases (MAPK) • MAPK phosphatases • Extracellular signal-regulated protein kinase (ERK) • c-Jun N-terminal kinase (JNK) • Inhibitor of kappaB-kinase (IKK) • Protein kinase-like endoplasmic reticulum (ER) eukaryotic initiation factor-2alpha kinase (PERK) • Protein kinase B (Akt) • Liver kinase B1 (LKB1) • Lipoapoptosis • Mammalian target of rapamycin (mTOR) • N-terminal Per-ARNT-Sim (PAS) kinase (PASK)

1 Introduction

Cells use protein phosphorylation to transduce information and intracellular signals (Johnson and Lewis 2001). Almost every cellular signaling pathway is known to be regulated by phosphorylation (Pawson and Scott 2005). Therefore, the action of protein kinases and protein phosphatases is essential for multiple physiological responses. On the one hand, protein kinase-mediated protein phosphorylation plays an essential role in a variety of cellular signaling (Cohen 2002). On the other hand, the activation of protein kinases often involves phosphorylation, regulation by N- or C-terminal domains or regulation by interacting partners (Busschots et al. 2012). In this regard, the catalytic and regulatory subunits of cyclic adenosine monophosphate (cAMP)-dependent protein kinases are highly dynamic signaling proteins. While it moves through its catalytic cycle, its catalytic subunit opens and closes. The core region of catalytic subunit is shared by all members of the protein kinase family, which is flanked by N- and C-terminal segments. Protein kinases are not only catalysts, but they are also scaffolds. In this respect, major function of protein kinases is to bind to other proteins. The catalytic subunit also interacts with its inhibitor proteins and the regulatory subunits (Taylor et al. 2005). Actually, the class of protein kinases is one of the largest recognized protein families which regulate greater number of biological processes by posttranslational phosphory-

lation of serine, threonine, and tyrosine residues (Manning et al. 2002a; Subramani et al. 2014). Manning et al. identified 518 putative protein kinase genes in human genome analysis (Manning et al. 2002b). Thirty-five per cent of 518 defined human serine (Ser)/threonine (Thr)/tyrosine (Tyr) kinases have known substrates (Yang et al. 2008). Each kinase displays its own unique substrate specificity, and a regulatory mechanism that may be modulated by association with other proteins (Owen et al. 1995).

2 Dual-Specificity Kinases

Protein kinases are classified by the target amino acid in their substrates. These are either known as Ser/Thr kinases or tyrosine kinases Tyr. However, a third group of protein kinases that can phosphorylate both Ser/Thr, as well as Tyr residues. This group of kinases has been known as dual specificity kinases (Dhanasekaran and Premkumar Reddy 1998). Under the guidance of various data, dual specificity kinases are divided into three groups: (1) kinases that show "true dual specificity" by phosphorylating Tyr- and Thr-residues of exogenous substrates; (2) kinases that exhibit dual specificity only through autophosphorylation; and (3) kinases that possess the structural motif characteristic of dual specificity kinases (Lindberg et al. 1992). Hereby, mitogen/extracellular-signal regulated kinase kinase (MEK) or simply mitogen-activated protein (MAP) kinase (MAPK) kinase (MKK) is a dual specificity kinase that phosphorylates extracellular signal-regulated protein kinase (ERK) on Tyr as well as Thr. Thus, MEK1/MKK1 and MEK2/MKK2 display the dual specificity kinase that phosphorylates ERK1 at Thr183 and ERK2 at Tyr185, respectively (Crews et al. 1992; Zheng and Guan 1993). The activators of p38 (MKK3 and MKK4), c-Jun N-terminal kinase (JNK) (MKK4), and ERK (MEK1 and MEK2) define independent MAPK signal transduction pathways. These MAPK isoforms are activated by dual phosphorylation on Thr and Tyr (Dérijard et al. 1995). Dual-specificity Tyr phosphorylation-regulated kinase 1A (Dyrk1A) is a proline-directed Ser/Thr kinase (Stk) that might be responsible for various biological pathways by phosphorylation of diverse substrate proteins such as transcription factors, splicing factors, and synaptic proteins (Park et al. 2009). Overexpressed human glycogen synthase kinase 3 (GSK3) in the skeletal muscle displays body weight gain because of an increase in fat mass (Pearce et al. 2004). Phosphorylation by Dyrk1A is a novel pathway for GSK 3-beta inactivation and that it has a potentially important role in the mechanism of obesity (Song et al. 2015). It has been identified that both the Thr- as well as Tyr-phosphorylating activities of the dual specificity kinases are required for the signaling of the respective signaling modules (Manning et al. 2002b). Substantially action of protein kinases on their substrates is often regulated by their own autoinhibitory segments. The C-terminus of the catalytic gamma-subunit of phosphorylase kinase contains two inhibitory sites. These two peptide segments resemble to sequences in phosphorylase b and these regions are potent inhibitors of phosphorylase kinase. As mentioned above all kinases recognize their protein substrates and no other protein kinase can duplicate the same reaction (Graves et al. 1999). Therefore, improper functioning of these enzymes due to mutations, mainly in the kinase domain, is often manifested in various human diseases (Choura and Rebaï 2011). Collectively, in human genome, 30–50% of proteins may undergo phosphorylation; improper functioning kinases may lead to various pathological conditions. Kinases do not always stably associate with its corresponding down-stream substrate, because the biochemical phosphorylation reaction is transient. It was shown that 85% of the kinases interact with one or more phosphoproteins. Recognition of 15,738 phosphorylation sites on 4195 phospho-proteins confirms above mentioned findings (Yang et al. 2008).

3 Dual-Specificity Phosphatases

Unlike the dual specificity kinases, a heterogeneous group of protein phosphatases removes phosphate groups from Tyr and Ser/Thr residues

on their substrate (Bakan et al. 2008). In this case dual-specificity phosphatases (DUSPs) are important signal transduction enzymes that regulate various cellular processes in coordination with protein kinases. These phosphatases dephosphorylate serine, threonine, and tyrosine residues in the same protein substrate (Ducruet et al. 2005). MAPKs, subsequent to phosphorylation, are responsible for the induction of a number of cellular responses, such as changes in gene expression, proliferation, differentiation, cell cycle arrest and apoptosis (Schaeffer and Weber 1999). Contrarily, MAPK phosphatases (MKPs) dephosphorylate and inactivate MAPKs. Dual-specificity Ser, Thr and Tyr phosphatases act on MAPKs. Dephosphorylation of the MAPKs is vital for their control. This is achieved by removal of phosphate groups from either the Thr residue or the Tyr residue, or both Theodosiou and Ashworth (2002). DUSPs are divided into six subgroups on the basis of sequence similarity; phosphatases of regenerating liver (PRLs), cell division cycle 14 (Cdc14) phosphatases, phosphatase and tensin homologues (PTENs) (deleted on chromosome 10), myotubularins, MKPs and atypical DUSPs (Patterson et al. 2009). MKPs dephosphorylate MAPK proteins, ERK, JNK and p38. MKP-1-deficient mice exhibit increased energy expenditure, and subsequently resistance to diet-induced obesity, that could occur through enhanced p38 MAPK-mediated activation of the transcriptional coactivator peroxisome proliferator-activated receptor gamma (PPARgamma) coactivator 1 alpha (PGC-1alpha) leading to increased skeletal muscle mitochondrial uncoupling protein-3 (UCP-3) expression. By contrast, MKP-1 overexpression inhibits PGC-1alpha activity in myoblasts in response to tumor necrosis factor-alpha (TNF-alpha), which implies that MKP-1 antagonizes PGC-1alpha activity in a MAPK-dependent manner. The fact that there are ten other MKPs, four of which reside in the nucleus, suggests that MKP-1 is a major nuclear-resident MKP that links MAPK signaling to PGC-1alpha function (Roth et al. 2009). Exercise reduces myocardial infarct size and increases phosphorylation of kinases such as protein kinase B (Akt), ERK 1/2, p70S6K, adenosine monophosphate (AMP)-activated protein kinase (AMPK) and GSK3beta. In this manner, the level of corresponding phosphatases PTEN, MKP-3 and protein phosphatase 2C (PP2C) decrease. Actually, mitochondrial permeability transition pores are increased by exercise. Regular exercise induces cardioprotection despite obesity and simultaneously restores kinase phosphorylations, decreases the level of phosphatases and increases resistance of mitochondrial permeability transition pores opening (Pons et al. 2013). The MAPKs are inactivated by MKPs either in the cytosol or nucleus. Nuclear regulation of the MAPKs by MKP-1 is essential for the management of metabolic homeostasis (Wu et al. 2006). MKP-4 expression is up-regulated during adipocyte differentiation in vitro and up-regulated during fasting in white adipose tissue in vivo. Overexpression of MKP-4 inhibits ERK and JNK phosphorylation and, to a lesser extent, p38 MAPK phosphorylation. MKP-4 also reverses the effect of TNF-alpha to inhibit insulin signaling and inhibits insulin-stimulated glucose uptake (Emanuelli et al. 2008). MKP-4 is frequently expressed in insulin-responsive tissues and the expression levels are up-regulated in insulin-resistant obese. Expression of MKP-4 in preadipocytes significantly blocks insulin-induced adipogenesis (Xu et al. 2003). On the other hand, in addition to inhibiting fatty acid oxidation, MKP-1 promotes hepatic lipogenic gene expression through PPARgamma. Hereby, MKP-1 regulates PPARgamma function which discloses a link MKP-1 and lipid droplet formation in the liver (Flach et al. 2011). Nuclear regulation of the protein kinases by protein kinase phosphatases is essential for the management of metabolic homeostasis (Wu et al. 2006).

4 Obesity and Protein Kinases

Very limited data have been published considering the kinase-phosphoproteins interactions in obesity. For instance, in some cases inactivation of GSK3beta by phosphorylation at specific residues is a primary mechanism. GSK3beta is directly phosphorylated by Dyrk 1A. Dyrk1A

transgenic animals show an inverse correlation with the effect of GSK3beta on obesity. GSK3beta activity is differentially regulated by phosphorylation at different sites in adipose tissue depending on the type of diet. Furthermore, overexpression of Dyrk1A suppresses the expression of adipogenic proteins including PPARgamma (Song et al. 2015).

Insulin receptor Tyr kinase phosphorylation of insulin receptor substrate-1 (IRS-1) at Tyr896 site is a necessary step in insulin stimulation. Eventually insulin-responsive glucose transporter 4 (GLUT4) is translocated to the cell surface. On the other hand, Ser337 and Ser636 of IRS-1 are also targets for phosphorylation by GSK3 and by JNK1, respectively, in metabolic syndrome. Diminished transmission of IRS-1 signaling and reduced insulin-stimulated translocation of GLUT4 seem to be the major cause of the insulin resistance that appear in obesity (Stuart et al. 2014). Additionally, in obese individuals, oxidized low-density lipoprotein (oxLDL) enhances coronary vasoconstriction by increasing the activity of PKC (Giardina et al. 2001) and inhibits glucose uptake through decreasing GLUT4 translocation to the plasma membrane without affecting GLUT4 gene expression. These findings are also associated with the impairment of insulin signaling. Specifically, in oxLDL-treated cells IRS-1 is highly degraded due to the enhanced Ser307 phosphorylation. This process was largely mediated by the activation of the inhibitor of kappaB-kinase beta (IKKbeta) and the JNK. Moreover, the activation of IKKbeta positively regulates the nuclear content of nuclear factor kappaB (NF-kappaB), by inactivating the inhibitor of NF-kappaB (IkappaB) alpha (Scazzocchio et al. 2009). Despite extensive sequence similarity, IKKalpha and IKKbeta have largely distinct functions, due to their different substrate specificities and modes of regulation. IKKbeta mediates the NF-kappaB activation by proinflammatory signaling cascades. However, IKKalpha mediates the activation of NF-kappaB in response to TNF-alpha and may also serve as the attenuator of IKKbeta-driven NF-kappaB activation (Häcker and Karin 2006). Actually oxidative stress and endoplasmic reticulum (ER) stress activate the JNK pathway which suppresses insulin biosynthesis (Kaneto et al. 2005). The stress-activated JNK has been recognized as a central mediator of insulin resistance. JNK mediates inhibitory phosphorylation of IRS. Suppression of the JNK pathway has been shown to improve insulin resistance and glucose tolerance (Li and Yu 2013). On the other hand, obesity related-insulin resistance together with glucagon stimulates hepatic glucose production. In this case, the molecular link between intracellular calcium and hepatic glucose production occur during the regulation of forkhead box protein O1 (FOXO1) nuclear transport via calcium/calmodulin dependent-protein kinase II (CaMKII) activation. CaMKII is activated in a calcium- and inositol 1,4,5-trisphosphate receptor (IP3R)-dependent manner by cyclic AMP (cAMP) and glucagon. Inhibition of CaMKII blocks nuclear translocation of FOXO1 by affecting its phosphorylation which impairs fasting- and glucagon/cAMP-induced glycogenolysis and gluconeogenesis. Consequently, blood glucose levels decrease (Ozcan et al. 2012). Downstream of the glucagon receptor is triggered by PKA-mediated activation of the ER calcium release channel. Channel opening results in release of calcium from ER stores, which then activates the cytoplasmic calcium-sensitive kinase, CaMKII. Furthermore, CaMKII subsequently activates the MAPK p38alpha, which phosphorylates FOXO1 and promotes its nuclear translocation. Nuclear FOXO1 induces target genes glucose-6-phosphatase catalytic-subunit (G6pc) and phosphoenolpyruvate carboxykinase 1 (PCK1) that are rate-limiting mediators for glycogenolysis and gluconeogenesis. CaMKII/p38-mediated pathway plays a critical role in obesity-associated insulin resistance in the liver. Thus, obesity-activated CaMKII/p38 suppresses insulin-induced Akt phosphorylation by activating the ER stress effector, activating transcription factor 4 (ATF4), which in turn induces the Akt inhibitor, through a putative endogenous inhibitor of insulin signaling, tribbles 3 (TRB3) (Ozcan et al. 2013). Fatty acids differentially regulate insulin resistance through ER stress-mediated induction of TRB3. Unlike the obesogenic diet

with lower unsaturated fat, widely used obesogenic diet containing high unsaturated fats fails to induce ER stress, TRB3 induction or insulin resistance (Geng et al. 2013).

Proteins must fold into their correct three-dimensional conformation in order to attain their biological function. Proteins are synthesized or folded continuously and this process that is greatly assisted by molecular chaperones which promote folding or prevent the aggregation of other proteins (Lee and Tsai 2005). Actually, the ER consists of an interconnected, membranous network that is the major site for the synthesis and folding of proteins (Winnay and Kahn 2011). Extracellular stimuli and changes in intracellular homeostasis can alter the protein-folding environment of the ER and cause the accumulation of misfolded or unfolded proteins (Han and Kaufman 2014). An imbalance between the abundance of synthesized proteins and the folding capacity of the ER leads to ER stress. The unfolded protein response (UPR) is an attempt to restore ER function by attenuating protein synthesis (Ogborn et al. 2014). Obesity/insulin resistance and subsequent type 2 diabetes are associated with ER stress and UPR activation in adipose tissue (Cazanave et al. 2011). Eventually, the accumulation of unfolded or improperly folded proteins in the ER lumen causes a state of ER stress. The subsequent UPR results in activation of three linked signal transduction pathways: RNA-dependent protein kinase (PKR)-like ER eukaryotic initiation factor-2alpha kinase (PERK), inositol-requiring ER-to-nucleus signaling protein 1 (IRE1) alpha, and activating a membrane-bound transcription factor 6 (ATF6) alpha (Ron and Walter 2007; Schröder and Kaufman 2005). Activation of the UPR depends on three ER stress sensor proteins, IRE1 alpha, PERK, and ATF6. Although the consequences of activation of these proteins are well-known, how these sensors detect ER stress remains unclear (Gardner et al. 2013). In fact, double-stranded RNA-dependent protein kinase R (PKR) by itself can respond to nutrient signals as well as ER stress. Hereby, PKR coordinates the activity of other critical inflammatory kinases such as the JNK (Nakamura et al. 2010). Furthermore, PKR may have a role in insulin sensitivity under normal physiological conditions, probably by modulating protein phosphatase 2A activity and Ser-Thr kinase phosphorylation (Carvalho-Filho et al. 2012). Moreover, PERK also phosphorylates eukaryotic initiation factor 2 (eIF2)-alpha subunit during ER stress and represses protein synthesis, which prevents further influx of ER client proteins. Indeed, eIF2 is an essential factor for protein synthesis. Its alpha-subunit contains the main target for phosphorylation, at Ser 51 position. The PERK pathway facilitates both the synthesis of ATF6 and trafficking of ATF6 from the ER to the golgi for intramembrane proteolysis and activation of ATF6. In fact, ATF6 is an ER stress-regulated transmembrane transcription factor. The cytosolic portion of ATF6 moves to the nucleus and causes the transcription of ER chaperones. In this case PERK depletion leads to reduced protein chaperone expression, disruptions of lipid metabolism, and enhances apoptosis (Teske et al. 2011). UPR activation with the perturbation of cellular lipid composition has important consequences in obesity and diabetes. Nevertheless, luminal unfolded protein stress-sensing domain-deficient IRE1alpha and PERK retain responsiveness to increased lipid saturation. Furthermore, direct sensing of the lipid composition of the ER membrane contributes to the UPR (Volmer et al. 2013). IRE1 expression is greater in obese versus non-obese individuals. Non-diabetic obese adults with vascular endothelial dysfunction have greater endothelial cells activation of UPR stress sensors, PERK and ATF6 (Kaplon et al. 2013). ER stress response through ATF6 also plays an important role in insulin resistance. Phospholipid metabolisms in the ER with perturbations in lipid storage/secretion and stress responses ultimately progress to obesity, diabetes and atherosclerosis (Lagace and Ridgway 2013). The ER resident PERK is necessary for Akt activation in response to ER stress. PERK is an intrinsic lipid kinase and gives priority to diacylglycerol as a substrate and generating phosphatidic acid. This activity of PERK correlates with the activation of mammalian target of rapamycin (mTOR) and phosphorylation of Akt at Ser473. Lipid kinase activity of PERK is regu-

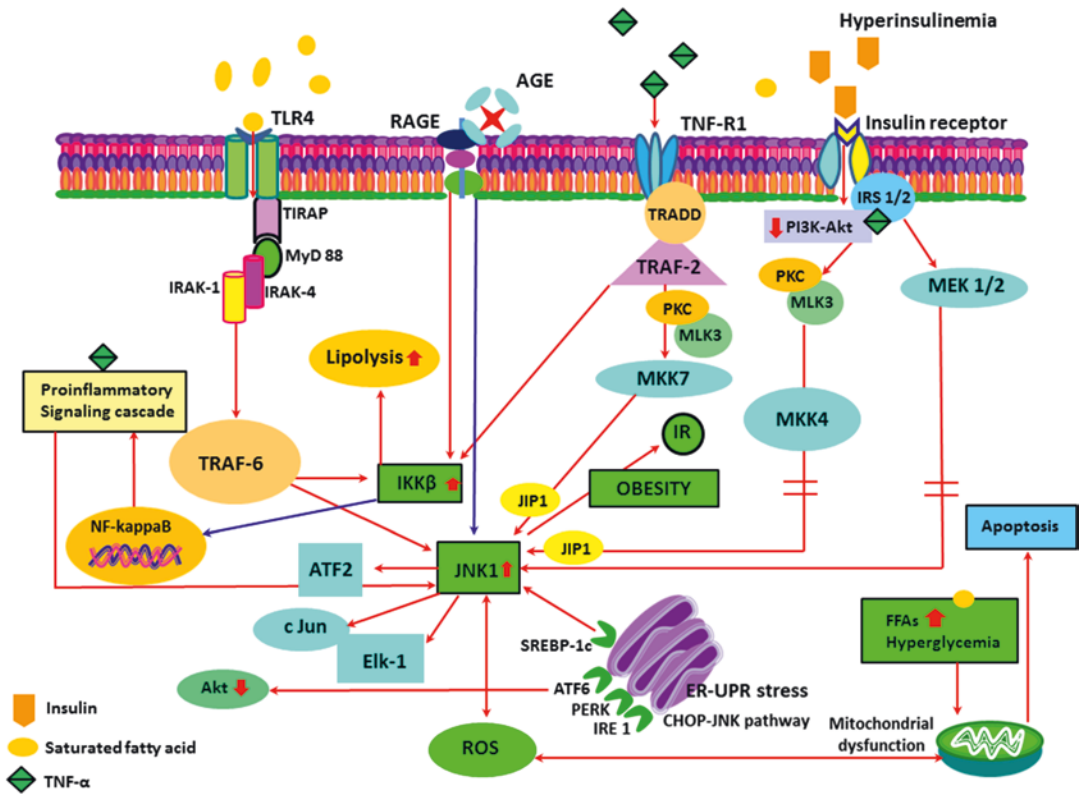

Fig. 5.1 Several potential mechanisms have been proposed in obesity-induced JNK1 activation. Firstly, exposure of cells to saturated fatty acids in high fat diet causes ER stress and induction of the unfolded protein response pathway. Secondly, toll-like receptor 4-mediated activation of IKKbeta and JNK leads to insulin resistance in diet-induced obesity. Thirdly, saturated fatty acids activate the JNK pathway by a mechanism that involves protein kinase C-mediated activation of the mixed-lineage protein kinase. This pathway requires the MAPKKs MKK4 and MKK7. Fourth, high fat diet-induced insulin resistance is associated with chronic low-grade inflammation and expression of inflammatory cytokines that can cause JNK activation. TNF-alpha overexpression is an important effector on several important sites of insulin action (*TLR4* toll-like receptor 4; *TIRAP* Adapter molecule associated with toll-like receptor; *MyD 88* Myeloid differentiation primary response gene 88; *UPR* Unfolded protein response; *ATF6* Activating a membrane-bound transcription factor 6; *ER* Endoplasmic reticulum; *JIP1* JNK-interacting protein 1; *PI3K* Phosphoinositide-3 kinase; *IRAK* Interleukin-1 receptor-associated kinase; *TRAF* TNF receptor associated factor; *IKK* Inhibitory-kappaB kinase; *JNK1* c-Jun N-terminal kinase 1; *ATF* Activating transcription factor; *ROS* Reactive oxygen species; *Elk-1* ETS domain-containing protein; *AGE* Advanced glycation end product; *FFA* Free fatty acid; *SREBP-1c* Sterol regulatory element-binding protein-1c; *RAGE* Receptor for advanced glycation end product; *NF-kappaB* Nuclear factor-kappa B; *PERK* Protein kinase (PKR)-like ER eukaryotic initiation factor-2alpha kinase; *IRE1* Inositol-requiring endoplasmic reticulum-to-nucleus signaling protein 1; *CHOP* CCAAT/enhancer-binding protein homologous protein; *SFA* Saturated fatty acid; *TNF-α* tumor necrosis factor-alpha; *TNFR1* TNF receptor-1; *TRADD* Tumor necrosis factor receptor type 1-associated DEATH domain adaptor protein; *TRAF2* TNF receptor-associated factor 2; *PKC* Protein kinase C; *MLK* Mixed-lineage protein kinase; *MAP* kinase kinase (*MKK*): Mitogen-activated protein kinase kinases MKK4 and MKK7; *IRS* Insulin receptor substrate; *IR* Insulin resistance; *MEK* Mitogen/extracellular-signal regulated kinase kinase; *Akt* Protein kinase B)

lated in a PI3K p85alpha-dependent manner (Bobrovnikova-Marjon et al. 2012). Deficiency of PERK attenuates free-fatty acids induced activation of IKKbeta. Suppression of IKKbeta decreases free-fatty acids induced inflammation and insulin resistance. Contrarily, overexpression of IKKbeta inhibits lipogenesis and promotes lipolysis (Jiao et al. 2011) (Fig. 5.1).

As mentioned above protein phosphorylation plays a major role in almost all insulin-regulated processes. As a member of the receptor tyrosine kinase (RTK) family, the insulin receptor engages the canonical PI3K/Akt (Manning and Cantley 2007), and mTOR pathways (Zoncu et al. 2011). PI3K/Akt pathway together with mTOR pathway may coordinate many of insulin's actions. A complex single-cell phosphorylation network comprising 37,248 phosphorylation sites on 5705 proteins of which, approximately 15% are insulin-regulated phosphorylation sites (Humphrey et al. 2013). While the phosphorylation of IRS-1 on Tyr residue is required for insulin-stimulated responses, the phosphorylation of IRS-1 on Ser residues has a dual role, either enhance or terminate the insulin effects (Gual et al. 2005). Excess of circulating free fatty acids in obesity causes insulin resistance by inhibiting insulin signaling through the activation of Ser-kinases, which promote a mechanism of Ser phosphorylation of IRS, leading to interruption of the downstream insulin receptor signaling. TNF-alpha, secreted by hypertrophic adipocytes and adipose tissue macrophages, also inhibits insulin resistance signaling by a double mechanism of Ser-phosphorylation and Tyr-dephosphorylation of IRS-1 (Capurso and Capurso 2012). Tyr phosphorylation of IRS-1 and its binding to PI3K are critical events in the insulin signaling cascade leading to insulin-stimulated glucose transport. Elevated plasma fatty acid concentrations are associated with reduced insulin-stimulated glucose transport activity as a consequence of altered insulin signaling through PI3K (Le Marchand-Brustel et al. 2003). Insulin activates PI3K, which catalyzes the synthesis of phosphatidylinositol (3,4,5)-triphosphate (PIP3) at the plasma membrane. In the presence of PIP3, the 3-phosphoinositide-dependent protein kinase 1 (PDK1) and Akt rapidly co-localize at the plasma membrane. Active Akt transduces insulin effects (Vanhaesebroeck and Alessi 2000). Activation of Akt phosphorylates FOXO1 and consequently excluded FOXO1 from the nucleus. The selective and irreversible degradation of phosphorylated FOXO1 prevents the re-entry of FOXO1 into the nucleus. At last, binding of insulin to its specific receptor initiates the proteasomal degradation of FOXO1 by the PI3K-Akt pathway (Matsuzaki et al. 2003). The interaction of insulin and growth factors with their receptors on the outer surface of a cell membrane leads to the activation of PI3K and generation of the PIP3 which is the second messenger at the inner surface of the cell membrane. These evidences indicate that the PDK1 phosphorylates and activates the AGC kinase members which are regulated by PI3K (Collins et al. 2005; Mora et al. 2004). AGC kinases are involved in diverse cellular functions and are potential targets for the treatment of human diseases such as cancer, diabetes, obesity, neurological disorders and inflammation. Although the AGC group is named after the definition of PKA, PKG, and PKC families, the group of AGC protein kinases includes more than 60 protein kinases in the human genome, classified into 14 families: PDK1, Akt/PKB, SGK, PKA, PKG, PKC, PKN/PRK, RSK, NDR, MAST, YANK, DMPK, GRK and SGK494 (Arencibia et al. 2013). The transcriptional regulation of adipogenesis is influenced by AGC family signaling kinases. In particular Akt overexpression increases differentiation of adipocyte, whereas its absence reduces adipogenesis (Bae et al. 2003). Binding of adenosine triphosphate (ATP) to Akt induces an intramolecular interaction between two phosphorylation sites of Akt and other domains in the protein. ATP hydrolysis and substrate phosphorylation permits dephosphorylation and inactivation of the kinase (Humphrey et al. 2013). There are 155 distinct phosphorylation sites in 77 mitochondrial phosphoproteins, including 116 phospho-serine, 23 phosphothreonine, and 16 phosphotyrosine residues. Analysis of kinase motifs revealed that many of these mitochondrial phosphoproteins are substrates for PKA, PKC, casein kinase II, and DNA-dependent protein kinases. Many of these mitochondrial phosphoproteins are involved in oxidative phosphorylation, tricarboxylic acid cycle, and lipid metabolism (Zhao et al. 2011).

The advent of genome-wide strategies revealed novel genetic factors with strong associations with obesity and diabetes (Körner et al. 2008). However, no significant interactions are

observed between genetic risk score and dietary intakes on body mass index (BMI) or obesity-related traits. Additionally macronutrient, fiber or total energy intake levels could not modify genetic susceptibility to obesity (Rukh et al. 2013). On the other hand, nutritional unbalance during fetal development may change the intrauterine environment and leads to altered gene expression with alterations in DNA or histone methylation. This event results in an increased susceptibility to obesity (Paquot et al. 2012). In this regard hypomethylation occurs in 2701 genes and hypermethylation occurs in 1070 genes subsequent to adipocyte differentiation. In addition, TNF-alpha, MAPK, and interleukin-8 (IL-8) are important for the formation of methylation. DNA methylation mechanisms may be involved in the regulation of differentiation process of human preadipocytes (Zhu et al. 2012). High fat diet stress causes a feed-forward cycle by increased adiposity and progressive inflammation in adipose tissue across generations. DNA hypomethylation of inflammation-associated genes in adipose tissue lead to epigenetically altered expression of toll-like receptor 1 (TLR1), TLR2 and linker for activation of T cells (Ding et al. 2014). Nutrient and energy inputs control mTOR complex 1 (mTORC1)-S6 kinase1 (p70 S6 kinase) signaling. S6Ks are key downstream effectors of the PI3K/PKB/mTORC1 signaling pathway. Eventually, mTOR and its effector S6K1 involve in specific pathological responses, including obesity. mTOR exists in two complexes: mTORC1, which is rapamycin-sensitive and phosphorylates S6K1 and initiation factor 4E binding proteins (4E-BPs), and mTORC2, which is rapamycin-insensitive and phosphorylates Akt (Dann et al. 2007). The mTORC1/S6K pathway regulates protein synthesis, cell growth and aging (Bilanges and Vanhaesebroeck 2010). S6K1 is sensitive to both insulin and nutrients. Amino acids negatively affect insulin signaling through mTOR/S6K1 phosphorylation of IRS-1. Therefore, infusion of amino acids into humans leads to S6K1 activation, subsequent inhibition of insulin-induced class 1 PI3K activation and insulin resistance. Hereby S6K1 may mediate deleterious effects, like insulin resistance (Um et al. 2006). Indeed, in the presence of excess amino acids or fat, S6K1 promotes the degradation of IRS-1 by constitutively phosphorylating IRS-1 at multiple sites (Bilanges and Vanhaesebroeck 2010).

In high fat diet-induced obesity, basal and insulin-stimulated Akt and atypical PKC activities are diminished in muscle, in contrast to liver. Despite elevated hepatic Akt activity, FOXO1 phosphorylation is impaired. In this manner Akt-dependent phosphorylation of glycogenic GSK3beta and lipogenic mTOR is elevated. Diminished Akt-dependent FOXO1 phosphorylation is associated with reduced scaffold protein, WD40/ProF-related Akt activity. In this case inhibition of hepatic atypical PKC restores Akt-dependent FOXO1 phosphorylation and decreases excessive expression of hepatic gluconeogenic and lipogenic enzymes (Sajan et al. 2014). FOXO is a key downstream effector of Akt action. Phosphorylation of the insulin kinase Akt1 decreases in steatosis. Without changing in IRS1 mRNA levels, the mRNA and protein levels of the FOXO1-dependent IRS2 increases progressively with the severity of steatosis. Upregulation of IRS2 is also associated with preserved activation of Akt2 which mediates the stimulating effect of insulin on de novo lipogenesis (Rametta et al. 2013). In this case, an important question may arise about the biological specificity of Akt. According to Bae et al. the Akt1/PKBalpha and Akt2/PKBbeta isoforms are uniquely adapted to preferentially transmit distinct biological signals, and this property is likely to contribute to the role of Akt/PKB in various processes (Bae et al. 2003). Actually in humans, fatty liver and steatohepatitis are associated with a progressive increase in the expression of gluconeogenic enzymes. Indeed, upon Akt signal suppression, FOXO1 directs the transcription of PCK1 and G6pc and promotes insulin resistance (Valenti et al. 2008). In fact, FOXOs modify a various sequences of cellular processes including the cell cycle, oxidative stress resistance, and aging. An extensive network of proteins involves in nuclear export, focal adhesion, and mitochondrial respiration. Loss of the mitochondrial UCP5 increases free radicals. The increased superoxide

content of cell induces JNK1 activity, which in turn affects FOXO localization through a compensatory dephosphorylation of Akt. Later nuclear FOXO increases expression of target antioxidant genes. Obesity and type 2 diabetes develop by connecting free radical defense and mitochondrial uncoupling to Akt/FOXO signaling (Senapedis et al. 2011).

Obesity may induce systemic oxidative stress and increase reactive oxygen species (ROS) in adipocytes. Biomarkers of oxidative damage are higher in individuals with obesity and correlate directly with BMI and the percentage of body fat (Pihl et al. 2006). Increase in intracellular ROS can promote PKCbeta activation (Aguiari et al. 2008). Activated PKCbeta induces growth factor adapter Shc (p66Shc) phosphorylation, thus allowing p66Shc to be recognized by Peptidyl-prolyl cis-trans isomerase NIMA-interacting 1 (Pin1), isomerized and transported into mitochondria, where p66Shc acts as ROS producer and so further increases intracellular ROS levels (De Marchi et al. 2013). Redox enzyme-activity of p66Shc is induced by insulin in adipocytes. p66Shc-generated peroxides reduce mitochondrial oxygen consumption and enhances triglyceride accumulation. In this case p66Shc promotes the signal-inhibitory phosphorylation of IRS1 by connecting it with mTOR effector S6K. This finding confirm that p66Shc is linked to obesity-induced during the state of excess feeding (Ranieri insulin resistance et al. 2010). As a generator of mitochondrial oxidant species and of signaling adaptor in the insulin receptor cascade, the p66Shc is a negative determinant of life span and healthy longevity in mammals (Ranieri et al. 2013). Actually, both atypical PKC and Akt activity are required for insulin-stimulated glucose transport. Defective muscle atypical PKC/Akt activation reflects both impaired activation of IRS-1 and PI3K. Abnormalities in muscle atypical PKC/Akt activation are observed in obesity and diabetes (Farese et al. 2005). One of the atypical members of the multifunctional PKC isoforms, PKC-lambda is a crucial mediator of insulin and also effects glucose transport in muscle and adipose tissue and lipid synthesis (Farese and Sajan 2010; Stretton et al. 2010). Insulin stimulated-PKC-lambda/zeta activity is reduced 57% in obese and 65% in diabetic subjects. Weight loss in obese subjects normalizes PKC-lambda/zeta activity and simultaneously increases IRS-1 phosphorylation and PI3K activity. Reduced insulin-stimulated PKC-lambda/zeta activity plays a role in the pathogenesis of insulin resistance in muscle of obese and type 2 diabetic subjects (Kim et al. 2003). The activity of PKC-delta, as well as ROS increase two-fold in high-fat diet adipocytes compared with control adipocytes. Moreover, PKC-delta activity was inhibited in high-fat diet adipocytes either by glucose deprivation or by treatment with the antioxidant N-acetyl-L-cysteine (Talior et al. 2003) (Fig. 5.1).

The MAPK cascade is a major signaling system by which cells transduce extracellular stimuli into intracellular responses (Blenis 1993). MAPKs serve as phosphorylation substrates for MKKs, whereas MAPK phosphatases reverse the phosphorylation and return the MAPK to an inactive state (Schaeffer and Weber 1999). Actually the MAPKs are activated by dual phosphorylation on Thr and Tyr in response to extracellular stimuli (Davis 1994). Furthermore, MAPK family of genes helps cells in sensing both extracellular and intracellular stimuli. Emerging data indicate that MAPKs have fundamental roles in the circadian biological clock (Goldsmith and Bell-Pedersen 2013). In the absence of acute stress, rhythmically activated MAPK signals to downstream effector molecules to regulate rhythmic expression of target genes of the pathway. When exposed to stress, clock regulation of MAPK signaling pathways coordinates major groups of genes to provide a growth and survival advantage to the organism (de Paula et al. 2008). G protein-coupled receptor 40 (GPR40) may not only participate in the control of insulin secretion by free fatty acids, but it might also play an important role in the control of beta-cell apoptosis by unsaturated free fatty acids. The activation of the ERK–MAPK pathway is promoted by unsaturated free fatty acids via GPR40. Subsequent increase in the early growth response protein 1 (Egr-1) represses the lipo-apoptotic effect (Zhang et al. 2007).

Guanosine-5'-triphosphate (GTP) binding protein binds the MAPK kinase kinase Raf-1, thereby translocating it to the plasma membrane and promotes its activation. Active Raf-1 phosphorylates and activates the MAPK/ERK (MEKs) 1 and 2, which in turn phosphorylate and activate ERK1 and ERK2 (Frost et al. 1997). MAPKs and IKK are suggested to link various conditions such as oxidative stress, ER stress, inflammation to develop insulin resistance in obese adipose tissue. Protein and mRNAs of p38 MAPK, ERK, JNK-1, and IKKbeta increase 1.5–2.5-fold in omental versus subcutaneous fat of severely obese women with BMI more than 32 kg/m^2. The phosphorylated (activated) forms of these kinases also increase to similar magnitudes as the total expression. Both obese and lean women reveale similar ERK2 and IKKbeta expression as well as their phosphorylated forms in fat depots. However, obese women exhibit 480% higher amount of the phosphorylated forms of p38MAPK and JNK in omental fat (Bashan et al. 2007). Contrarily high-fat diet increases NF-kappaB activation, which leads to a sustained elevation in IKK epsilon activity in adipocytes and adipose tissue macrophages (Chiang et al. 2009). The MAPK-activated protein kinases (MKs) family comprises six related kinases; ribosomal S6 kinases (RSKs), the mitogen- and stress-activated kinases (MSKs), the MAPK-interacting kinases (MNKs), MK2 and MK3 (formally termed as MAPKAP-K2 and -3), and MK5 (formally termed as MAPKAP-K5). The MKs mediate wide range of biological functions in response to mitogens and stress stimuli (Roux and Blenis 2004). MAPKs are activated by phosphorylation on T and Y residues within a T(X)Y phosphorylation motif, where (X) can be Glutamic acid (Glu-E), Proline (Pro-P), or Glycine (Gly-G). In this respect three groups of MAPKs are identified based on their dual phosphorylation motifs, TEY, TPY, and TGY, which are termed ERK1/2, JNK, and p38, respectively (Abe et al. 1996). Typically cell surface receptors, Tyr kinases and G protein-coupled receptors transmit activating signals to the ERK1/2 module and a significant amount of ERK1/2 accumulates in the nucleus (Chen et al. 1992; Gonzalez et al. 1993). p38 MAPK module may localize in both the nucleus and cytoplasm. p38 is activated in macrophages, neutrophils, and T cells by numerous extracellular mediators of inflammation. JNK activation requires dual phosphorylation on Tyr and Thr residues within a conserved Thr-Pro-Tyr (TPY) motif. Like ERK1/2 and p38, the JNKs may re-localize from the cytoplasm to the nucleus following stimulation (Mizukami et al. 1997).

Obesity causes chronic low-grade inflammatory responses that lead to activation of stress pathways including the JNK1 that play critical roles in the etiology of obesity-induced insulin resistance (Hotamisligil 2006; Shoelson et al. 2006; Weston and Davis 2007; White 2003). In obesity, the pro- and anti-inflammatory effects of adipokines and cytokines through intracellular signaling pathways mainly involve the NF-kappaB and JNK systems as well as the IKK-beta. MAPK and ERK pathways, which lead to signal transducer and activator of transcription 3 (STAT3) activation, are also important in the production of pro-inflammatory cytokines (Gil et al. 2007). MAP4K4 silencing in adipocytes enhances the expression of lipogenic enzymes and simultaneous increases triglyceride and fatty acids. JNK1 and JNK2 depletion causes the opposite effects. Furthermore, high expression of MAP4K4 fails to activate endogenous JNK, while MAP4K4 depletion does not attenuate JNK activation by TNF-alpha (Danai et al. 2013).

In fact, both TNF-alpha and free fatty acids are potent regulators of JNK and IKK activity. Absence of JNK1 results in decreased adiposity, significantly improved insulin sensitivity in obesity (Hirosumi et al. 2002; Yuan et al. 2001). By contrast, JNK activation and lipoapoptosis are greater during exposure to the saturated FFAs (Malhi et al. 2006). Indeed, JNK can be activated by saturated FFAs through TNF-alpha-independent mechanisms. In this regard JNK is a major contributor to FFA-induced cellular insulin resistance (Nguyen et al. 2005). Substantially, obesity is strongly associated with increased activation of the JNK. Therefore, on a high-fat diet, the dominant-negative JNK (ap2-dn-JNK) mice displays a marked reduction in weight gain, fat mass, and size of the adipocytes accompanied by

lack of both JNK1 and JNK2 in their adipose tissue (Zhang et al. 2011). Similarly, JNK1 deficiency in adipose tissue suppresses high-fat diet-induced insulin resistance in the liver. On the other hand, JNK1 is a component of a metabolic stress signaling pathway that regulates IL-6 expression in adipose tissue (Sabio et al. 2008). MAP4K4 upstreams JNK signaling as a novel negative regulator of insulin-stimulated glucose transport in adipocytes. However, MAP4K4 suppresses adipocyte lipogenesis independent of JNK. In this respect, MAP4K4 inhibits adipose lipogenesis by suppression of sterol-regulated element binding protein-1 in an AMPK- and mTOR-dependent but JNK-independent mechanism (Danai et al. 2013).

Suppressor of cytokine signaling 3 (SOCS-3) expression is increased in only adipose tissue of obese mice. SOCS-3 appears as a TNF-alpha target gene in obesity, and antagonizes insulin-induced IRS-1 Tyr phosphorylation (Emanuelli et al. 2001). Interestingly, SOCS-3 regulation by fatty acids also depends on JNK activity (Nguyen et al. 2005). Increased IKKalpha/beta and JNK serine phosphorylation contribute to increasing IRS-1 serine phosphorylation. IRS-1 concentration remains unchanged although IRS-1 Tyr phosphorylation is decreased and IRS-1 Ser phosphorylation is increased in obese animals (Zolotnik et al. 2012). JNK associates with IRS-1 and phosphorylates IRS-1 mainly at Ser307. JNK-mediated phosphorylation of Ser307 inhibits insulin-stimulated Tyr phosphorylation of IRS-1. That means that phospho-Ser307 blocks the interaction between the IRS-1 phosphotyrosine binding domain and the insulin receptor (Aguirre et al. 2000). However, the direct binding of JNK to IRS1 is not required for its activation by insulin, whereas direct binding is required for Ser307 phosphorylation of IRS1 (Lee et al. 2003). Actually JNK1 phosphorylates the adapter protein IRS1 at an inhibitory site that can block signal transduction by the insulin receptor (Aguirre et al. 2000). JNK1 may therefore directly induce insulin resistance (Weston and Davis 2007).

Obesity-induced insulin resistance is predominantly mediated by JNK1. The balance between JNK1 and JNK2 isoform expression influences total JNK activity. Resultant JNK activity is a critical determinant of inflammatory cytokine production in obesity (Tuncman et al. 2006). Four potential mechanisms of obesity-induced JNK1 activation have been proposed. First, high fat diet or exposure of cells to saturated fatty acids causes ER stress (Hotamisligil 2010) and induction of the UPR pathway leading to JNK1 activation (Hotamisligil 2008) to regulate insulin action by a mechanism that requires the double-stranded RNA-dependent protein kinase (PKR) in adipose and liver tissues (Nakamura et al. 2010). Second, TLR4-mediated activation of IKKbeta, JNK and insulin resistance in diet-induced obesity (Shi et al. 2006; Tsukumo et al. 2007). Third, saturated fatty acids activate the JNK pathway by a mechanism that involves PKC-mediated activation of the mixed-lineage protein kinase (MLK). This pathway requires the MAPKKs MKK4 and MKK7 (Jaeschke and Davis 2007) and subsequent JNK activation mediated by the JNK-interacting protein 1 (JIP1) scaffold protein which binds components of the JNK signaling module in the obese adipose tissue (Jaeschke et al. 2004). Loss of PKC, MLK3, MKK4, or MKK7 expression prevents FFA-stimulated JNK activation (Jaeschke and Davis 2007). Fourth, high fat diet-induced insulin resistance is associated with chronic low-grade inflammation and expression of inflammatory cytokines that can cause JNK activation (Davis 2000) including TNF-alpha overexpression as an important effector on several important sites of insulin action (Uysal et al. 1997) (Fig. 5.1).

JNK regulates the cell function through phosphorylating activator protein-1 (AP-1) complex proteins, including c-Jun and Jun B (Davis 2000). The JNK-MAPKs are encoded by three genes, of which two, JNK1 and JNK2, are expressed ubiquitously including hepatocytes. MAPK kinase kinases converge on the MAPK kinases MKK4 and MKK7, which preferentially phosphorylate JNK on Tyr185 and Thr183, respectively (Weston and Davis 2007). Consequently, JNK1 is activated by the MAPK kinases MKK4 and MKK7 (Tournier et al. 2001). JNK1 phosphorylates c-Jun; JNK2 lacks this function and may even

block c-Jun phosphorylation (Sabapathy et al. 2004). The ability of JNK over-activation to impair insulin signaling is the principal risk factors for non-alcoholic fatty liver disease (NAFLD) development—obesity and insulin resistance (Aguirre et al. 2000). In diet-induced and genetic models of obesity, JNK activity is increased in adipose tissue, muscle and liver (Hirosumi et al. 2002). The ability of JNK2 to oppose JNK1 phosphorylation of c-Jun suggests that the effect of a loss of JNK2 may be compensated by increased JNK1 function. Increased hepatic JNK, c-Jun and AP-1 signaling occur in parallel with the development of lipid over accumulation and hepatitis (Schattenberg et al. 2006). JNK1 isoform has primarily been implicated in the development of obesity and insulin resistance (Hirosumi et al. 2002), although JNK2 isoform is also involved in metabolic regulation. However, JNK1 is fully expressed because of regulatory crosstalk between the two isoforms (Tuncman et al. 2006).

Central melanocortin stimulation increases white adipose tissue lipolysis (Shrestha et al. 2010). The melanocortin-4 receptor (MC4R) is involved in regulating energy intake and expenditure (Tao 2010). More than 150 MC4R mutations have been identified in obese patients of different ethnic origins (Tao 2009). In addition to the conventional Gs-stimulated cAMP pathway, the MC4R also activates MAPKs, especially ERK1/2. The decreased basal or ligand-stimulated ERK1/2 signaling might contribute to obesity pathogenesis caused by mutations in the MC4R gene (He and Tao 2014). Stored lipids in adipocytes are mobilized by the melanocortin system. Lipid droplets are surrounded by phosphorylated hormone-sensitive lipase in alpha-melanocyte-stimulating hormone (alpha-MSH)-stimulated adipocytes. Alpha-MSH-activated MC5R signals through the cAMP/PKA and MAPK/ERK1/2 pathways in adipocytes. PKA is necessary for hormone-sensitive lipase and perilipin 1 activation and lipolysis regulation. ERK1/2 inhibition strongly interferes with the release of nonesterified fatty-acid (NEFA)s. The intracellular triglyceride levels are decreased after MC5R activation, whereas restored after ERK1/2 inhibition. That means, these kinases are involved in NEFA re-esterification and lipolysis regulation (Rodrigues et al. 2013). Proteasomal degradation of IkappaB proteins liberates IkappaB-bound NF-kappaB transcription factors, which translocate to the nucleus to drive expression of target genes. IKKbeta, mediate phosphorylation of IkappaB protein leads to NF-kappaB activation by proinflammatory signaling cascades (Häcker and Karin 2006).

Maternal diet-induced obesity leads to offspring cardiac hypertrophy, which is independent of offspring obesity but is associated with hyperinsulinemia-induced activation of Akt, mammalian target of rapamycin, ERK, and oxidative stress. Offspring are hyperinsulinemic and display increased insulin action through Akt, ERK, and mTOR. p38MAPK phosphorylation also increases (Fernandez-Twinn et al. 2012). Offspring of diet-induced obese dams have disrupted liver metabolism and develop NAFLD prior to any differences in body weight or body composition. Oxidative damage and mitochondrial dysfunction may cause the progression of fatty liver in these offspring (Alfaradhi et al. 2014). Oxidative stress acts as a link between free fatty acid and hepatic insulin resistance. Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase 3 (NOX3) is the predominant source of palmitate-induced ROS generation. NOX3-derived ROS may drive palmitate-induced hepatic insulin resistance through JNK and p38 MAPK pathways (Gao et al. 2010). Furthermore, elevated levels of palmitate, a predominant saturated fatty acid in diet and fatty acid biosynthesis, alter cellular function. AMPK might have a role in the palmitate-mediated regulation of clock genes (Lee and Kim 2013). Palmitate and oleate induces JNK activation and Akt inhibition which results in decreased phosphorylation of FOXO1 following nuclear localization and leading to the reducing of insulin. Palmitate and oleate stimulated apoptosis through p38 MAPK, p53 and NF-κB pathway (Yuan et al. 2010). Despite equal cellular steatosis, lipid-induced apoptosis and JNK activation are greater during exposure to saturated versus monounsaturated free fatty acids. Inhibition of JNK, pharma-

cologically as well as genetically, reduces saturated free fatty acid-mediated hepatocyte lipoapoptosis. JNK-dependent lipoapoptosis is associated with activation of Bax, a known mediator of mitochondrial dysfunction (Malhi et al. 2006).

Although lipid accumulation is a central event in the development of obesity and type2 diabetes, the mechanisms of lipid accumulation are not known completely. Hypertrophy of adipocytes and the development of a chronic sub-clinical inflammation in adipose tissue are the characteristic features of obesity. In this case dysfunctional hypertrophic adipocytes and a pro-inflammatory environment contribute to insulin resistance. AMPK may not only influence metabolism in adipocytes, but also act to suppress this pro-inflammatory environment (Bijland et al. 2013). Eventually AMPK activation prevents the mTORC1 activation, ER stress response, and lipid accumulation (Li et al. 2014b). AMPK is a Ser/Thr protein kinase that works as a central sensor of metabolic signals and responds to low glucose levels via a high AMP/ATP ratio. Activated, AMPK switches on catabolic pathways to produce ATP while simultaneously shutting down energy-consuming anabolic processes (Lee and Kim 2013). Activation requires phosphorylation of Thr172 within the catalytic subunit of AMPK by Stk11 (Carling 2005). Two AMPK kinases have been identified that mediate phosphorylation of the AMPK alpha catalytic subunit at Thr172. However, changes in adenine nucleotide concentrations do not directly regulate liver kinase B1 (LKB1 or Stk11) and Ca^{2+}/calmodulin-dependent protein kinase kinase beta (CaMKK beta). In this manner AMP and adenosine diphosphate (ADP) binding to the AMPK gamma subunit inhibit dephosphorylation of Thr172 in the presence of LKB1 (Bijland et al. 2013). Two transcription factors, PPAR gamma and CCAAT/enhancer-binding protein a (C/EBPalpha) are considered master regulators of adipogenesis (Farmer 2006). LKB1 deletion markedly reduces the levels of IRS1, PPAR gamma, CCAAT/ C/EBP alpha, and phosphorylated AMPK. The markers of the adipogenesis fatty acid binding protein (Fabp) 4 and fatty acid synthetase (FAS), as well as those of C/EBPa and PPARgamma significantly decrease in LKB1-deficient cells. Consequently, LKB1 controls IRS1-dependent adipogenesis via AMPK in white adipose tissue (Zhang et al. 2013). Actually obesity is an important disorder of lipid metabolism. Cyclin-dependent kinase 8 (CDK8) and its regulatory partner cyclin C (CycC) are key repressors on lipogenic gene expression, de novo lipogenesis, and lipid accumulation. In this case insulin is the principal stimulator of lipogenesis through the activation of the sterol regulatory element binding proteins (SREBP)-1c (Zhao et al. 2012). Hyperinsulinemia is associated with increase in SREBP-1 mRNA in adipose tissue (Boden et al. 2013). CDK8 can directly phosphorylate the T402 site of SREBP-1c and promotes its degradation. CDK8 functions as a novel kinase of SREBP-1c. GSK-3β, which functions downstream of insulin signaling, was previously reported to be involved in phosphorylation of SREBP proteins in its C-terminal domain, including T426 of SREBP-1a (the site corresponding to T402 of SREBP-1c) (Bengoechea-Alonso and Ericsson 2009). It is not uncommon that the same Thr or Ser residue of a protein can be phosphorylated by multiple kinases (Zhao et al. 2012).

AMPK directly phosphorylates at least two proteins to induce rapid suppression of mTORC1 activity, the TSC2 tumour suppressor and the critical mTORC1 binding subunit raptor (Shaw 2009).

Additionally, MARK4, also known as Par-1d/MarkL1, is a member of the AMPK-related family of kinases. MARK4 deficiency causes hyperphagia, hyperactivity, and hypermetabolism and enhances insulin-stimulated Akt phosphorylation, leading to protection from diet-induced obesity, insulin resistance and its related metabolic complications through up-regulation of brown fat activity (Sun et al. 2012). The increases in ER stress response and lipid accumulation are associated with activation of mTORC1 signaling. Inhibition of mTORC1 signaling attenuates the ER stress response and lipid accumulation (Li et al. 2014b). Regardless of type, nutrient overload is associated with the development of obesity, insulin resistance, and type 2 diabetes. Thus,

excess amino acids reduce AMPK phosphorylation, upregulates Notch1 expression through the activation of STAT3, and also impairs the insulin-stimulated phosphorylation of Akt Ser473 and IRS-1 Tyr612. Consequently, mTORC1/STAT3/Notch1 signaling pathway is activated subsequent to AMPK activity suppression by high-protein diet and insulin resistance develops. In this case activation of AMPK prevents amino acid-induced insulin resistance through the suppression of the mTORC1/STAT3/Notch1 signaling pathway (Li et al. 2014a). Despite the roles of mTORC1 in promoting protein synthesis and inhibiting autophagy in response to nutrients, it emerges as a central regulator of lipid homeostasis (Ricoult and Manning 2013). mTORC1 integrates systemic signals with local signals (Dibble and Manning 2013). There is close signaling interplay between mTORC1 and two other protein kinases, AMPK and Unc-51-like kinase 1 (ULK1). This kinase triad collectively senses the energy and nutrient status of the cell (Dunlop and Tee 2013). The Ser/Thr kinase ULK1 is a mammalian homolog of Atg1, part of the Atg1 kinase complex, which is the most upstream component of the autophagy. AMPK directly phosphorylates, ULK1 on several sites and this modification is required for ULK1 activation after glucose deprivation. In contrast, when nutrients are plentiful, the mTORC1 complex phosphorylates ULK1, preventing its association and activation by AMPK (Egan et al. 2011).

In addition to cellular growth, AMPK and mTOR also regulate lipid metabolism and adipogenesis. However, they may play differential roles in white adipose tissue and brown adipose tissue development (Fernández-Veledo et al. 2013). Actually differentiation of brown adipocytes employs different signaling pathways from white adipocytes. Sequential activation of p38 MAPK and LKB1-AMPK-tuberous sclerosis complex 2 (TSC2) as well as significant attenuation of ERK1/2 and mTOR-p70 S6 kinase 1 (p70S6K1) activation is demonstrated through the brown adipocyte differentiation process. In this case AMPK has a critical role in controlling the mTOR-p70S6K1 signaling cascade (Vila-Bedmar et al. 2010). The interaction between AMPK with hypothalamic lipid metabolism and other metabolic sensors, UCP-2, the mTOR and the deacetylase sirtuin 1 (SIRT1), are responsible from the hypothalamic control of feeding and energy expenditure (Blanco Martínez de Morentin et al. 2011). Leptin, insulin, glucose and alpha-lipoic acid have been shown to reduce food intake by lowering hypothalamic AMPK activity, whereas ghrelin and glucose depletion increase food intake by enhancing hypothalamic AMPK activity (Lee et al. 2005). Hypothalamic FoxO1 expression is reduced by the anorexigenic hormones insulin and leptin. FoxO1 is an important regulator of food intake and energy balance. Nuclear FoxO1 stimulates the transcription of the orexigenic neuropeptide Y (NPY) and Agouti-related protein (AgRP) through the PI3K/Akt signaling pathway and suppresses the transcription of anorexigenic pro-opiomelanocortin (POMC) by antagonizing the activity of STAT3 (Kim et al. 2006). Insulin-induced export from the nucleus into the cytoplasm as well as phosphorylation is an efficient modification of FoxO1 by the covalent attachment of one or more ubiquitin molecules (Matsuzaki et al. 2003). These molecular events can repress FoxO1-induced orexigenic signals in the hypothalamus. Diet-induced obesity alters alpha2-AMPK in muscle and hypothalamus and STAT3 in hypothalamus and impairs further effects of leptin on these signaling pathways. Defective responses of AMPK to leptin may contribute to resistance to leptin action on food intake and energy expenditure in obese states (Martin et al. 2006).

Chronic mTOR inhibition attenuates the upregulation of lipid uptake, lipoprotein lipase expression, and fat enlargement induced by PPARgamma activation in both subcutaneous white adipose tissue and in brown adipose tissue, which result in hyperlipidemia. Indeed, mTOR is a major regulator of adipose tissue lipoprotein lipase-mediated lipid uptake (Blanchard et al. 2012). Deletion of PPARgamma impairs alternative macrophage activation, and predisposes these animals to development of diet-induced obesity, insulin resistance, and glucose intolerance (Odegaard et al. 2007). Genes involved in insulin sensitivity, glucose transport, and

insulin receptor signaling, PPARgamma, GLUT-2/GLUT-4 and IRS-1/IRS-2 are up-regulated by omega-3-polyunsaturated fatty acids (omega-3-PUFAs), respectively. Moreover, omega-3-PUFAs increase adiponectin, an anti-inflammatory and insulin-sensitizing adipokine, and induce AMPK phosphorylation, a fuel-sensing enzyme (González-Périz et al. 2009). Eventually, omega-3 PUFAs negatively regulate macrophage inflammation by deacetylating NF-kappaB, which acts through activation of AMPK/SIRT1 pathway (Xue et al. 2012). Thus, NF-kappaB signaling stimulates glycolytic energy flux during acute inflammation, whereas SIRT1 activation inhibits NF-kappaB signaling directly by deacetylating the p65 subunit of NF-kappaB complex. SIRT1 stimulates oxidative energy production via the activation of AMPK, PPARalpha and PGC-1alpha simultaneously and repress the chronic inflammation (Kauppinen et al. 2013). Obesity is fundamentally caused by cellular energy imbalance and dysregulation. Like AMPK and mTOR, Per-ARNT-Sim (PAS) kinase is also a nutrient responsive protein kinase and important for proper regulation of glucose metabolism in mammals at both the hormonal and cellular level (Hao and Rutter 2008). Its sequence contains a C-terminal Ser/Thr kinase domain and an N-terminal PAS domain (Manning et al. 2002b). Interestingly, PGC-1 expression and AMPK and TOR activity are not affected in PAS kinase deficient mice (Hao and Rutter 2008). PAS kinase overexpression itself mimics the induction of preproinsulin promoter activity by high glucose concentrations. Conversely, RNA interference (RNAi)-mediated depletion of PAS kinase suppresses glucose-induced preproinsulin up-regulation (da Silva Xavier et al. 2004). PAS kinase knockout mice are protected from obesity, liver triglyceride accumulation, and insulin resistance when fed with a high-fat diet (Grose and Rutter 2010). Actually, fatty acids impair the glucose responsiveness of insulin gene transcription (Poitout and Robertson 2008). Similarly, the glucose-induced expression of PAS kinase mRNA and protein levels are reduced in the presence of saturated fatty acid. Additionally, inhibition of ERK1/2, but not of Akt, partially prevents the inhibition of insulin gene expression in the presence of palmitate or ceramide (da Silva Xavier et al. 2004). As mentioned above, dual specificity protein kinases and dual specificity phosphatases have great importance in developing obesity, but there is a need for more information about the signaling pathways with their downstream targets and direct biological regulation.

5 Conclusion

The underlying causes of obesity and metabolic syndrome are cellular lipid and glucose imbalance or dysregulation, islet dysfunction, inflammation, and genetic or environmental risk factors. Maintaining a balance between lipid synthesis and catabolism is of great importance in the prevention of obesity. Protein kinases involved in lipid metabolism may become a potential target for the treatment of obesity-related diseases. Further research on the effects of protein kinases is required to determine their functions as a target for treating metabolic diseases, particularly obesity.

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Fat Cell and Fatty Acid Turnover in Obesity

6

Atilla Engin

Abstract

The ratio of free fatty acid (FFA) turnover decreases significantly with the expansion of white adipose tissue. Adipose tissue and dietary saturated fatty acid levels significantly correlate with an increase in fat cell size and number. Inhibition of adipose triglyceride lipase leads to an accumulation of triglyceride, whereas inhibition of hormone-sensitive lipase leads to the accumulation of diacylglycerol. The G0/G1 switch gene 2 increases lipid content in adipocytes and promotes adipocyte hypertrophy through the restriction of triglyceride turnover. Excess triacylglycerols (TAGs), sterols and sterol esters are surrounded by the phospholipid monolayer surface and form lipid droplets. Following the release of lipid droplets from endoplasmic reticulum, cytoplasmic lipid droplets increase their volume either by local TAG synthesis or by homotypic fusion. The number and the size of lipid droplet distribution is correlated with obesity. Obesity-associated adipocyte death exhibits feature of necrosis-like programmed cell death. NOD-like receptors family pyrin domain containing 3 (NLRP3) inflammasome-dependent caspase-1 activation in hypertrophic adipocytes induces obese adipocyte death by pyroptosis. Actually adipocyte death may be a prerequisite for the transition from hypertrophic to hyperplastic obesity. Major transcriptional factors, CCAAT/enhancer-binding proteins beta and delta, play a central role in the subsequent induction of critical regulators, peroxisome-proliferator-activated receptor gamma, CCAAT/enhancer-binding protein alpha and sterol regulatory element-binding protein 1, in the transcriptional control of adipogenesis in obesity.

Collectively, in this chapter the concept of adipose tissue remodeling in response to adipocyte death or adipogenesis, and the complexity of lipid

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droplet interactions with the other cellular organelles are reviewed. Furthermore, in addition to lipid droplet growth, the functional link between the adipocyte-specific lipid droplet-associated protein and fatty acid turn-over is also debated.

Keywords

Obesity • Fat cell • Adipocyte • Free fatty acids • Adipose triglyceride lipase (ATGL) • Hormone sensitive lipase (HSL) • Perilipin (PLIN) • Lipotoxicity • Lipid droplets • Peroxisome proliferator-activated receptor-gamma (PPAR-gamma) • Triacylglycerol (TAG) • Sterol regulatory element-binding protein

1 Introduction

Synthesis, storage, and turnover of triacylglycerols (TAGs) in adipocytes are critical cellular processes to maintain lipid and energy homeostasis in mammals. TAGs are stored in metabolically highly dynamic lipid droplets, which are subjected to fragmentation and fusion under lipolytic and lipogenic conditions, respectively (Paar et al. 2012). The mean residence time of lipids in lipid droplets is 1.6 years, whereas the mean survival of adipocytes is 9.5 years. This implies that triglycerides are approximately replaced six times during the life span of the adipocyte. During this period lipid storage and mobilization is dynamically regulated. Furthermore, in obese individuals, the rate of triglyceride storage and the mean residence time of lipids in lipid droplets are markedly increased compared to non-obese individuals (Arner et al. 2011). In this context, white adipose tissue plays a central role in the development of obesity-associated comorbidities. A reduction in adipocyte number without a change in energy balance would result in larger fat cells, but not less in total adipose mass (Rosen and Spiegelman 2006). Six hundred and thirty-six individuals' fat distribution-related a subset of homeotic genes, HOX genes, HOXC9 and HOXC10 mRNA expression have been investigated in paired abdominal subcutaneous and omental adipose tissue samples. HOXC9 and HOXC10 may play an important role in the development of obesity, adverse fat distribution, and subsequent alterations in whole-body metabolism and adipose tissue functions (Brune et al. 2016). The contributions of different adipose tissue depots to the metabolic complications are different. People who have an upper body fat distribution around the abdomen are at greater risk of disease than those who tend to store fat in their lower body around the hips and thighs (Dam et al. 2016). Additionally, upper-body subcutaneous fat has a higher lipolytic activity than the lower-body fat depot in both women and men. Free fatty acids (FFAs) released by the visceral fat depot contribute only a small percentage of systemic FFA delivery. Hence upper-body subcutaneous fat is the dominant contributor to circulating FFAs and the source of the excess FFA release in upper-body obesity (Koutsari and Jensen 2006). The ratio of FFA turnover/lipolysis decreases significantly with increases in the degree of obesity. Lipid oxidation could only account for 50% of the FFA disappearance rate (Lillioja et al. 1986). Furthermore, increased adipocyte number in obesity has life-long effects on adipose tissue homeostasis and white adipose tissue mass. Primary cells from the adipose tissue stromal fraction can proliferate and differentiate into functional, mature adipocytes to reconstitute a fully functional adipose depot (Rodeheffer et al. 2008). Even after obese individuals undergo severe weight loss, elevated adipocyte number is maintained (Spalding et al. 2008). Therefore, besides the size and amount of adipocytes, fat

cell death, function of the lipid droplets, and adipogenesis should be debated in the scope of the fat cell and fatty acid turnover of obesity.

2 Size and Number of Adipocytes in Obesity

Actually fat cell size is a significant predictor of the cardio-metabolic disorders related to obesity. Adipocyte hypertrophy in the visceral fat compartment may represent a strong marker of limited hyperplasic capacity in subcutaneous adipose tissues, which in turn is associated with the presence of numerous cardio-metabolic disorders (Laforest et al. 2015). Expansion of white adipose tissue occurs either through increased lipid filling within existing mature adipocytes to enlarge adipocyte size or increased differentiation of adipocyte precursor cells to increase the number of adipocytes. Former is defined as hypertrophic adipose tissue growth and latter is defined as hyperplastic adipose tissue growth or adipogenesis (Joe et al. 2009; Wang et al. 2013). Determining omental weight as well as mean adipocyte size and number of 55 obese patients revealed that increase in visceral adipose tissue mass is predominantly dependent on adipocyte number rather than adipocyte size (Arner et al. 2013). In a previous study, Drolet et al. showed that adipocyte size of both subcutaneous and omental fat was increased in obese women, whereas hyperplasia was predominant in the subcutaneous fat depot. Thus mRNA levels of CCAAT/enhancer-binding protein (CEBP)-alpha, Peroxisome proliferator-activated receptor-gamma (PPAR-gamma) 2, sterol-regulatory-element-binding protein (SREBP) 1c and the other genes related to lipid metabolism are found to be significantly higher in subcutaneous adipocytes when compared with the omental fat tissue (Drolet et al. 2008). Indeed, preadipocyte differentiation is promoted via upregulation of PPAR-gamma and CEBP-alpha and inhibition of Glycogen synthase kinase 3 (GSK3)alpha/beta and beta-catenin signaling (Sohn et al. 2015). Additionally, the transcription factor SREBP 1c is highly expressed in adipose tissue and plays a central role in adipocyte development along with the generation of endogenous PPAR-gamma ligand and the expression of several genes which are critical for lipid biosynthesis (Payne et al. 2010). Women with omental adipocyte hypertrophy have significantly lower amount of subcutaneous adipose tissue glucose transporter type 4 (GLUT4) mRNA, higher subcutaneous CEBP-beta mRNA expression as well as higher mRNA expression of omental perilipin (lipid droplet-associated protein; PLIN), compared to women with omental adipocyte hyperplasia. Women with subcutaneous adipocyte hypertrophy have lower mRNA expression of GLUT4, higher mRNA expression of CEBP-beta, lower plasma adiponectin concentrations compared to women with subcutaneous adipose tissue adipocyte hyperplasia (Michaud et al. 2014). Actually adiponectin released by both omental and subcutaneous adipocytes are similar in lean individuals; however, in obese or visceral obese women, adiponectin release from omental adipocytes significantly reduces (Drolet et al. 2009). Furthermore, women characterized by omental adipocyte hypertrophy have higher plasma and very-low-density lipoprotein (VLDL) triglyceride levels as well as a higher total-to-high-density lipoprotein (HDL) cholesterol ratio compared with women characterized by omental adipocyte hyperplasia. In these cases, 10% enlargement of omental adipocytes significantly increases the risk of hypertriglyceridemia independent of body composition and fat distribution. In addition, 10% increase in visceral adipocyte number also markedly enhances the risk of hypertriglyceridemia (Veilleux et al. 2011). Fat cell enlargement is associated with insulin resistance even in non-diabetic individuals independent of the body mass index (BMI) (Lundgren et al. 2007). Moreover, in addition to insulin, catecholamines also involve in the regulation of adipocyte volume. The lipolytic effect of catecholamines is more pronounced in the abdominal fat depots (Engfeldt and Arner 1988). Thus, in non-obese individuals alpha2-adrenergic receptors do not have a significant effect on subcutaneous adipose tissue lipolysis during the high circulating adrenaline concentrations. However, in the preperitoneal adipose tissue depot, alpha2-

adrenergic receptor tone plays an important role for the lipolytic rate (Simonsen et al. 2008). In subjects with upper-body obesity, the lipolytic action of catecholamines changes according to the depot localization of adipocytes. While the effect of beta 2-adrenergic receptors decreases, the increased activity of alpha 2-adrenergic adrenoceptors combined with hormone sensitive lipase (HSL) deficiency inhibits the lipolytic effect of catecholamines in subcutaneous adipocytes. However increased activity of beta 3-adrenergic receptors is coincidental with the decreased activity of alpha 2 adrenoceptors and augments the lipolytic response in visceral adipocytes (Arner 1995). Subcutaneous abdominal fat cells are significantly larger and their cellular cholesterol content is greater than omental adipocytes of morbidly obese patients. Higher amount of high-density lipoprotein uptake in these patients is the result of differences in adipocyte size rather than differences in the cholesterol-to-triglyceride ratio (Despres et al. 1987). Furthermore, large adipocytes express more adipocytokine and show high amount of oleic acid via stearoyl-CoA desaturase upregulation (Matsubara et al. 2009). In overweight/obese patients, polyunsaturated fatty acid accumulation is coincidental with reduced adipocyte size according to the depot localization. In contrast, adipose tissue and dietary saturated fatty acid levels significantly correlate with an increase in fat cell size and number (Garaulet et al. 2006). There are important differences in fatty acid composition between the depot localizations. Saturated fatty acids are higher, whereas monounsaturated fatty acids are lower in perivisceral fat pad than in subcutaneous fat (Garaulet et al. 2001).

Activation of PPAR-gamma and stimulation of its target genes are prerequisite for the metabolic features specific to adipocytes, such as fatty acid uptake, TAG synthesis and storage or adrenergically stimulated lipolysis. Additionally the G0/G1 switch gene 2 (G0S2) expression is also upregulated by PPAR-gamma during adipogenesis (Heckmann et al. 2013). Schweiger et al. showed that adipose triglyceride lipase (ATGL) is required for efficient lipolysis in the basal and stimulated state of adipocytes. Inhibition of ATGL leads to an accumulation of triglyceride, whereas inhibition of HSL leads to the accumulation of diacylglycerol (DAG). In human adipocytes ATGL and HSL have rate-limiting roles in triglyceride and DAG hydrolysis, respectively. Furthermore, G0S2 expression is concomitantly upregulated with ATGL. Overexpression of G0S2 in the presence of the HSL inhibitor almost completely abolishes lipolysis (Schweiger et al. 2012). Thus, the increase in G0S2 protein expression attenuates ATGL activity and decreases lipolysis in human adipocytes. Therefore, cell size inversely correlates with G0S2 mRNA expression in both subcutaneous and omental adipose depots. G0S2 mRNA expression is 75% higher in subcutaneous adipose depots compared to omental adipose tissue (Skopp et al. 2016). G0S2 blocks lipolysis through direct interaction and inhibition of the triglyceride hydrolase activity of ATGL. Binding between the hydrophobic domain of G0S2 and the patatin-like domain of ATGL results in lipolytic inhibition in adipocytes (Yang et al. 2010). G0S2 protein levels increase in mesenteric fat depots of high-fat diet-fed mice. Overexpression of G0S2 in mesenteric white adipose tissue reduces basal lipolysis and increases PLIN protein levels in adipocytes. Otherwise increased basal lipolysis in mesenteric adipocytes is parallel by decreased PLIN content (Wueest et al. 2012). Endogenous G0S2 is simultaneously recruited to lipid droplets through direct interaction with ATGL. Hence cytosolic pools of ATGL are identified as G0S2-bound and unbound forms (Yang et al. 2010). In adipocytes, both G0S2 and ATGL also occur in association with an endoplasmic reticulum-related membrane compartment, from which they are recruited onto lipid droplets upon adrenergic stimulation (Yang et al. 2010). Translocation of G0S2-unbound ATGL leads to acute activation of triglyceride hydrolysis. Upon prolonged adrenergic stimulation, G0S2 is downregulated thereby releasing G0S2-bound ATGL for sustained lipolysis (Heckmann et al. 2013). Human G0S2 is upregulated during adipocyte differentiation and inhibits ATGL activity in a dose-dependent manner. In this manner, G0S2 regulates human lipolysis by affecting both enzyme activity and

intracellular localization of ATGL in adipogenesis (Schweiger et al. 2012). On the other hand, G0S2 protein could be degraded in response to tumor necrosis factor-alpha (TNF-alpha) stimulation. TNF-alpha also significantly decreases PPAR-gamma protein levels. Degradation of PPAR-gamma almost completely abolishes the binding of PPAR-gamma to the G0S2 promoter in adipocytes that are treated with TNF-alpha. Thus, proteasomal degradation of PPAR-gamma and the reduction of G0S2 content are permissive for prolonged TNF-alpha-induced lipolysis (Jin et al. 2014). Collectively, G0S2 increases lipid content in adipocytes and promotes adipocyte hypertrophy through the restriction of triglyceride turnover. Eventually the majority of alterations exhibited by adipose overexpression of G0S2 are due to reduction of lipolytic capacity through the inhibition of ATGL (Heckmann et al. 2014). As usual, ATGL is highly expressed in adipose tissue of humans. This enzyme exhibits high substrate specificity for TAG and is also associated with lipid droplets. Inhibition of ATGL markedly decreases total adipose acylhydrolase activity (Zimmermann et al. 2004). Actually ATGL activity is highly dependent on the association with comparative gene identification-58; α/β hydrolase domain-containing protein 5, ABHD5 (CGI-58) in adipose tissue. CGI-58 is an essential component for the lipolytic breakdown of cellular lipid depots (Lass et al. 2006). CGI-58 does not compete with G0S2 in binding to ATGL, and G0S2 possess the capacity to prevent ATGL-mediated lipid droplet turnover in the presence of CGI-58 (Lu et al. 2010).

Furthermore, genetic inactivation of ATGL also leads to ectopic TAG accumulation in multiple tissues. In this case the reduced availability of ATGL-derived FFAs increase glucose consumption and improve glucose tolerance and insulin sensitivity (Haemmerle et al. 2006). ATGL activity is also controlled by lipid intermediates which are generated during lipolysis. Inhibition of the major lipolytic enzymes ATGL and HSL by long-chain acyl-CoAs is an effective feedback mechanism for controlling lipolysis and protecting cells from lipotoxic concentrations of fatty acids and fatty acid-derived lipid metabolites (Nagy et al. 2014). ATGL is a key regulator of TAG lipolysis and whole body energy metabolism at rest and during exercise. However, ATGL Ser404 phosphorylation is not related to increases in adenosine monophosphate (AMP)-activated protein kinase (AMPK) activity, and no interaction was found between AMPK and ATGL (Mason et al. 2012). Activation of AMPK induces dose-dependent apoptotic cell death, inhibition of lipolysis, and downregulation of PPAR-gamma and CEBPalpha. Thus, AMPK may diminish adiposity via reduction of fat cell number (Dagon et al. 2006).

FFAs are directly taken up into subcutaneous fat in the post-absorptive state, in approximately 4% and 8% of men and women, respectively. Five versus 10% of FFAs released by subcutaneous fat cells are subsequently taken up directly and independent of the lipoprotein lipase. Direct FFA uptake pathway is more efficient in redistribution of subcutaneous adipose tissue fat in women compared to the men (Shadid et al. 2007). Eventually, in the postabsorptive state approximately 9% and 3% of systemic FFAs are directly re-stored in subcutaneous fat in women and men, respectively. In this regard plasma FFA concentrations are the best predictor of direct FFA storage rates (Koutsari et al. 2011). Considering per gram of adipose tissue lipid, direct FFA storage rates are equal in small, medium and large cells from upper to lower adipose depots. Nevertheless, storage rate of per cell is much greater in large than small adipocyte (Rajjo et al. 2014). However, mitochondrial dysfunction of adipocyte in obesity is related to overall adiposity rather than individual adipocyte hypertrophy (Yin et al. 2014). Thus, mitochondrial respiratory capacities of adipocytes are inversely associated with BMI but are independent of cell size (Fischer et al. 2015). Although mitochondrial content of adipocyte is not significantly different between obese and non-obese volunteers, mitochondrial oxidative capacity of adipocyte is reduced in obese compared with non-obese adults and this difference is not due to cell size differences (Yin et al. 2014). Obesity is associated with dysfunctional adipose tissue, which is unable to expand further to store excess dietary lipids. Increased fluxes of

plasma FFAs lead to ectopic fatty acid deposition in insulin-dependent organs and lipotoxicity (Tumova et al. 2016). Impairment of insulin action in adipocytes may contribute to metabolic disorders in adipose tissue, since insulin stimulates synthesis and storage of TAG and inhibits lipolysis. Actually adipose tissue hypoxia powerfully inhibits insulin action in adipocytes. The inhibition leads to an increase in lipolysis and cell death in large adipocytes (Yin et al. 2009). Increased fat cell size is highly associated with insulin resistance and may represent the failure of the adipose tissue mass to expand and therefore to accommodate an increased energy influx. Ectopic fat deposition is the result of synergistic effects of increased dietary intake, decreased fat oxidation and impaired adipogenesis (Heilbronn et al. 2004). Contrariwise, depletion of the fat-specific protein, FSP27 by small interfering RNA (siRNA) in white adipocytes results in the formation of numerous small lipid droplets, increases lipolysis, and decreases TAG storage (Nishino et al. 2008). On the other hand, FSP27 belongs to the cell death–inducing DNA fragmentation factor (DFF45)-like effector (CIDE) family of proteins. Furthermore, CIDE-A and CIDE-B genes are encode homolog proteins with DFF45. CIDE-A and CIDE-B activate apoptosis in mammalian cells, which is inhibited by DFF45 but not by caspase inhibitors (Inohara et al. 1998). CIDE-A expression is decreased two-fold in obese humans. Human adipocyte depletion of CIDE-A by RNA interference stimulates lipolysis and increases TNF-alpha secretion by a post-transcriptional effect (Nordström et al. 2005). Besides its localization to lipid droplet surface, FSP27-positive staining is observed at the site where two lipid droplets form close contact with adipocytes. The locally concentrated FSP27 or CIDE-A at lipid droplet-lipid droplet contact sites (LDCSs) may provide a tethering force for stable lipid droplet contact that is required for efficient lipid transfer. The mode of lipid droplet growth adopted by FSP27 and CIDE-A is different from membrane fusion, as the former involves CIDE-mediated focal contact of pairing lipid droplets and directional net lipid transfer between lipid droplets with size disparity (Gong et al. 2011). Eventually CIDE-A and CIDE-C are predominantly expressed in adipocytes. Both of them localize on the surface of lipid droplets and are particularly enriched at LDCSs to promote atypical lipid droplet fusion and growth by lipid exchange and transfer in adipocytes (Jambunathan et al. 2011). Enlarged lipid droplets have decreased lipolysis due to decreased surface area/volume. Indeed, FSP27 negatively regulates lipolysis and promotes triglyceride accumulation (Puri et al. 2007).

3 Lipid Droplets

Lipid droplets have a hydrophobic core of neutral lipids and are surrounded by a phospholipid monolayer which consists of over a hundred different phospholipid molecular species (Penno et al. 2013). Lipid droplets are coated by PLIN family and other structural proteins which include lipogenic enzymes, lipases and membrane-trafficking proteins (Carr and Ahima 2016). The most abundant surface proteins of lipid droplets are PLIN1, adipophilin (PLIN2), tail-interacting protein of 47 kDa (TIP47 or PLIN3), S3-12 (PLIN4), and OXPAT (for a PAT family protein expressed in oxidative tissues, Myocardial Lipid Droplet Protein or MLDP and Lipid Storage Droplet Protein 5 or PLIN5). These proteins collectively have been referred as the PAT family of proteins which is generally defines the three of them; PLIN, Adipophilin, and TIP47 (Brasaemle 2007). Nascent lipid droplets emerge with a coat composed of S3-12, TIP47, and adipophilin. The non-lipid droplet pools of S3-12, adipophilin, and TIP47 constitute a ready reservoir of coat proteins to permit rapid packaging of newly synthesized TAG (Wolins et al. 2005). The most abundant lipid droplet surface proteins are the adipocyte specific PLIN, the ubiquitous adipocyte differentiation-related protein (ADRP) and TIP47. All of them actively participate in the control of lipid storage and mobilization (Blouin et al. 2010). Initially lipid droplets are formed within membranes of the endoplasmic reticulum, later they are separated from endoplasmic reticulum membranes to become distinct structures.

However, final steps of neutral lipid synthesis are catalyzed by enzymes that reside in the endoplasmic reticulum (Brasaemle 2007). The earliest detectable deposits of neutral lipid collect in tiny dispersed structures at the periphery of adipocytes; both S3-12 and TIP47 co-localize to these minute structures. These droplets were distinct from those droplets surrounded by PLIN. The composition of lipid droplet coat proteins changes with the expanding of lipid droplets. The formation of S3-12-coated droplets is insulin-dependent and require glucose and fatty acids that can be incorporated into TAG (Wolins et al. 2003, 2005). In this event, initially the nascent lipid droplets form peripherally and then they migrate toward the perinuclear storage droplets. Wolins et al. proposed that nascent droplets enlarge as they move centrally, perhaps by synthesis of new TAG directly in the droplet rather than by droplet fusion. The PLIN-Adipophilin-TIP47 proteins are recruited to the nascent droplet surface from preexisting pools as they move centrally over time. The lack of fusion in this process reveals that nascent lipid droplets enlarge by synthesis of TAG on the droplets themselves (Wolins et al. 2005). Actually the lipid droplet monolayer surface is an area of endoplasmic reticulum membrane leaflet, but it is modified by its unique content of PLIN. Microperoxisomes are also closely associated with lipid droplets, however they are not in contact with the lipid droplet surface layer (Blanchette-Mackie et al. 1995). In adipocytes, circular and elongated membranous structures at the periphery of the lipid droplet but external to its surface layer are identified as microperoxisomes. Endoplasmic reticulum foci for the biosynthesis of phospholipid and TAG may simultaneously meet the requirements of proliferating peroxisomes for membrane components of developing lipid droplets. PLIN is associated only with TAG accumulation in endoplasmic reticulum and is not found in peroxisomes (Blanchette-Mackie et al. 1995). Lipid droplet-protein associated intermediate-sized filament, vimentin creates a spherical, cage-like structures by surrounding lipid globules. Various stages of emerging lipid globules are accompanied with PLIN as linking protein between lipid droplets and vimentin (Heid et al. 2014). Virtually both PLIN A and vimentin is important for lipid droplet stability. However, these proteins are significantly reduced in lipid droplets of the obese adipose tissue (Ding et al. 2012).

In terms of number and the size distribution of lipid droplets, they are correlated with obesity as well as other pathologies linked with fat accumulation (Rizzatti et al. 2013). The TAG-rich lipid droplets of adipocytes provide the largest storage depot for energy in the form of esterified fatty acids. Lipolysis of adipocyte TAG also releases glycerol that is transported to liver for metabolism by either gluconeogenesis or glycolysis (Brasaemle 2007). Microsomal triglyceride transfer protein (MTP) is essential for the assembly of triglyceride-rich lipoproteins. MTP is expressed in adipose tissue of humans and plays a key role in lipid droplet formation and turnover. MTP and PLIN2 are found on the same droplets; however, MTP does not co-localize with PLIN2. Inhibition of MTP activity has no effect on the movement of triglyceride out of the cell either as a lipid complex or via lipolysis (Love et al. 2015).

It is thought that one of the growth options of lipid droplets in size is the fusion process by which a larger lipid droplet is obtained by the merging of two smaller lipid droplets. The number and size of lipid droplets are influenced by the catabolism and the absorption of lipids or interaction with other organelles (Boschi et al. 2014). The size of lipid droplets is regulated by modes of lipid droplet growth including rapid/homotypic as well as slow/atypical lipid droplet fusion, and is identified by key proteins; fat-specific protein 27 (FSP27), seipin, fat storage inducing transmembrane protein 2 (FITM2) and PLIN1 and lipids; phosphatidylcholine and phosphatidic acid (Yang et al. 2012). Fusion involves an initial step in which the two adjacent membranes become continuous, followed by the slower merging of the neutral lipid cores to produce a single spherical lipid droplet. These fusion events are accompanied by changes to the lipid droplet surface organization (Murphy et al. 2010). Thus, phosphatidylcholine has a major role in stabilizing the lipid droplet surface and preventing lipid droplets coalescence, whereas

phosphatidic acids may facilitate lipid droplets coalescence of contacting lipid droplets, resulting in the formation of lipid droplets may reach up to 50 times the normal volume in cells (Fei et al. 2011). Phosphocholine cytidylyltransferase (CCT) activation on surfaces of expanding lipid droplets increases the flux through the Kennedy pathway, which generates phosphatidylcholine to coat growing lipid droplets and prevent their coalescence. Phosphatidylcholine acts as a surfactant to prevent lipid droplet coalescence, which otherwise yields large, lipolysis-resistant lipid droplets and triglyceride accumulation (Krahmer et al. 2011). Actually choline/ethanolamine phosphotransferase-1 (CEPT1) is the terminal enzyme in the Kennedy pathway of phospholipid synthesis. High fat feeding and obesity induces CEPT1. Indeed, in obese human muscle CEPT1 mRNA was inversely correlated with insulin sensitivity (Funai et al. 2016).

TAG is synthesized by two distinct diacylglycerol acyltransferase (DGAT) enzymes; DGAT1 and DGAT2. Both DGAT enzymes reside in the endoplasmic reticulum, but DGAT2 also co-localizes with mitochondria and lipid droplets. DGAT2 is capable of catalyzing TAG synthesis and promote its storage in cytosolic lipid droplets independent of its localization (McFie et al. 2011). FSP27 and Cidea (one of the CIDE proteins) are enriched at a particular sublipid droplet location, at the LDCS. The enrichment of FSP27 at LDCSs is a critical first step for FSP27-mediated lipid droplet growth. Once FSP27 and Cidea are enriched at LDCSs, rapid lipid exchange among contacted lipid droplet pairs occurres. The transfer of neutral lipids from smaller to large lipid droplets at FSP27-positive LDCS forms larger lipid droplets. In this event FSP27 may provide a tethering force for stable lipid droplet attachment and recruit unidentified proteins to form a complex at the LDCS (Gong et al. 2011). Eventually CIDE proteins, localize to lipid droplets and endoplasmic reticulum, control lipid metabolism in adipocytes through regulating AMPK activity and influencing lipogenesis or lipid droplet formation (Gong et al. 2009; Yonezawa et al. 2011). CIDE proteins appear to play a unique role in the control of the sizes of cytosolic lipid droplets in various cell types. Interestingly, CIDE proteins are localized to the surface of cytosolic lipid droplets and endoplasmic reticulum (Gong et al. 2009). In fact, FSP27-mediated enlargement of lipid droplets consists of two independent steps. Initially FSP27 mediates a rearrangement of small lipid droplets to form clusters, and then it promotes the formation of enlarged droplets by fusing the clustered droplets. FSP27 depletion in adipocytes causes fragmentation of lipid droplets and increases lipolysis (Puri et al. 2007).

The mean ratio of stiffness of the lipid droplets over cytoplasm stiffness is in the range of 2.5–8.3. This indicates that lipid droplets mechanically distort their intracellular environment (Shoham et al. 2014). The increase in cellular structural stiffness can potentially influence the localized deformations of adjacent adipocytes and may accelerate intracytoplasmic lipid production to form even larger and more tightly packed intracellular lipid droplets. The peak strain energy density at the plasma membrane of the adipocytes due to the levels of lipid accumulation in the neighboring cells is large enough to affect intracellular pathways to produce more lipid contents and deformation-induced differentiation in adipocytes (Ben-Or Frank et al. 2015).

Additionally, reactive oxygen species (ROS) levels are higher in high-lipid containing cells. In this respect, ROS levels are proportional to lipid droplet levels. It has been shown that 15% of cells with the highest lipid droplet levels accumulate more than 15% of the overall lipids, and also have 53% more ROS than the mean of the adipocyte population. This indicates that lipid droplet compartmentalization reduces toxic lipid metabolites in most of the adipocytes. Thereby high-lipid containing cells are an advantage for the remaining cells of adipose tissue (Herms et al. 2013). PLIN5 is essential for maintaining lipid droplets by antagonizing lipases. Lipid droplets in turn prevent excess ROS production by sequestering fatty acid from excessive oxidation and hence suppress oxidative stress (Kuramoto et al. 2012). Indeed, PLIN5 already present in the cytosol and is rapidly recruited to the surface of a nascent pool of lipid droplets. This protein reg-

ulates lipid droplet TAG hydrolysis in a protein kinase-A dependent manner. Eventually PLIN5 takes part in protection against cellular lipotoxicity by transiently entrapping bioactive lipids in lipid droplets (Wang and Sztalryd 2011). On the other hand, a similar mechanism is activated for limiting lipid storage. The components of vesicle-mediated coat protein complex I (COPI) transport complex regulate the PLIN, adipocyte differentiation related protein, tail interacting protein of 47 kDa (PAT) composition at the lipid droplet surface, and promote the association of ATGL with the lipid droplet surface to mediate lipolysis (Beller et al. 2008). In addition, the adenosine diphosphate (ADP)-ribosylation factor 1 (Arf1)/COPI protein machinery also regulates lipid droplet morphology. In this case Arf1/COPI proteins localize to cellular lipid droplets and bud nano-lipid droplets from larger cellular lipid droplets. Eventually lipid droplet surface tension decreases and bridges from lipid droplet to the endoplasmic reticulum are impaired. Conversely Arf1/COPI deficient cells have increased amounts of phospholipids on lipid droplets (Wilfling et al. 2014).

Large lipid droplets filled with triglycerides are enriched with caveolin-1. The caveolin-1 lipid droplet pool is organized as multi-protein complexes containing cavin-1, with similar dynamics as those found in caveolae. Caveolins mainly locate around intracellular lipid droplets. Nevertheless, caveolin enrichment of lipid droplets is present in all adipose localizations. This indicates that there is a connection between lipid droplet size and caveolin association. Indeed, despite comparable total adipocyte caveolin-1 expression between subjects, caveolin-1 concentration on lipid droplets is positively correlated with fat cell size in obese human adipocytes (Blouin et al. 2010). The specific enrichment of caveolins in enlarged lipid droplets results from an active pathway rather than trapping of caveolins to lipid storage organelle (Blouin et al. 2008). As a key regulator of membrane traffic, Rab protein and as an integral part of membrane protein, caveolin provides a functional link between the cell surface and lipid droplets. Consequently, lipid droplet interactions with each other (homotypic) or with other organelles (heterotypic) in adipocytes or in non-adipocytic cells may involve inter-organelle membrane contact sites or a hemifusion type mechanism to facilitate lipid transfer (Murphy et al. 2009). Actually Rab-8a is a direct interactor and regulator of FSP27 in mediating lipid droplets fusion in adipocytes (Wu et al. 2014). FSP27 (CIDE-3 in humans) plays a critical role in lipid metabolism by regulating lipid droplet size and lipid storage (Lee et al. 2013). Indeed, FSP27 suppresses lipolysis and thereby enhances triglyceride accumulation in adipocytes. The lipolytic actions of TNF-alpha, interleukin-1beta (IL-1beta), and interferon-gamma (IFN-gamma) are accompanied by marked decreases in FSP27 expression and lipid droplet size in adipocytes (Ranjit et al. 2011). Focally enriched FSP27 at the LDCS mediates lipid droplet growth and creates single-chambered lipid droplets (Gong et al. 2011). Contrariwise the formation of numerous small lipid droplets increases lipolysis, and decreases TAG storage (Nishino et al. 2008). PLIN1 is another adipocyte-specific lipid droplet-associated protein. PLIN1 also interacts with the FSP27 and markedly increases FSP27-mediated lipid exchange, lipid transfer and lipid droplet growth. Functional cooperation between PLIN1 and FSP27 is required for efficient lipid droplet growth in adipocytes (Sun et al. 2013).

During adipogenesis, SREBP-1, SREBP-1 stimulates lipogenic gene expression. In this case, PPAR-gamma enhances PLIN gene expression which, results in generating lipid droplets to store TAG in adipocytes. Lipid droplet biogenesis may create the endoplasmic reticulum microenvironment favorable for SREBP-1 activation (Takahashi et al. 2013). Furthermore, nuclear SREBP-1 increases in response to PLIN-mediated lipid droplet generation. However, adipose tissue PLIN deficiency attenuates lipid droplet formation via reduced SREBP-1 activation with the suppressed lipogenic gene expression. Lipid accumulation decreases. Conversely PLIN-mediated lipid droplet biogenesis stimulates SREBP-1 target gene expression and subsequent TAG synthesis, which further enhances lipid droplet formation (Takahashi

et al. 2013). Insulin/c-Jun N-terminal kinase2 (JNK2) pathway mostly regulates expression of genes involved in lipid metabolism, including SREBP-1. Depletion of JNK2 attenuates insulin-induced upregulation of SREBP-1c target lipogenic enzymes, leading to reduced de novo fatty acid synthesis. Actually SREBP-1c is an insulin/JNK2-regulated gene, so that the JNK2/SREBP-1c pathway mediates insulin-induced fatty acid synthesis, which may lead to enlargement of lipid droplets in human adipocytes (Ito et al. 2013). PLIN5 is a marker for PPAR activation and fatty acid oxidation. Ectopic expression of PLIN5 promotes fatty acid-induced TAG accumulation, long-chain fatty acid oxidation. It also participates in the regulation of ATGL activity and/or translocation (Wolins et al. 2006). The amount of ATGL and HSL that binds to the surfaces of lipid droplets is controlled by the adipophilin and PLIN A protein composition of the lipid droplets, respectively (Listenberger et al. 2007; Sztalryd et al. 2003). Thus, PLIN A is phosphorylated by protein kinase A (PKA) in response to lipolytic stimuli. Phosphorylation of PLIN A is essential for HSL translocation and stimulation of lipolysis (Londos et al. 2005). Thus, phosphorylation of HSL requires promotion of lipolysis of triglyceride substrates packaged in lipid droplets. Recruitment of HSL to the PAT protein scaffold on lipid droplets is insufficient to increase lipolysis without activation of PKA (Wang et al. 2009). PLIN sequesters abhydrolase domain containing 5 (ABHD5) in the basal state, thereby basal lipolysis is suppressed subsequent to the inhibition of ATGL activation. PKA activation leads to PLIN phosphorylation, which has two parallel effects. First, PLIN phosphorylation frees ABHD5 to activate ATGL on lipid droplets with and without PLIN. Second, PKA activation promotes the rapid translocation of HSL to PLIN-containing lipid droplets, which is partially dependent on PLIN phosphorylation (Granneman et al. 2007). In brief, the lipolytic action of HSL at the lipid droplet surface requires PKA-dependent PLIN phosphorylation (Miyoshi et al. 2006). PLIN A also regulates ATGL-dependent lipolysis and defines serine 517 as the PLIN A-protein kinase A site, essential for this regulation. Actually the phosphorylation of PLIN A by PKA alters the conformation of PLIN A at the lipid droplet surface to facilitate lipolysis (Miyoshi et al. 2007). Furthermore, ATGL activity is highly dependent on association with co-activator comparative gene identification-58 (CGI-58) (Lass et al. 2006). ATGL and CGI-58 are found to be co-localized at the surface of lipid droplets (Lu et al. 2010). Interaction of ATGL with CGI-58 increases TAG lipase activity (Watt and Steinberg 2008). PKA-mediated phosphorylation of CGI-58 also plays a role in the dispersion of CGI-58 from the PLIN scaffold and increases the availability of CGI-58 to bind to ATGL in adipocytes (Sahu-Osen et al. 2015). Indeed, the PKA-mediated phosphorylation of two carboxyl-terminal serine residues of PLIN A facilitates the release of CGI-58 from PLIN. CGI-58 is sequestered on the PLIN scaffold and PLIN suppresses lipolysis. Eventually PKA phosphorylation sites on PLIN are necessary for releasing CGI-58. Furthermore, phosphorylation of either site Ser 492 or Ser 517 of PLIN mediates the PKA-dependent interaction of CGI-58 with ATGL and, thus promotes lipolysis in fat cells (Granneman et al. 2009). PKA activation also increases the translocation of HSL to PLIN A-containing droplets and increases the colocalization of ATGL with its coactivator CGI-58 (Granneman et al. 2007). PLIN5 or OXPAT also has been shown to interact with ATGL. PLIN5 binds ATGL or CGI-58 but not both simultaneously. The association of PLIN5-CGI-58 complexes on lipid droplet surfaces is more stable than PLIN5-ATGL complexes, and oleic acid treatment selectively promotes the interaction of PLIN5 and CGI-58 (Granneman et al. 2011).

Fat cell lipolysis, the cleavage of triglycerides and release of fatty acids and glycerol, ensures survival during prolonged food deprivation, but all these paradoxically increase in obesity. Principally, HSL and ATGL control lipolysis in adipocytes. However, PLIN family proteins of the lipid droplet surface are master regulators of lipolysis. They protect or expose the triglyceride core of the droplet to lipases (Wang et al. 2008). Thus PLIN targets peripheral lipid storage drop-

lets, which are the sites of attack by HSL. Thereby, PLIN and HSL are continuously colocalized following lipolytic activation (Moore et al. 2005).

The mammalian target of rapamycin (mTOR) C1 inhibitor, rapamycin enhances the isoproterenol-stimulated phosphorylation of HSL on Ser-563 (a PKA phosphorylation site) however it has no effect on the isoproterenol-mediated phosphorylation of PLIN. Thereby inhibition of mTORC1 signaling synergizes with the beta-adrenergic-cyclicAMP(cAMP)/PKA pathway to augment phosphorylation of HSL to promote hormone-induced lipolysis. In other words, mTORC1 signaling suppresses lipolysis, and supports the TAG storage (Soliman et al. 2010). Likewise, leptin induces the formation of lipid droplets in macrophages in a phosphatidylinositol 3-kinase (PI3K)/mTOR pathway-dependent manner via adipose differentiation-related protein-enriched lipid droplets. The mTOR inhibitor, rapamycin inhibits leptin-induced lipid droplet formation by inhibiting leptin-induced adipose differentiation-related protein accumulation in macrophages. Thus PI3K/mTOR is an important signaling pathway for leptin-induced cytoplasmic lipid body biogenesis (Maya-Monteiro et al. 2008). Hence, simultaneous inhibition of mTOR and leptin signaling by rapamycin exhibits an anti-obesity effect (Deepa et al. 2013). Furthermore, apoptosis inhibitor of macrophage (AIM) induces lipolysis in a manner distinct from that of hormone-dependent lipolysis, without activation or augmentation of lipases. AIM diminishes lipid droplet-coating proteins including FSP27 and PLIN leading to lipolysis in adipocytes (Iwamura et al. 2012).

As mentioned above, both HSL and PLIN are phosphorylated directly by PKA (Anthonsen et al. 1998; Su et al. 2003). PKA-dependent PLIN phosphorylation either directly or indirectly facilitates PLIN interaction with lipid droplet-associated HSL. Thus, the lipolytic action of HSL at the lipid droplet surface requires PKA-dependent PLIN phosphorylation (Miyoshi et al. 2006). Actually lipolysis is differently impaired between fat depots in human obesity. PLIN A expression is a critical element in adipocyte lipolysis. PLIN protein level may contribute to differences in basal lipolysis and in adipocyte size between fat depots. Thus regulation of lipid accumulation may vary in subcutaneous or omental adipocytes (Ray et al. 2009). In this regard, the regulation of lipolysis is achieved by coordinated actions of many lipid droplet-associated proteins such as PLIN, HSL, ATGL, and its activator protein, CGI-58. Although micro-lipid droplets catch the fatty acid moiety of triglyceride from pre-existing lipid droplets during lipolysis, fatty acid re-esterification is blocked. Glycerol release is significantly reduced, whereas release of FFAs is enhanced (Hashimoto et al. 2012). Otherwise lipid droplets undergo to an active cycle of lipolysis and re-esterification to form micro-lipid droplets. TAG synthesis for lipid droplet formation and expansion occurs in the endoplasmic reticulum and on lipid droplets. TAG is transferred between lipid droplets during lipid droplet fusion. Furthermore, interaction of lipid droplets through the endoplasmic reticulum and mitochondria facilitates lipid transfer and lipid droplet expansion (Khor et al. 2013). During the remodeling of lipid droplets, micro-lipid droplets formation is an important feature of lipolysis in adipocytes. Treatment with insulin and fatty acids results in the re-formation of lipid droplets and return to the basal state. Insulin-dependent reformation of large lipid droplets may involve two distinct processes: microtubule-dependent homotypic fusion of micro-lipid droplets and expansion of individual micro-lipid droplets (Ariotti et al. 2012). The choice of fusion process requires the alpha-soluble N-ethylmaleimide-sensitive factor adaptor protein receptor (SNAP23), which is also involved in the insulin-dependent translocation of a glucose transporter to the plasma membrane. SNAP23 is a link between increased lipid droplet accumulation and development of insulin resistance (Olofsson et al. 2008).

Upon phosphorylation of four serine; Ser552, Ser554, Ser649 and Ser650, the human HSL translocate to the lipid droplet to participate in lipolysis. PKA-induced phosphorylation provides a conformational change to expose hydrophobic groups on HSL, which facilitates HSL binding to its substrate, lipid. Thus, phosphorylated HSL is found to have a closer interaction

with phospholipid vesicles than unphosphorylated ones (Contreras et al. 1998; Krintel et al. 2009). In particular, Ser649 and Ser650 are located in the vicinity of a lipid binding region and that PKA phosphorylation controls the accessibility of this region (Krintel et al. 2008).

In fact, lipid droplets are not simple lipid storage depots and contain various species of neutral lipids and are surrounded by a monolayer of phospholipid with embedded peripheral proteins (Martin and Parton 2006). Cytoplasmic lipid droplets increase their volume by homotypic fusion or localized TAG synthesis. According to energy requirement of the cell, lipid stores are eventually remobilized and metabolized into TAGs, diacylglycerols or monoacylglycerols, as well as FFAs. Lipid droplets transport their lipids to other cellular organelles by interacting with these organelles including endoplasmic reticulum, endosomes, peroxisomes and mitochondria. In this context "Transient Inter-Compartmental Contact Sites" mediate lipid traffic between various organelles and lipid droplets (Beller et al. 2010; Zehmer et al. 2009). PLIN is not only the major substrate for cAMP-dependent protein kinase A, but also has a dual action on lipid droplets by promoting triglyceride storage or triglyceride lipolysis. Under basal conditions, PLIN restricts the access of cytosolic lipases to lipid droplets and thus promotes TAG storage. In times of energy deficit, PLIN is phosphorylated by PKA and facilitates maximal lipolysis by HSL and ATGL (Brasaemle 2007). The lipolytic effect of endoplasmic reticulum stress is coincidental with the elevated cAMP production, in addition to the enhanced PKA and extracellular signal regulated kinase (ERK)1/2 activity. Actually endoplasmic reticulum stress stimuli do not alter the levels of HSL and ATGL but cause Ser 563 and Ser 660 phosphorylation of HSL and increase its translocation from the cytosol to lipid droplets (Brasaemle et al. 2000; Deng et al. 2012). Endoplasmic reticulum stress does not alter the level of PLIN proteins but increases its phosphorylation. The lipolytic response is arrested when the effect of PLIN phosphorylation is attenuated on PKA inhibition (Deng et al. 2012).

Proteomic analyses revealed that lipid droplets have a small component, guanosine triphosphatase (GTPase) Rab18, on the lipid droplet coat in human adipose tissue (Martin and Parton 2008). Rab18 production increases during adipogenic differentiation. Furthermore, insulin induces the recruitment of Rab18 to the surface of lipid droplets via PI3K. Hence, Rab18 is a common mediator of lipolysis and lipogenesis (Pulido et al. 2011). Under basal conditions, Rab18 overexpression provokes a 38% increase in triglyceride content of lipid droplet. Reduction in Rab18 expression does not modify basal lipogenic activity but abrogates insulin-stimulated lipogenesis. Rab18 mRNA levels are higher in both omental and subcutaneous fat of obese individuals (Pulido et al. 2011).

4 Adipocyte Death

Almost 10% of fat cells are renewed annually at all adult ages and levels of BMI. Neither adipocyte death nor generation rate is altered in early onset obesity. This suggests that a tight regulation is evident in fat cell number during adulthood (Spalding et al. 2008). Adipocyte death may be a prerequisite for the transition from hypertrophic to hyperplastic obesity in adipose tissue (Faust et al. 1978). Compared with the other fat depots, visceral depots are the prevalent sites of adipocyte death and macrophage infiltration. Indeed, visceral adipocytes display a greater susceptibility to cellular death (Murano et al. 2008). More than 90% of all macrophages infiltrated in white adipose tissue of obese humans are localized to the site of dead adipocytes. Up to 15 macrophages aggregate around each dead adipocyte to form crown-like structure and scavenge the residual free adipocyte lipid droplets. Obesity-associated adipocyte death exhibits feature of necrosis-like programmed cell death. Furthermore, crown-like structures formation around PLIN-negative adipocytes is a marker of adipocyte death (Cinti et al. 2005). Eventually degenerating adipocytes release lipid droplets into the extracellular space and the lipid-like materials extruded from degenerating adipocytes

is detected in neighboring macrophages (Giordano et al. 2013). Hence death of hypertrophic adipocytes triggers macrophage recruitment around dead adipocyte remnants (Giordano et al. 2013). Expression of both TNF-alpha and IL-6 protein by adipose tissue macrophages are arranged in crown-like structures around the remnant of lipid droplets and dead adipocytes. Later stages of adipose tissue remodeling are associated with phenotypic changes in adipose tissue macrophage subsets in which CD11b is reduced and CD68 is increased. These findings are consistent with the susceptibility of adipose tissue to obesity-associated adipocyte death (Strissel et al. 2007). Actually TNF-alpha regulates lipolysis, in part, by decreasing PLIN protein levels at the lipid droplet surface (Souza et al. 1998). Adipose tissue contains a heterogeneous array of cells including preadipocytes and adipocytes along with resident and inflammatory macrophages (Neels and Olefsky 2006). The obesity-induced recruitment of the inflammatory adipose tissue macrophage subtypes to macrophage clusters of adipose tissue is originated from the circulation. So these macrophage clusters are not related to the conversion of resident M2a macrophages to M1 adipose tissue macrophages (Lumeng et al. 2008). Actually FFAs-induced lysosomal permeabilization and subsequent cathepsin B activation occurs in hypertrophied adipocytes at the early stages of high fat diet induced-obesity. This event is preceded by M1 type macrophage infiltration into hypertrophied adipose tissue. Eventual adipocyte death in this tissue is mainly dependent on mitochondrial dysfunction with the resultant increase of ROS and release of cytochrome c into the cytosol (Eguchi and Feldstein 2013). Upon stimulation of caspase-8 dimerization, numerous adipocytes lose immunoreactivity for PLIN. All PLIN negative, dead adipocytes are surrounded by crown-like structures. In these areas during the time course of inflammation three steps are accomplished. Firstly, neutrophil recruitment is followed by macrophages. Afterwards, macrophages are transformed from macrophage surface glycoproteins binding to galectin-3 (Mac-2; galectin-3) negative state into galectin-3 positive state. Finally, crown-like structures are formed (Murano et al. 2013). Galectin-3 is expressed on the surface of human monocytes and that the level of cell surface galectin-3 increases progressively as these cells differentiate into macrophages (Liu et al. 1995). More than 90% of the galectin-3 positive macrophages that infiltrate adipose tissue of obese humans are localized in crown-like structures (Cinti et al. 2005). In fact galectin-3 is a chemoattractant for macrophages (Sano et al. 2000), and contributes to macrophage phagocytosis (Sano et al. 2003). Thus, the chemoattractant activities of galectin-3 facilitate and prolong macrophage recruitment, aggregation, and function at the sites of adipocyte death. Adipocyte death rate dramatically increases in hypertrophic adipocytes. Obesity-associated adipocyte death exhibits morphological features of necrosis and the leukocyte-eliciting profile of apoptosis. Thus, free lipid droplets of dead adipocytes act as persistent sites of macrophage fusion, lipid uptake, and multinucleate giant cells formation (Cinti et al. 2005). Actually apoptotic cells secrete chemotactic factors that stimulate the attraction of macrophages. The activation of caspase-3 in the apoptotic cell is required for the release of these chemotactic factors. The putative chemoattractants have been identified as the phospholipid and lysophosphatidylcholine (Lauber et al. 2003). In particular, lysophosphatidylcholine is assumed to act as "find-me" signals for the attraction of phagocytes. Additionally, lysophospholipids and G-protein-coupled receptor are the crucial receptor/ligand system for the attraction of phagocytes to apoptotic cells (Peter et al. 2008). In human adipocytes, death receptors Fas (CD95 or apoptosis antigen 1; APO-1) ligand (FasL), TNF receptor 1 (TNF-R1), TNF-related apoptosis-inducing ligand receptors 1 and 2 (TRAIL-R1 and TRAIL-R2) are expressed, so the apoptosis can be induced by specific ligands (Fischer-Posovszky et al. 2004). Increased adipocyte cell death has been proposed as an initial event that contributes to macrophage infiltration. Thus Fas and FasL protein expression are significantly increased in the hypertrophied adipocytes of obese mice fed a high fat diet compared to control diet (Alkhouri et al. 2010). Fas and FasL complex activates Fas signaling pathway resulting

in activation of caspase 8, which subsequently cleave and activate the effector caspases, mainly caspase 3 (Guicciardi and Gores 2009). Increase of proapoptotic proteins, tBid and Bax, decrease of anti-apoptotic protein (Bcl-XL) results in adipocyte apoptosis which occurs in association with the increase in macrophage infiltration and up-regulation of pro-inflammatory genes. In this respect Fas and FasL are significantly highly expressed in omental compared to subcutaneous adipose tissue. FasL expression most strongly correlates with adipocyte size and adipose tissue macrophage infiltration. Insulin-sensitive obese individuals have significantly lower Fas and FasL expression than insulin-resistant obese individuals (Blüher et al. 2014).

Under nutritional excess, induction of endoplasmic reticulum stress stimulates the unfolded protein response signaling (Hummasti and Hotamisligil 2010). The expression of glucose-regulated/binding immunoglobulin protein (GRP78), a central component of the unfolded protein response (UPR), protein kinase R (PKR)-like endoplasmic reticulum kinase (PERK) phosphorylation, and JNK activity are all significantly increased in adipose tissue of obese mice compared to lean controls (Ozcan et al. 2004). In this condition adipocytes exhibit signs of stress; including hypertrophy and hypertrophy-associated mechanical stress, compositional changes of lipids, hypoxia, disruption of mitochondrial function, production of ROS, apoptotic signaling and, increase in fatty acid release (Gregor and Hotamisligil 2007; Hotamisligil 2010; Opie and Walfish 1963). Actually energy imbalances due to nutritional excess lead to the storage of excess energy in adipocytes. This event results in both adipocyte hypertrophy and hyperplasia. Furthermore, excessive energy accumulation in adipocytes is also associated with mitochondrial stress and disruption of endoplasmic reticulum function. In this case, oxidative stress can be induced by itself or adipocyte associated inflammatory macrophages (Codoñer-Franch et al. 2011). Subsequent to adipocyte death, intracellular contents of degenerating adipocytes disseminate in the extracellular space. During this period, increased antioxidant enzymes in obese fat pads suggest that structural changes leading to adipocyte death are accompanied by oxidative stress. Crown-like structures are also correspond to remnants of hypertrophic adipocytes (Giordano et al. 2013). The stress signaling pathways, JNK and mitogen activated protein kinases (MAPK)/ERK, are relatively suppressed in subcutaneous adipose tissue thereby these adipocytes become more resistant to oxidative stress. Similarly, pre-adipocytes from subcutaneous adipose tissue are significantly more resistant than visceral-derived adipocytes to cell death caused by oxidative stress (Liu et al. 2015). Under stress conditions the complex metabolic changes triggered by ectopic expression of mitochondrial uncoupling protein 1 (UCP1) in the white adipose tissue are associated with the activation of AMPK, a metabolic master switch, in adipocytes (Rossmeisl et al. 2004).

During differentiation or lipid accumulation, adipose tissue undergoes to inflammasome and caspase-1 activation. An increase in fat mass causes upregulation and activation of caspase-1 that directs adipocytes toward a more insulin-resistant phenotype (Stienstra et al. 2010). NACHT, LRR and PYD domains-containing protein 3 (NLRP3) inflammasome-dependent caspase-1 activation in hypertrophic adipocytes induces obese adipocyte death by pyroptosis which is a pro-inflammatory programmed cell death. Eventually the mechanisms causing adipocyte death are not linked to the obese condition but may correlate directly to adipocyte size (Giordano et al. 2013). Mainly inflammasomes are responsible for the activation of pyroptosis type of cell death, (Abais et al. 2015). Indeed NLRP3 inflammasomes sense the lipotoxicity-associated increases in intracellular ceramide which, induces caspase-1 cleavage in macrophages and adipose tissue (Vandanmagsar et al. 2011).

5 Adipogenesis

Adipogenesis is determined by the balance between uptake of fatty acids from plasma into adipocytes, intracellular fatty acid oxidation

versus esterification of fatty acid into triglycerides, lipolysis of triglycerides by intracellular lipases, and secretion of fatty acids from adipocytes. High fat diet-induced adipogenesis is aggravated by stimulating lipoprotein lipase (LPL) activity and attenuated by inhibiting LPL activity (Voshol et al. 2009). LPL is the rate-limiting enzyme for the hydrolysis of the triglyceride core of circulating triglyceride-rich lipoproteins, chylomicrons, and VLDL. LPL is regulated at transcriptional, posttranscriptional, and posttranslational levels in a tissue-specific manner (Wang and Eckel 2009). LPL synthesized in parenchymal cells and transported to the luminal surface of the surrounding vascular endothelium. The molecular and metabolic microenvironment associated with functional, mature blood vessels promotes preadipocyte differentiation and adipose tissue formation (Fukumura et al. 2003). It is fact that, a PPAR response element and also a SREBP has been identified in the human LPL promoter (Schoonjans et al. 1996, 2000). Thus a transcriptional network with PPARgamma and its heterodimeric partner retinoid X receptor (RXR) mediates an appropriate transcriptional response to changing fatty acid concentrations by fine tuning the levels of enzymes involved in metabolic processes associated with fatty acid and lipid metabolism (Hamza et al. 2009). On the other hand, small adipocyte factor 1 (Smaf1) displays enrichment at adipocyte lipid droplets and it is a novel small protein endogenous to adipocytes. Also Smaf1 expression is closely tied to PPARgamma-mediated signals and the adipocyte phenotype (Ren et al. 2016). The development of new adipocytes from pluripotent precursors involves a complex and tightly orchestrated programme of gene expression (Rosen and MacDougald 2006). The major transcriptional factors of this process are C/EBPbeta and C/EBPdelta. They play a central role in the subsequent induction of PPARgamma, C/EBPalpha and SREBP-1 (Fajas et al. 1998). In particular, PPARgamma and C/EBPalpha function as critical regulators of adipogenesis. First of all, activation of C/EBPalpha expression requires PPARgamma activity. Although C/EBPbeta is also required, is not sufficient alone to initiate C/EBPalpha expression during the early phase of adipogenesis. This process requires a PPARgamma-dependent degradation of histone deacetylase by the 26 S proteasome (Zuo et al. 2006). However, the cyclin D1 have an important role in the regulation of PPARgamma-mediated adipocyte differentiation through recruitment of histone deacetylases. While normal physiological levels of cyclin D1 inhibit adipocyte differentiation, overexpression of cyclin D1 promotes cellular proliferation (Fu et al. 2005). These data indicate that C/EBPbeta and C/EBPdelta have a synergistic role in terminal adipocyte differentiation. The induction of C/EBPalpha and PPARgamma does not always require C/EBPbeta and C/EBPdelta, but co-expression of C/EBPalpha and PPARgamma is not sufficient for complete adipocyte differentiation in the absence of C/EBPbeta and C/EBPdelta (Tanaka et al. 1997). Galectin-3, a beta-galactoside-binding lectin is elevated in obesity and directly activates PPARgamma and leads to adipocyte differentiation. However lack of galectin-3 significantly reduces adipocyte differentiation and also decreases the expression of PPARgamma, C/EBPalpha, and C/EBPbeta (Baek et al. 2015). In addition, IL-4 primed tissue macrophages have efficient phagocytic activity and accumulate lipid from dying fat cells and upregulate expression of arachidonate 15-lipoxygenase (Alox15) (Lee et al. 2016). Although IL-4 signaling shows anti-inflammatory responses, Alox15 may either promote or inhibit an anti-inflammatory phenotype of IL-4-stimulated human macrophages in a context-dependent manner (Namgaladze et al. 2015). Eventually non-inflammatory removal of adipocyte remnants and coordinated generation of PPARgamma ligands by M2 macrophages provide localized adipogenic signals to support de novo brown/beige adipogenesis (Lee et al. 2016). Thus, IL-4 inhibits adipogenesis by downregulating the expression of PPARgamma and C/EBPα-alpha. Additionally, IL-4 promotes lipolysis by enhancing the activity and translocation of HSL in mature adipocytes. Consequently, IL-4 exhibits pro-lipolytic features by inhibiting adipocyte differentiation and lipid accumulation as well as by promoting lipolysis in mature adipocytes to decrease lipid deposits (Tsao et al. 2014).

Actually C/EBP factors may directly influence lipogenesis by controlling the early induction of the key lipogenic enzyme DGAT2 during adipogenesis. Inhibition of C/EBPbeta expression in differentiating preadipocytes reduces DGAT2 expression. Otherwise DGAT2 expression is maintained at high levels during the later stages of adipogenesis (Payne et al. 2007). The three SREBP isoforms, SREBP-1a, SREBP-1c and SREBP-2, have different roles in lipid synthesis. Of these, overexpression of the nuclear form SREBP-1c in adipose tissue inhibits adipocyte differentiation, whereas nuclear SREBP-1a overexpression in adipose tissue causes the hypertrophy of fully differentiated adipocytes (Horton et al. 2003). In this case adipocyte determination and differentiation-dependent factor 1 (ADD1) binds to two distinct DNA sequences which are associated with adipocyte development, and the sterol regulatory element expression (Kim and Spiegelman 1996). Eventually simultaneous expression of ADD1/SREBP1 increases the transcriptional activity of PPARgamma (Kim et al. 1998). ADD1/SREBP1 and PPARgamma are induced in early adipogenesis. ADD1/SREBP1-mediated transcriptional activity of PPARgamma increases the percentage of cells undergoing differentiation (Kim and Spiegelman 1996).

The hyperplastic growth of white adipose tissue results in the formation of new adipocytes. Since mature adipocytes are post-mitotic, adipocyte hyperplasia in adults requires that new adipocytes to be produced from the differentiation of precursor cells. Hence primary cells from the adipose tissue stromal fraction can proliferate and differentiate into functional, mature adipocytes to reconstitute a fully functional adipose depot (Rodeheffer et al. 2008). The progressive obesity is characterized by marked early enlargement of fat cell size and fat cell hyperplasia (Cleary et al. 1979). Once the adipocytes reach their maximal size, proliferation of new adipocytes is stimulated (Faust et al. 1978). Fat mass enlargement in obese patients is associated with an increased Ki-67+ progenitor cell population together with a new fraction of small adipocytes and increased cell death (Maumus et al. 2008). Hence nutritional excess without a rise in energy expenditure promotes adipocyte hyperplasia. The rise in adipocyte number is triggered by signaling factors that induce conversion of mesenchymal stem cells to preadipocytes that differentiate into adipocytes. Phosphorylation of the transcription factor C/EBPbeta by MAPK and GSK3beta is critical in this event (Tang and Lane 2012). On the other hand, Akt2 is required for the activation of adipocyte precursor cells in obesogenic adipogenesis. Human visceral white adipose tissue expansion is primarily controlled by adipocyte hyperplasia. In this context Akt2 is effective in the expansion of white adipose tissue through increased adipocyte number in human obesity (Jeffery et al. 2015). It is concluded that Akt2 is mandatory for the regulation of preadipocyte and adipocyte number (Fischer-Posovszky et al. 2012). mTOR/p70S6K pathway acts downstream of the PI3K/Akt pathway in mediating the adipogenesis of human mesenchymal stem cells (Yu et al. 2008). Akt2 is a positive regulator for the preadipocyte proliferation, whereas overexpression of the nicotinamide adenine dinucleotide $(NAD)^+$-dependent protein deacetylase sirtuin-1 (SIRT1) attenuates adipogenesis. In differentiated fat cells, upregulation of SIRT1 triggers lipolysis and loss of fat (Fischer-Posovszky et al. 2012; Picard et al. 2004). Differentiating SIRT1-silenced preadipocytes exhibit enhanced mitotic clonal expansion accompanied by reduced levels of p27, as well as elevated levels of C/EBPbeta and c-Myc. Eventually SIRT1 controls the cell number and functional integrity of adipocytes through c-Myc regulation (Abdesselem et al. 2016). In addition to c-Myc regulation, C/EBPalpha also regulates Sirt1 expression during adipogenesis by directly binding to the SIRT1 promoter (Jin et al. 2010). Eventually adipogenesis is balanced by positive regulation of Akt2 and negative regulation of SIRT1 through crosstalk between C/EBPalpha and insulin–stimulated PI3K/Akt pathways (Pang et al. 2013).

Thus, adipogenesis takes palace in two stages; firstly, as mentioned above mesenchymal stem cells turn into preadipocyte with terminal differentiation. Adipogenic stimuli induce terminal differentiation in preadipocytes through the activation of PPARgamma. The coordination of

PPARgamma with C/EBP transcription factors maintains adipocyte gene expression (Cristancho and Lazar 2011). In this case, C/EBP-beta activates a single unified pathway of adipogenesis involving its stimulation of PPARgamma expression, which then activates C/EBPalpha expression by removing histone deacetylases-1 from the promoter region for degradation (Zuo et al. 2006). During adipocyte differentiation, C/EBPbeta is induced to activate the expression of C/EBPalpha and PPARgamma (Guo et al. 2015). Galectin-3 is elevated in obesity and directly activates PPARgamma and initiates adipocyte differentiation. However lack of galectin-3 significantly reduces adipocyte differentiation and also decreases the expression of PPAR-gamma, C/EBPalpha, and C/EBPbeta (Baek et al. 2015). Under normal physiological conditions, PPARgamma is mainly expressed in adipose tissue and prevents the lipotoxicity by two ways. Firstly, it regulates the development of fat cells and their capacity for lipid storage. Secondly, PPARgamma coactivator-1alpha is a coactivator of PPARgamma that induces the differentiation of preadipocytes to brown adipocytes and enhances the oxidation of fatty acids via increasing mitochondrial capacity (Medina-Gomez et al. 2007). Carbohydrate response element binding protein (ChREBP) regulates de novo lipogenesis in adipocytes. ChREBPbeta expression in human adipose tissue predicts insulin sensitivity (Herman et al. 2012). The levels of ChREBP mRNA and protein show dramatic increases during the differentiation of human omental and subcutaneous preadipocytes (Hurtado del Pozo et al. 2011). ChREBP is expressed in both nuclear and cytoplasmic compartments of preadipocytes; the cytoplasmic level of ChREBP increases by 50% on day seven of differentiation into mature adipocytes (Pompei et al. 2012).

6 Conclusion

The progressive obesity is characterized by marked early enlargement of fat cell size and subsequent fat cell hyperplasia. Adipocyte death rate dramatically increases in hypertrophic adipocytes. In this context visceral adipocytes display a greater susceptibility to cellular death. Nutritional excess without a rise in energy expenditure promotes adipocyte hyperplasia. Adipogenic stimuli induce differentiation in preadipocytes through the activation of PPARgamma. The coordination of PPARgamma with C/EBP transcription factors maintains adipocyte gene expression. Increased adipocyte number in obesity has life-long effects on white adipose tissue mass. Even after obese individuals undergo weight loss, elevated adipocyte number is maintained. Although lipid droplet formation and adipogenesis is an earlier protective response against the excessive nutrition, later they lead to hypertrophic and hyperplastic growth of white adipose tissue, respectively.

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7 Adipose Tissue Function and Expandability as Determinants of Lipotoxicity and the Metabolic Syndrome

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Abstract

The adipose tissue organ is organised as distinct anatomical depots located all along the body axis and it is constituted of three different types of adipocytes: white, beige and brown which are integrated with vascular, immune, neural and extracellular stroma cells. These distinct adipocytes serve different specialised functions. The main function of white adipocytes is to ensure healthy storage of excess nutrients/energy and its rapid mobilisation to supply the demand of energy imposed by physiological cues in other organs, whereas brown and beige adipocytes are designed for heat production through uncoupling lipid oxidation from energy production. The concert action of the three type of adipocytes/tissues has been reported to ensure an optimal metabolic status in rodents. However, when one or multiple of these adipose depots become dysfunctional as a consequence of sustained lipid/nutrient overload, then insulin resistance and associated metabolic complications ensue. These metabolic alterations negatively affects the adipose tissue functionality and compromises global metabolic homeostasis. Optimising white adipose tissue expandability and its functional metabolic flexibility and/or promoting brown/beige mediated thermogenic activity counteracts obesity and its associated lipotoxic metabolic effects. The development of these therapeutic approaches requires a deep understanding of adipose tissue in all broad aspects. In this chapter we will discuss the characteristics of the different adipose tissue depots with respect to origins and

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precursors recruitment, plasticity, cellular composition and expandability capacity as well as molecular and metabolic signatures in both physiological and pathophysiological conditions.

Keywords
White adipose tissue • Brown adipose tissue • WAT browning • Obesity • Expandability • Fibrosis • Inflammation • Lipotoxicity

1 Introduction

Obesity and its associated metabolic dysfunctions, including type 2 diabetes and cardiometabolic complications, conform the Metabolic Syndrome (MetS); without any doubt one of the most important public health problems worldwide, which threatens not only our quality of life but also the financial stability of global economies. Despite substantial research, health policies, financial incentives/disincentives and widely accepted mutilating surgical treatments, the evidence is that these strategies have not been as successful as expected. This outcome urges to look for complementary new therapeutic strategies and more effective social policies directed not only to treat/prevent obesity but more urgently to palliate its devastating associated cardiometabolic comorbidities.

Whereas targeting obesity requires focusing on the mechanisms controlling energy balance, namely factors determining food intake and energy dissipation, when targeting the cardiometabolic complications of obesity, the focus on the white adipose tissue (WAT) becomes essential. WAT is a key metabolic organ that contributes to the metabolic functional flexibility by efficiently storing the surplus of fuel and by quickly mobilising lipids/energy to supply peripheral organs to meet their functional demands. However, in the situational context of the current obesity epidemic, the demands imposed on the expandability and functionality of WAT are paramount, in many cases overwhelming WAT capacity to store and mobilise fat appropriately in obese patients.

Despite its apparent simplicity, WAT is more than just an organ made of large adipocytes filled with big drops of lipids. In fact, WAT exhibits marked cellular heterogeneity, rich vascularisation and innervation, and as would be expected from an endocrine organ serving a complex hormonal homeostatic system, WAT is highly integrated with other metabolically relevant tissues (Wronska and Kmiec 2012). Moreover, to accommodate the overload of nutrients associated with obesity, WAT has to endure multiple cellular and structural remodelling processes aiming at optimising its expansion. This requires the coordinated and titrated increase in adipocyte size (hypertrophy) and/or number (hyperplasia) (Wang et al. 2013) to meet the lipid storage demands determined by the individual energy balance. This remodelling is coupled with appropriate recruitment/infiltration of immune cells (Lolmede et al. 2011) to facilitate the development and reorganisation of the vasculature/extracellular matrix (ECM) required to reach adequate storage capacity, oxygenation and mobilisation of fuel and waste metabolites (Cao 2013; Sun et al. 2013).

Sustained fat mass accretion is not infinite. The adaptive mechanisms enabling adipose tissue expansion can be exhausted resulting in blockade of further WAT expansion and associated chronic subclinical WAT inflammation. Failure of WAT to expand also impairs its functional flexibility to quickly shift between storage and mobilisation of lipids and it is associated with abnormal secretion patterns of adipokines, which further impairs lipid handling, adipocyte precursor differentiation, and promotes fibrosis deposition and WAT stiffness. At whole body level, the dysfunction of WAT leads to insulin

resistance, not only in WAT, but also in peripheral important metabolic organs such as liver or muscle through mechanisms that involve ectopic deposition of nutrients/fat causing lipotoxicity.

The '**adipose tissue expandability hypothesis'** provides an intellectual framework linking obesity to its metabolic complications by positing that in the context of positive energy balance, it is not the absolute fat mass that determines the appearance of metabolic complications, but instead the inability of WAT to further expand and appropriately accommodate energy surplus. In some way, this suggests that the "metabolic problem of obesity" is not determined by how obese someone is but rather by their inability to become fatter by efficiently storing fat in WAT. Hence, the problem seems to be in the mismatch between WAT storage ***demands*** required by positive energy balance and storage space ***supply determined*** by adipose tissue expandability. This is resolved initially through procurement of ectopic unhealthy storage in other organs whose dysfunction maps the scope of the metabolic syndrome (e.g. NAFLD, muscle insulin resistance, β cell failure, etc.).The complexity governing the WAT expandability derives in part from the cellular and functional heterogeneity that characterises its different anatomic depots. The largest WAT depots, which are more accessible and consequently more investigated, are the subcutaneous (SAT) and visceral adipose tissue (VAT) depots. In principle, an appropriate plasticity and expandability capacity of the WAT depots seem crucial to prevent metabolic dysfunctions (Arner et al. 2010). Since the VAT enlargement is more prevalent and frequently associated with deleterious metabolic conditions, this is the depot that has been "*demonised*" as determinant for the Metabolic Syndrome. Supporting this concept, it has been shown that engraftment of SAT or removal of VAT in obese murine models reversed the deleterious effects of obesity ameliorating carbohydrate metabolism (Foster et al. 2011; Hocking et al. 2015). Moreover, promoting SAT expansion improved lipid buffering and metabolic status (Kim et al. 2007; Jensen 2008). However, from the perspective of the adipose tissue expandability hypothesis, the fact that expansion of visceral fat is associated with metabolic complications, does not necessarily imply that the original problem may reside in this depot. In fact, another possibility is that expansion of the intrabdominal depot may be an adaptation to a primary defect of the subcutaneous fat pad, resulting in preferential visceral deposition analogous to other ectopic deposition (Ali et al. 2011; Britton and Fox 2011). This perspective is supported by data showing metabolic dysfunction related to defective storage capacity in subcutaneous adipose tissue (Alligier et al. 2013) as well as a beneficial metabolic response associated with expansion of the SAT depot (Jensen 2008; Kim et al. 2007).

An alternative solution to the mismatch between adipose tissue storage demand and storage supply is to ***decrease the storage demands***. This can be accomplished by dieting but also by promoting energy dissipation through lipid oxidation strategies. In fact, several studies indicate that the detrimental metabolic effects caused by dysfunctional white adipocyte mediated lipid spill over can be limited by the pro-oxidative anti-lipotoxic activity of another type of AT, the brown adipose tissue (BAT) (Laurila et al. 2016; Liu et al. 2015).

BAT is another type of adipose tissue, which differs from WAT pads at morphological/molecular level (i.e. vascularisation (Cinti 2009; Xue et al. 2010), innervation (Murano et al. 2009; Rosell et al. 2014), cellularity (Meyer et al. 2013; Prunet-Marcassus et al. 2006; Roberts-Toler et al. 2015), and adipogenic potential (Hames et al. 2015; Meyer et al. 2013; Prunet-Marcassus et al. 2006; Zhang et al. 2014), as well as by virtue of its unique thermogenic capacity. This depot is the main site of non-shivering thermogenesis (Peirce et al. 2014) in rodents and infants. Its main function is to produce heat to maintain body temperature and it is of special relevance in small mammals such as rodents to survive periods of nocturnal and hibernating cold as well as for human newborn/infants to overcome the cold stress of birth (Cannon and Nedergaard 2004).

However, increasing the caloric intake in animals also stimulate brown adipose tissue activity (Rothwell and Stock 1979). Conversely, there is evidence that murine models of brown adipose tissue ablation or impaired function develop obesity (Bachman et al. 2002; Lowell et al. 1993; Whittle et al. 2012). What is known as *canonical brown fat* forms discrete depots and it is activated by the sympathetic nervous system (SNS) through β-adrenergic stimulation of brown adipocytes, which leads to energy dissipation in the form of heat. This function is mediated by the mitochondrial uncoupling protein-1 (UCP-1). There is another type of UCP-1-expressing multilocular fat cells, identified also as 'beige' or 'brite' (brown-in-white) adipocytes, which in rodents can be observed interspersed within SAT white adipocytes particularly upon β-adrenergic stimulating conditions such as chronic cold exposure (Jia et al. 2016). Humans are also susceptible to browning of their WAT. There is evidence of beige cells in the omental adipose tissue depot of patients affected by pheochromocytoma, a rare catecholamines-secreting tumour (Frontini et al. 2013). Beiging is also observed in the SAT of extensively burned victims probably due of the chronic elevated levels of noradrenaline in their blood following severe adrenergic stress response (Sidossis et al. 2015).

Thus, increasing the mass and activation of BAT and beige cells represents one potential therapeutic approach to eliminate the surplus of energy and prevent lipotoxicity. This approach has been suggested as treatment/prevention of obesity and as a strategy to prevent diabetes. This idea is mainly supported by studies performed in rodent models (Liu et al. 2015; Stanford et al. 2013) and recent findings indicate its applicability in humans (Cypess et al. 2009, 2012; Huttunen et al. 1981; Van der Lans et al. 2013). However, obese individuals present BAT atrophy, which is more evident in the presence of increased visceral fat, ageing and hyperglycaemia (Cypess et al. 2009). This suggests that defective BAT in obese patients may contribute to the development of obesity and associated metabolopathologies. However, what cannot be ruled out in these individuals is that excessive white fat-mediated thermo-insulation could have induced BAT regression.

The intellectual framework incorporating the ***concepts of lipotoxicity and defective adipose tissue expandability/functionality*** justifies two main therapeutic strategies to improve metabolic dysfunctions linked to obesity progression and development of related complications: (1) the amelioration/optimisation of adipose tissue (AT) plasticity/functionality to facilitate fat deposition and prevent peripheral lipotoxicity and (2) to decrease the lipotoxic burden by decreasing the demand for storage capacity through the promotion of thermogenesis by activation of pre-existing brown adipocytes and/or recruitment and differentiation of brown or brown-like adipocyte precursors (Peirce et al. 2014).

However, the success of these approaches is hampered by the still limited knowledge regarding the identity/origin of adipocytes from different AT depots and the scarce information available about how obesity-related changes in cellularity/fibro-inflammation influence WAT plasticity. Thus a better understanding of the molecular mechanisms and cellular mediators that control white and brown AT plasticity, expansion and activity is essential.

In this chapter we focus on the current understanding of the origins of the adipose organ and its specific depots, on the identity of white/brown/brite adipocyte progenitors; on how depot-specific vascularisation and fibro-inflammation crosstalk with adipogenesis and cell hypertrophy/hyperplasia and how obesity adipose tissue dysfunction can cause depot-specific lipotoxic signatures. Moreover, we will discuss BAT plasticity and how obesity-associated environmental cues can be targeted to ameliorate tissue activation and global metabolic homeostasis. In doing all this, we will include recent insights highlighted by lineage-tracing studies in mice and genetic/genomics data obtained from human studies as well as the newest technologies and cellular models essential to address the adipose tissue function and obesity at translational level.

2 Adipose Tissue (AT) Development and Function in Early Life

The appearance and development of WAT in humans occurs between the 14th to the 24th week of gestation (Poissonnet et al. 1984) and follows an anterior to posterior, rostral to caudal and dorsal to ventral direction of development similarly to other species (Crandall et al. 1997). The earliest structures identified are the fat lobules in areas where capillary proliferation is more active. At the beginning of the third trimester, at about 28th weeks of gestation, adipose tissue is already present in the six principal fat depots i.e. head, neck, thorax, abdomen, upper and lower limbs. At these early stages, no difference in AT is observed between genders (Poissonnet et al. 1984). However, fat cell number and size varies among depots (Poissonnet et al. 1988) (Fig. 7.1a). Recently, Wang et al. using their 'AdipoChaser' mouse model, engineered with an inducible adipocyte-tagging system for temporally controlled detection of mature adipocytes and identification of newly formed adipocytes, identified the developmental timing of subcutaneous and visceral adipose tissue development in mice. The SAT adipocytes commitment and differentiation occurred in early embryogenesis in both genders, (i.e. between E14 and E18) and the number of SAT adipocytes remained relatively stable postnatally. Conversely, the epididymal adipocytes preferentially differentiated after birth (Wang et al. 2013) over a relatively long period of time i.e. over 20 days after birth (Han et al. 2011). Using a different experimental approach taking advantage of transgenic mouse models engineered for a transient or permanent fluorescent labelling of cells expressing Pref-1, a preadipocyte marker, it was shown that adipocyte precursors could be detected as early as E10 and become lipid loaded by day E17.5 in the subcutaneous region, whereas visceral VAT develops after birth (Hudak et al. 2014).

With respect to the origin of brite/beige cells, Contreras et al., showed their recruitment in mice occurs in subcutaneous AT of 129S1/SvImJ mice at approximately P10, when at room temperature, and disappears spontaneously at around P30. This regression strongly depends on the genetic background of the mouse strain. However, beige cells can re-emerge later on in response to cold or to β_3-adrenergic agonists (Contreras et al. 2014) (Fig. 7.1a). Maintenance of these beige adipocytes in WAT depots may enable a quick thermogenic response under changing environmental or nutritional conditions.

Analysis of *canonical brown fat* development, by lineage-tracing studies using Engrailed-1 (marker of embryonic dermomyotome region) (*En1*)-CreERT-inducible mice crossed with Rosa-floxed Stop-*LacZ* mouse) revealed that BAT becomes visible in mouse embryos at E14.5 (Atit et al. 2006). However, the divergence between myoblast and BAT from a communal precursor already occurs between stage E9.5 and 11.5 (Lepper and Fan 2010). In humans, BAT is detectable at birth, in early childhood and also in adult individuals (Cypess et al. 2009; Van Marken Lichtenbelt et al. 2009). Moreover, Di Franco et al. reported the presence of human brown fat in the intrascapular region of human foetuses of 9 to 12 weeks of age as demonstrated by the observation in situ of multilocular cells strongly expressing UCP1 (Di Franco et al. 2016) (Fig. 7.1a).

An important goal during foetal fat development, particularly in precocious thermoregulators, is that sufficient UCP1 is present at birth to enable effective thermoregulation when first exposed to low temperature in the extrauterine environment. The full activation of UCP1 at birth is coordinated with a dramatic increase in lipolysis and mobilisation of lipids from white fat depots to supply enough fuel (Symonds et al. 2003).

2.1 Post-natal Adipose Tissue Growth

WAT adipocyte *cell size* rapidly increases after birth and seems to reach adolescent values within the first year of life. Morphometric analyses performed on various subcutaneous AT sites indicate that fat cells enlarge significantly during the first 6 months of life. During the first year of life

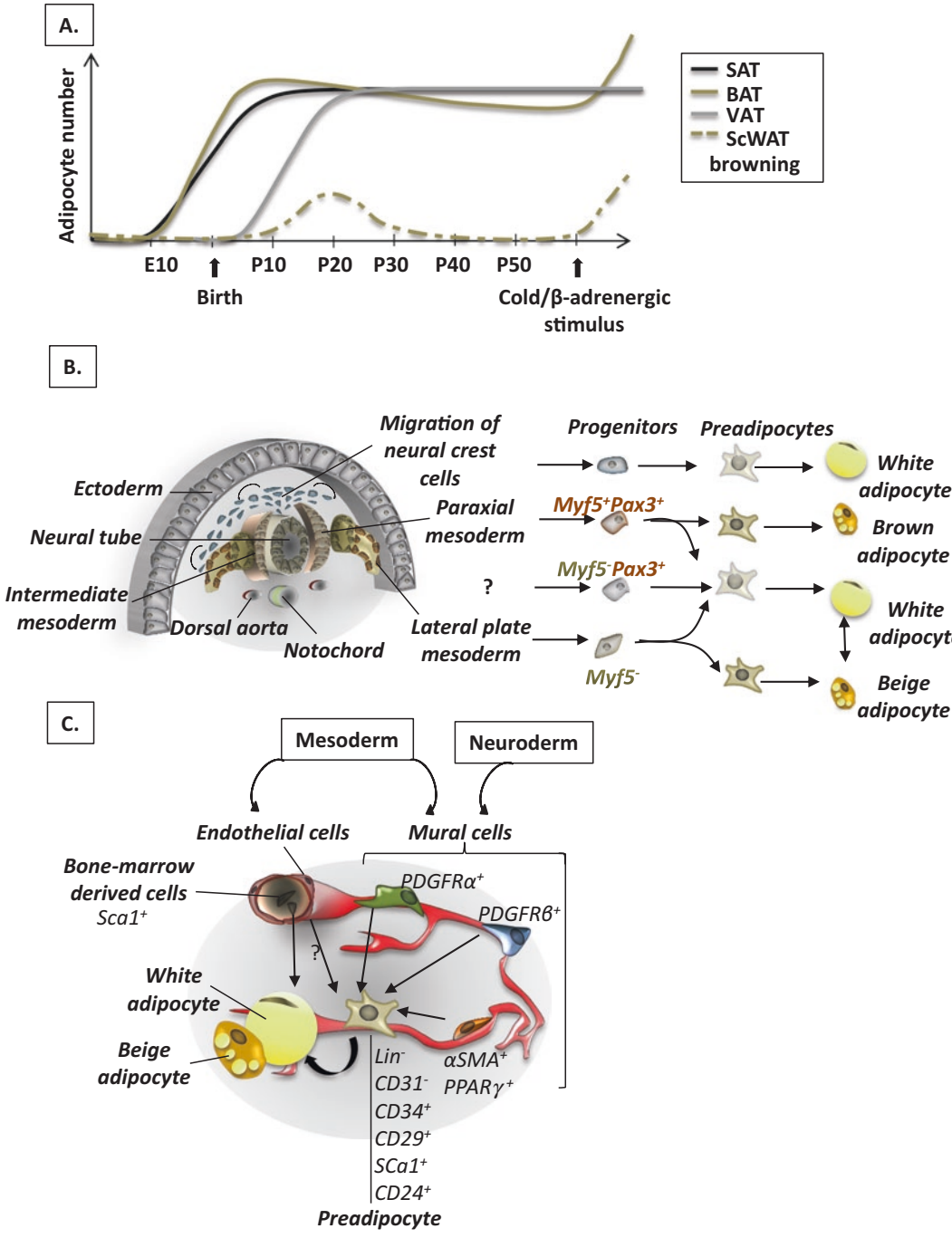

Fig. 7.1 Adipose tissue development and origins of white/brown/beige adipocytes. (**a**) Temporal development of white, brown and beige adipose from embryogenesis to early life. Development of brown adipose tissue (BAT) and subcutaneous adipose tissue (SAT) starts around E14. Visceral fat (VAT) appears at birth. Beige adipocytes can be observed in SAT between 10 and 30 days of age, and in adult animals following β-adrenergic stimulation. (**b**) Embryonic origin of white and brown adipocytes. Rodent brown adipocytes originate from paraxial mesoderm derived precursors MYF5^{+}PAX3^{+}, while white adipocytes derive from a MYF5^{-} lateral plate derived lineage. However, white adipocyte have also been described to originate from MYF5^{+}PAX3^{+}, MYF5^{-}PAX3^{+} and neural crest precursors. Beige adipocytes may derive from MYF5^{-} precursors or directly from white adipocyte trans-differentiation. (**c**) Vascular origin of white, brown and beige adipocytes. Different populations of precursors located within the vascular niche have been identified. Among them, pericyte-like cells expressing PDGFRβ. Adipocytes can also derived from differentiation of bone-marrow derived precursors. However, a direct endothelial origin of adipocyte is still matter of debate

the number of adipocytes does not rise significantly despite the body fat % increasing considerably between birth and 6 months, period after which the rate of % increase falls steadily until the end of the first year. From the beginning of the second year fat percentage seems to decrease until 6–8 years of age (less in female than in males) and the total number of adipocytes becomes fixed towards the end of childhood. From year one of age to puberty the size of fat cells remains fairly stable, whereas the number of adipocytes increases progressively. Puberty is a key event in the adipose tissue history being characterised by a substantial increase in AT cell size and total number of cells (Poissonnet et al. 1988).

3 Adipocyte Progenitors

3.1 Embryonic Lineage Determination

Adipose tissue is generally regarded as having a mesodermal origin (Carobbio et al. 2013b) (Fig. 7.1b). Lineage-tracing approaches have established more precisely the origins of white and brown as well as brite adipocytes with respect to the embryonic primary tissue, concluding that *brown adipocytes* and *myocytes* share common progenitors originated in the *paraxial mesoderm* and being characterised by the expression of specific markers i.e. MYF5, PAX3, PAX7 and MYOD (Peirce et al. 2014; Sanchez-Gurmaches and Guertin 2014; Timmons et al. 2007). With respect to *white adipocytes*, they were believed to derive from the *lateral plate mesoderm* (Carobbio et al. 2013b) and preferentially from MYF5$^-$ precursors. However, this view was recently challenged by Sanchez-Gurmaches et al., by demonstrating that the *Myf5-driven* conditional deletion of *Pten* caused overgrowth of BAT and also, a mixed pattern of paradoxical growth of specific white fat depots and loss of others (Sanchez-Gurmaches et al. 2012). Subsequent lineage-tracing studies from the same group confirmed the presence of MYF5$^+$ and PAX3$^+$ precursors in WAT, suggesting heterogeneity in white adipocyte origin being derived from both, MYF5$^+$/PAX3$^+$ and MYF5$^-$/PAX3$^+$ progenitors (Fig. 7.1b). Whether adipocyte can derive from MYF5$^-$/PAX3$^-$ lineages requires further investigation (Sanchez-Gurmaches and Guertin 2014).

Of note, a subset of white adipocytes localised in the salivary glands and ears originate from the neural crest (NC). Billon et al., using a *Sox10*-Cre/Rosa26-YFP mouse model that constitutively labelled NC-derived cells, demonstrated that the NC also contributes to the adipose tissue lineage during normal development (Billon et al. 2007). This observation was confirmed by another cell fate tracing study showing that the earliest wave of mesenchymal progenitors in the mouse embryo originate from SOX1$^+$ neuroepithelial precursors, in part through a NC intermediate stage (Takashima et al. 2007).

3.2 VAT and SAT have Distinct Origins

The existence of discrete WAT depots located in different parts of the body and the evidence of precursors with distinct genetic signature raises an important question: whether adipose tissue depots have specific genetic origins. A recent study showed that VAT but not SAT, originates from cells expressing the Wilms' tumour 1 gene (*Wt1*) in late mouse gestation. *Wt1* continues to be expressed in VAT progenitors also in adults. The same study showed that VAT is lined by mesothelium and provided evidence that this structure was the source of adipocytes in VAT depot (Chau et al. 2014). Conversely, Sanchez-Gurmaches et al., proved that the majority of the progenitors and mature subcutaneous white adipocytes in adult C57Bl/6 mice are labelled by *Prx1*-Cre, whereas very few to none brown nor VAT adipocytes are labelled (Sanchez-Gurmaches et al. 2015). Consistent with the selective green labelling of the SAT cells in Prx1-Cre;Rosa26RmTmG mice reported in the study by Sanchez-Gurmaches, the conditional deletion of the SHP-2 phosphatase, a key adipogenic factor, by Lapinski et al., using the same Prx1-cre mouse, resulted in mice preferentially lacking the subcutaneous fat pad (He et al. 2013; Lapinski et al. 2013).

3.3 Tissue-resident Adipocyte Progenitors

Multiple studies have identified several early WAT and BAT resident progenitors in adult mouse tissues. Using FACS analysis, Rodheffer et al., isolated a subpopulation of WAT precursors from the stromal vascular fraction (SVF). The adipogenic potential of these cells was confirmed in vitro and in vivo after transplantation in lipoatrophic A-Zip mice. These WAT precursors were identified as $Lin^-/CD34^+/CD29^+/Sca\text{-}1^+/CD24^+$ and could form white fat when subcutaneously transplanted (Rodeheffer et al. 2008) (Fig. 7.1c).

Several studies investigating the spatial and temporal association between adipogenesis and blood vessel formation identified adipose precursor niches located close to the growing vasculature, suggesting their likely endothelial origins. Nishimura et al. developed a confocal microscopy method for three dimensional visualisation of living intact adipose tissue. They observed that adipocyte differentiation occurs within cell clusters containing multiple cell types, including endothelial cells and stromal cells that express CD34 and CD68 (Nishimura et al. 2007). Other lineage-tracing studies using the endothelial marker VE-cadherin or the pre-adipocyte marker ZFP423 indicated that white and brown adipocytes could also originate from endothelial precursors (Gupta et al. 2012; Tran et al. 2012). Moreover, pre-adipocytes $perilipin^+/adiponectin^+$ identified in 16.5 day old embryonic WAT, were found to proliferate forming clusters closely related to the growing adipose vasculature until birth, being characterised by expressing stem cell markers such as CD24, CD29 and PDGFRα (Hong et al. 2015) (Fig. 7.1c). In this study, a small proportion of pre-adipocytes seemed to derive from $PDGFR\beta^+$ mural cells. Altogether, this work indicates that adipocytes originate from endothelial precursors also at embryonic stage. While adult adipose tissue progenitors from mural lineage were found i.e. $SMA^+/PPAR\gamma^+$ originating in the vascular niche, Jjang et al., also observed that adipocyte precursors (APs) expressing PPARγ were important for the development of the AT (Jiang et al. 2014) (Fig. 7.1c). Despite this evidence the existence of an endothelial adipose precursor niche is still challenged by other cell fate mapping studies, for instance, the use of the endothelial markers Cdh5 and Tie2. $Cdh5^+$ cells *were traced using Cdh5-Cre: mT/mG* and failed to show any Cdh5-derived adipocyte progenitors within the SVF. Similar results were obtained with *Tie2-Cre* (Berry and Rodeheffer 2013). This discrepancy may be due to the specific endothelial markers used to trace the origin of the adipocytes, as these markers may be expressed by different subpopulations of endothelial precursors. Further investigation is required to elucidate this point.

3.4 Non tissue-resident Adipocyte Progenitors

White adipocytes in adults are typically derived from tissue resident mesenchymal progenitors. However, this paradigm has been recently challenged by the observation of de novo production of adipocytes whose origins are in bone marrow (BM) derived cells in mice (Fig. 7.1c). Upon transplantation of the bone marrow of an AdipoQ-luciferase engineered mouse model into a wild type acceptor mouse, Gavin et al., demonstrated the existence of a subpopulation of adipocytes originated from the BM of the transgenic mouse (Gavin et al. 2016). Another study reported a gradual increase of $Sca\text{-}1^+$, which is a stem cell marker for the hematopoietic lineage (Gawronska-Kozak et al. 2014), in enlarging fat depots of wt mice fed a HFD. The contribution of the hematopoietic lineage to the adipocyte population seems plausible in humans as shown by Ryden et al., in their study where BM transplanted subjects were followed up for several decades and BM/PBSC-derived circulating progenitor cells were found to contribute to the WAT adipogenesis of these patients. This was more evident in subjects with excess fat mass and only analysed in the subcutaneous AT (Ryden et al. 2015). Moreover, cell fate tracing using LysMcreROSAflox/STOP mice with LacZ expression restricted to the myeloid lineage highlighted the presence of labelled

mature adipocytes in WAT, indicating that a subpopulation of mature adipocytes in WAT arises from myeloid progenitor cells (Majka et al. 2010). However, the haematopoietic origin of adipocytes still remains controversial since another lineage-tracing study using a *Vav1*-Cre; R26R-*mTmG* knock-in model (*Vav1* being a proto-oncogene expressed in the haematopoietic and lymphoid systems) failed to show fluorescence in adipocyte progenitors and mature adipocytes from the different WAT pads (Berry and Rodeheffer 2013).

3.5 Is there a Brite/beige Adipocyte Lineage?

Recent evidence from adult mice suggests that beige adipocytes are originated either by transdifferentiation from white adipocytes and/or by proliferation and differentiation from either "bipotential" white/beige progenitors or specific beige precursors (Lee et al. 2012; Wang et al. 2013) (Fig. 7.1b, c). In support of the first hypothesis, beige adipocytes can arise from white adipocyte when chronically exposed in vitro to PPARγ agonists (Petrovic et al. 2010). Also, using transgenic mice in which UCP-1$^+$ cells are constitutively or transiently labelled with fluorescent markers, beige adipocytes recruited after cold exposure were identified to derive directly from white adipocytes (Lee et al. 2015). Even though in humans cold exposure has not yet been established to induce AT browning (Van der Lans et al. 2013), a recent study reported the presence of beige adipocytes in SAT of burned victims (Sidossis et al. 2015). In these patients, progressive recruitment of UCP-1-expressing multilocular adipocytes was observed in serial SAT biopsies likely originated from interconversion of mature white cells.

Evidence supporting the existence of specific precursors for white and beige adipocytes comes from the study by Lee et al., again using the 'AdipoChaser mouse model', to demonstrate that cold-recruited brown-like cells originate by clonal expansion from a specific precursor (Wang et al. 2013). This is consistent with another study reporting that beige adipocytes arise de novo in WAT in response to adrenergic stimulation as demonstrated by tracking WAT browning using BrdU accumulation (Lee et al. 2012). Other cell fate mapping approaches have identified self-renewing PDGFRα-expressing precursors as an important source of newly formed beige adipocytes; these progenitors seem to be 'bi-potential', being able to differentiate into both, beige and white adipocytes, in vitro (Lee et al. 2012). In response to β3 -adrenergic agonist (CL316, 243) treatment, all multilocular adipocytes arising in the posterior SAT originate from Prx1-Cre expressing cells. This proves that Prx1-Cre labelling, while selective for SAT, does not distinguish between recruited beige adipocytes and pre-existing white adipocytes. Thus, Prx1-Cre could be expressed in an early precursor pool able to generate both, subcutaneous beige and white fat cells. However, this does not exclude that Prx-1 could also be expressed in separate progenitor pools producing these different white and beige cell types (Sanchez-Gurmaches et al. 2015). In humans, native CD45$^-$/CD34$^+$/CD31$^-$ cells were initially defined as white fat precursors (Elabd et al. 2009; Sengenes et al. 2005) but when further selected for the cell surface marker MSCA 1, they displayed the potential to become white and beige adipocytes upon stimulation with specific stimuli (Esteve et al. 2015).

Beige fat cells could also originate from specifically dedicated precursors. In support of this, a study focusing in the in vitro characterisation of the adipogenic potential of immortalised WAT- and BAT-derived precursors, demonstrated that a subpopulation of white APs differentiated preferentially into beige adipocytes (Wu et al. 2012). This indicates the presence in WAT of distinct subtypes of adipocyte precursors, differing in their capacity to generate beige adipocytes. This heterogeneity is probably due to differences in the lineage origins of the cells. Results supporting this conclusion come from a study showing that PAX3$^-$ or MYF5$^-$ adipocyte progenitors isolated from WAT are more prone to differentiate into brown-like cells when compared to PAX3$^+$ or MYF5$^+$ precursors, respectively (Liu et al. 2013; Sanchez-Gurmaches and Guertin 2014).

Brite/beige cells also possess a smooth muscle-like gene expression profile. Following 2 weeks of cold challenge, constitutive and inducible Cre drivers under the control of the Myh11 promoter (a selective marker for smooth muscle-like cells) labelled approximatively 10% of brite adipocyte in the SAT. Thus, a subpopulation of brite adipocytes may originate from smooth muscle-like cells (Abdennour et al. 2014).

In adult humans, the presence of recruitable beige cells in WAT surrounding phaeochromocytomas (pheos) (Frontini et al. 2013) suggests the browning potential of adipocyte precursors isolated from inducible BAT (B-ASC) found close to pheos *vs.* the progenitors from subcutaneous fat of the same patient, as only B-ASCs were able to differentiate into beige cells, suggesting an independent origin of the two AT depots (Di Franco et al. 2014).

The previous data provides evidence supporting the three different origins of beige cells i.e. transdifferentiation, white/beige "bipotential" progenitors and existence of a specific dedicated precursor. These possibilities are not exclusive. Also it may be possible that the origins of beige adipocytes may be WAT depot-specific or depending on the stimuli used to induce their recruitment, etc. Further investigations are required to answer this question.

3.6 Increased Adipogenic Differentiation Potential of Adipocyte Precursors in Subcutaneous Adipose Tissue

The anatomical localisation of different AT depots seems to be an important determinant of the proliferation and differentiation potential of their SVF and precursors, both in humans and rodents. Of note, SVF cells isolated from human and rodent SAT show greater adipogenic differentiation capacity compared to VAT (Adams et al. 1997).This is in agreement with higher expression levels of regulators of adipogenesis such as CEBPα or FABP4, as well as greater response to PPARγ agonists in subcutaneous *vs.* visceral fat. Consistently, TZD mediated activation of PPARγ enhanced fat storage preferentially in SAT (Smith et al. 2005) whereas rare loss-of-function PPARγ mutations predominantly cause a reduction in subcutaneous (particularly gluteal) fat (Semple et al. 2006). In addition, *adipocyte precursors* from subcutaneous AT display high expression levels of pro-adipogenic genes i.e. *PPAR, CEBPA*, *BMP2*, *BMP4* and *DKK2* and show greater differentiation potential compared to those AP from visceral AT in response to classical adipogenic induction (Macotela et al. 2012). This could be, at least in part, due to the intrinsic differences between VAT *vs*. SAT precursors. Of relevance, visceral APs exhibit a more 'mesenchymal stem cell (MSC)'-like phenotype characterised by higher expression of MSC markers e.g. *LIF*, *CTGF* and *MGP* as well as adipogenic inhibitors such as *GATA2* and *TGFB2*. It has been reported that the expression of the transcription factor Islet1 is restricted to the SVF of VAT and that in animals and humans its expression correlates with leanness (Li et al. 2008). Recently has been also demonstrated that Islet1 is able to inhibit 3T3-L1 pre-adipocytes differentiation in vitro in part via down regulation of Bmp4 (Ma et al. 2014). Moreover, clonogenic assays and in vivo BrdU studies conducted in adult C57BL/6 mice showed that the number of adipocyte precursors is eightfold greater in subcutaneous *vs.* visceral adipose tissue (Jeffery et al. 2015; Joe et al. 2009), indicating increased proliferation capacity of SAT.

4 Adipose Tissue Depot-Specific Features

The identity of the each adipose tissue depot is defined by specific features of their structure, morphology and function. The two major and more widely studied types of WAT, are the *visceral fat* depot located in the abdominal cavity and mediastinum and *subcutaneous fat* depot located in the hypodermal layer of the skin (Fig. 7.2a). With respect to canonical BAT depots, in mice they are preferentially identified in the interscapular region, and also in the axillar and cervical area and around the aorta. However

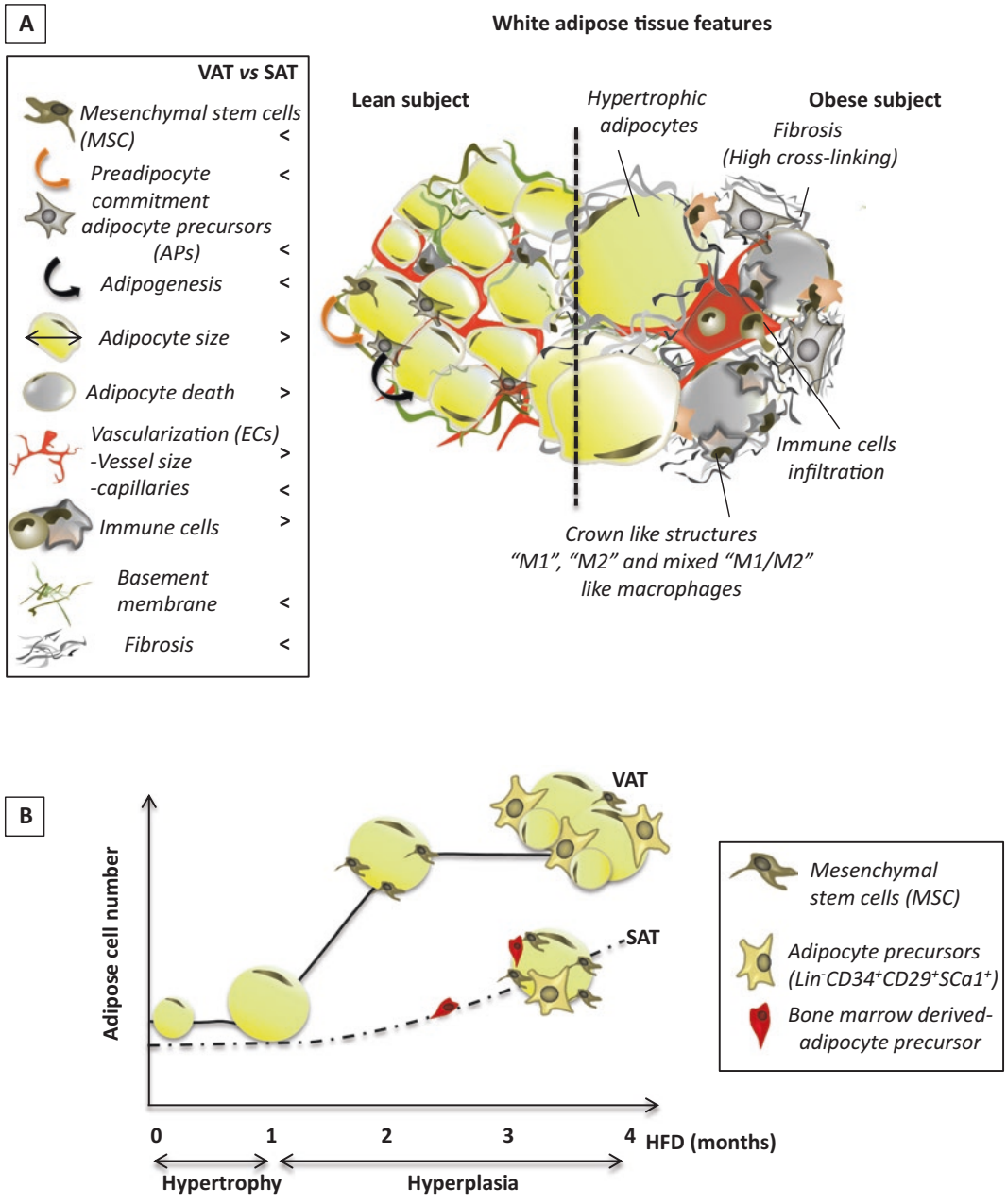

Fig. 7.2 White adipose tissue remodelling in obesity and differences among depots. (**a**) WAT undergoes cellular and structural remodelling in obesity characterized by (1) impairment of adipogenesis, (2) hypertrophy and inflammation of adipocytes, (3) decreased capillary density associated with vascular dysfunctions, (4) accumulation of immune cells such as macrophages organised in crown-like structures and (5) activation of fibroblasts and APs leading to fibrosis deposition and decreased tissue plasticity. Differences between VAT and SAT are presented in the legend (VAT *vs.* SAT). (**b**) Adipose tissue expansion through hyperplasia and hypertrophy during the development of obesity in diet-induced obese mice (HFD). The differences between depots are represented in association with their ability to recruit and differentiate adipocyte progenitors

brown-like cells can also be found as interspersed foci in skeletal muscle and within WAT (Cinti 2012). In human infants the canonical BAT depot is well developed and contributes to maintain body temperature and to warming the blood flow to key organs. In the adult human discrete canonical BAT depots persist, predominantly in the cervical supraclavicular, mediastinal, paravertebral, suprarenal and peri-renal areas (Cypess et al. 2009). Recent studies have also highlighted structural characteristics in humans BAT depots whose adipocytes display a phenotype closer to rodent beige/brite cells than to murine canonical brown fat (Wu et al. 2012).

Analysis of WAT depots also revealed that visceral fat is more vascularised (Villaret et al. 2010) than SAT, although exhibiting less capacity for new vascular sprouting (Gealekman et al. 2011). VAT is also more innervated than SAT (Rosell et al. 2014) and contains a larger number of inflammatory and immune cells (Bruun et al. 2005; Kranendonk et al. 2015). Moreover, VAT pre-adipocytes exhibit less differentiation potential (Macotela et al. 2012), which leads to an increased proportion of large adipocytes (Skurk et al. 2007). VAT cells are also more sensitive to adrenergic stimulation (Arner et al. 1990; Hellmer et al. 1992), to lipolysis (Lafontan and Langin 2009) and more prone to develop insulin resistance (Abate et al. 1995; Frayn 2000) than SAT adipocytes. Conversely, SAT adipocytes more avidly take up TGs and free fatty acids from the circulation (Marin et al. 1992; Misra and Vikram 2003), at least in healthy individuals. A prerequisite to maintain appropriate adipocyte and tissue structure is the presence of a permissive external connective tissue framework including extracellular matrix (ECM) such as collagens and proteoglycans. The presence of this scaffold reduces the impact of external forces on the adipocytes and ensures their structural and functional integrity (Mariman and Wang 2010). VAT and SAT have different composition of ECM. More specifically, SAT presents higher secretion of THSB1/2, type 1 collagen, SPARC, TIMP1, and lower secretion of laminin (b/c), fibronectin, type 6 collagen and TGFβ1 than VAT (Mori et al. 2014; Roca-Rivada et al. 2015). Increased levels of type VI collagen and TGFβ promote fibrosis formation and recruitment of pro-fibrotic macrophages and correlate inversely with insulin sensitivity (Spencer et al. 2010; Sun et al. 2013). Moreover, ECM composition influences WAT biomechanical properties, and lipid storage and cellular expansion capacity of the AT. Alkhouli et al., tested the force/extension and stress/relaxation characteristic of SAT *vs.* VAT in humans, and observed that SAT has greater capacity to expand and recover from mechanical deformation than VAT (Alkhouli et al. 2013). The quantitative and qualitative pattern of adipokine and cytokine secretion is also specific for each WAT depot. Whereas SAT releases larger amount of adiponectin (Drolet et al. 2009) and leptin (Van Harmelen et al. 1998), VAT releases more pro-inflammatory cytokines such as IL-6 (Fontana et al. 2007). In mice, several studies have demonstrated the greater browning potential of SAT *vs.* VAT upon cold exposure (Barreau et al. 2016; Jia et al. 2016) or adrenergic stimulus (Contreras et al. 2014) (Fig. 7.2a).

4.1 Hypertrophy *vs.* Hyperplasia in SAT and VAT

This is not a trivial question since the adipocyte cell size may be a key determinant of adipocyte dysfunction. In a situation of positive energy balance, there is an increased demand for lipid storage, and to accommodate the excess lipids, the storage capacity can be increased either by increasing adipocytes size (hypertrophy) and/or by increasing adipocyte number (hyperplasia) (Virtue and Vidal-Puig 2010) (Fig. 7.2b). In obesity, WAT expansion through adipocyte hypertrophy has been associated with increased glucose intolerance, hyperinsulinaemia, type 2 diabetes, cardiometabolic complications independently from total fat mass (Weyer et al. 2000). However, while Skurk et al., reported that adipose depots with larger adipocytes exhibit increased inflammation and susceptibility to cell death (Skurk et al. 2007), Hoffstedt et al., showed that in their cohort of morbidly obese woman there was no correlation between adipose cellularity and

inflammatory markers (Hoffstedt et al. 2010). This lack of association was also observed by McLaughlin et al. (Mclaughlin et al. 2010). Considering the differences with respect to adipocyte size and precursors pool in SAT *vs.* VAT, it is not unexpected that their plasticity is differently affected by an overload of nutrients. Moreover, adipose tissue hyperplasia in SAT and VAT has been significantly associated with improved glucose, insulin and lipid profile compared with adipose hypertrophy in any or both depots (Hoffstedt et al. 2010). In agreement with the ***adipose tissue expandability hypothesis***, the percentage of small adipocyte is greater in SAT and omental VAT in healthy non-diabetic individuals than in obese diabetic patients (Fang et al. 2015). A recent time course experiment in mice fed a HFD also highlighted inter-depot differences in immune cell composition in relation to WAT enlargement (Van Beek et al. 2015). This study also revealed a certain depot hierarchy or order of expansion with gonadal VAT being the first depot expanding during the initial phase of obesity, followed by the SAT and mesenteric VAT. More specifically, when mice reached a body weight of 40 g, their gonadal VAT stopped expanding, whereas SAT and mesenteric VAT continued to grow. This original work was performed in C57BL/6J mice, however given the metabolic differences among strains it cannot be ruled out strain specific peculiarities in their order/hierarchy of adipose depots expansion. The exhaustion of the ability of gVAT to expand coincided with increased adipocytes death and AT inflammation i.e. augmented formation of crown-like structures (Strissel et al. 2007). Another study in mice has suggested that visceral fat mass increases predominantly through adipocyte hypertrophy, whereas hyperplasia is predominantly observed in SAT (Joe et al. 2009). The lower differentiation potential of VAT progenitors and the fact that VAT adipocytes are more prone to cell death than in SAT, could explain why VAT enlarges preferentially though hypertrophy, while SAT expands through hyperplasia facilitated by greater precursors number and/or activity (Tchoukalova et al. 2010). This finding reinforces the concept that the abundance of adipocytes/precursors in SAT is a key determinant of SAT expandability and functionality.

The identification of MSCs and pre-adipocytes with proliferative and adipogenic potential in human adult WAT is relatively recent. For instance, ^{14}C birth-dating experiments indicate that the amount of WAT adipocytes increases during childhood and adolescence, while the number stays relatively stable in adulthood, independently of BMI. However, in human obesity there is now evidence for precursor proliferation (Spalding et al. 2008). In fact the number of adipocytes is greater in obese *vs.* lean subjects, even following severe weight loss, suggesting that the increase in adipocytes generation in obesity has lifelong effects on AT homeostasis and mass. Moreover, a reduction in self-renewing precursors division, which happens primarily in SAT and compromises its expansion suggests that metabolic complications such as insulin resistance ensues primary following the impairment of SAT function (Kim et al. 2014). Oppositely, a study carried out in obese human subjects showed that augmented VAT mass also involves an increase in adipocytes number rather than only adipocyte size (Arner et al. 2013). Two lineage tracing studies performed independently also supported these findings, demonstrating greater hyperplasic capacity in VAT with respect SAT particularly at the onset of obesity when insulin resistance is less evident (Jeffery et al. 2015; Wang et al. 2013).

Using the 'AdipoChaser' mouse model, Wang et al. observed that during the first month of an HFD challenge, the initial major contributor to AT expansion is hypertrophy. However, in case of a prolonged exposure to HFD (i.e. more than 1 month), a wave of adipogenesis is initiated preferentially in gonadal AT, whereas only a negligible effect is detected in SAT (Fig. 7.2b). Similarly, taking advantage of their adiponectin-CreER; mT/mG mouse model to identify newly formed adipocytes, Jeffery et al. observed increased generation of adipocytes exclusively in VAT after 8 weeks of HFD. According to BrdU analysis, this was accompanied by an increase in proliferation of precursors after the first week on HFD in VAT, but not in SAT. Taken together, these results sug-

gest that despite characteristically increased cellular proliferation in SAT under physiological conditions, in the context of obesity, adipogenesis may occur predominantly in VAT in the context of a failing SC depot. Of note, Hoffstedt et al., demonstrated that in morbidly obese woman, large fat cells in their visceral region are linked to dyslipidaemia, correlating quite significantly with plasma levels of total cholesterol, LDL, LDL cholesterol, TG and apolipoprotein B. Conversely, they found that large subcutaneous adipocytes are important for insulin and glucose abnormalities, considering that MO woman with hypertrophic SAT display higher HOMA index, plasma insulin and glucose levels as well as lower insulin-induced glucose disposal (Hoffstedt et al. 2010). The same group showed that SAT morphology can predict improvements in insulin sensitivity after both short term/moderate and long term/pronounced weight reduction in obese subjects. Moreover the subjects displaying SAT hypertrophy before weight loss show greater improvement in insulin sensitivity with respect to weight-matched individuals with hyperplasia (Eriksson-Hogling et al. 2015).

4.2 Brown Adipose Tissue Plasticity

The brown adipose tissue also expands during cold exposure and in response to "cafeteria" diet feeding (Bukowiecki et al. 1982). In murine models, upon 7 days cold exposure, BAT increases in mass through hyperplasia, while the fat mass of subcutaneous and epididymal WAT transiently decreases as a reflection of increased lipolysis and lipid supply to BAT. In these experiments, the cold exposure was associated with an increase in the phosphorylation of mitogen-activated protein kinase (p42/p44) Erk1 and Erk2, which protects brown adipocytes from apoptosis and promotes cell survival (Jia et al. 2016; Lindquist and Rehnmark 1998). Similarly, Rodriguez et al., demonstrated that 3 months feeding with a "cafeteria diet" induced brown adipose tissue hypertrophy increasing its thermogenic capacity in male rats, whereas female BAT was hypertrophied, but did not show signs of increased thermogenesis. This indicates that in female, BAT was already activated with respect of males, suggesting a gender dimorphism with respect to BAT activation (Roca et al. 1999; Rodriguez et al. 2001).

4.3 Where Do the Newly Formed Adipocytes Come from in Obesity?

Whereas Increased proliferation of total SVF cells in WAT after long-term HFD feeding has been reported in a few studies (Hudak et al. 2014; Joe et al. 2009), Jeffery et al. focused on the events occurring in a short term nutritional challenge. They observed that in mice fed a HFD for 8 weeks, new adipocytes were formed in VAT, accompanied by the activation and proliferation of $Lin^{-}Sca1^{+}CD29^{+}CD34^{+}$ ($CD24^{+}$ and $CD24^{-}$) adipose progenitors through the PI3K–Akt2 dependent pathway (Jeffery et al. 2015). This study confirmed that new adipocytes generation starts shortly after the beginning of HFD feeding (i.e. 1 week) and that this event occurs before the production of signals derived from hypertrophic adipocytes reflecting exhausted storage capacity, at least in VAT. Interestingly, while Akt2 is required for HFD induced adipogenesis, Akt2 is not required during the development of WAT, suggesting that there are distinct molecular mechanisms involved in obesogenic *vs.* developmental adipogenesis (Jeffery et al. 2015). Another study by Joe et al. showed augmented frequency of $BrdU^{+}$ Lin^{-} $Sca1^{+}CD34^{+}$ in HFD vs. chow challenged mice in SAT. In contrast only a minor increasing trend in VAT was observed (Joe et al. 2009).

Although it is generally assumed that newly formed adipocytes originate from resident preadipocyte or mesenchymal progenitors, Crossno et al. found that a subset of *bone-marrow-derived* circulating progenitors can also contribute to adipogenesis in WAT. They detected a small population (2–7%) of GFP^{+} adipocytes arising from the transplantation of GFP^{+} bone-marrow-derived cells into mice (Crossno et al. 2006). Furthermore, this adipogenic contribution was increased up to 8–25% by PPARγ agonist treatment or HFD

challenge. A recent study including 65 human subjects that underwent allogeneic bone marrow or peripheral blood stem cell transplantation showed that ~10% bone marrow derived cells contributed to SAT adipocyte count, a contribution that increased up to 2.5-fold in obese individuals. These numbers were determined by taking advantage of genomic differences inherent to donor and recipient cells and performing both bulk and single-cell analyses (Ryden et al. 2015). Moreover, Vishvanath et al. investigated mural cell lineage contribution to newly formed adipocytes in obesity. Using their "MuralChaser" mouse model, they uncovered adipose perivascular cells as developmental precursors of adipocytes formed in obesity, with adipogenesis and progenitor abundance regulated in a depot dependent manner (Vishvanath et al. 2016).

4.4 Developmental Gene Expression Is Associated with Pathological Metabolic Traits in Obesity

Combined gene expression analysis of adipocyte- and preadipocyte-containing fractions from intraabdominal and subcutaneous AT of mice, demonstrated coordinated depot-specific differences in the expression of several genes involved in *embryonic development* and pattern specification. Similar depot-specific differences in the expression of developmental genes are also observed in human SAT *vs*. VAT. This suggests that genetically programmed developmental differences in adipocytes and precursors characteristic of specific depots, may contribute to obesity, body fat distribution and define unique depot-specific functional roles (Gesta et al. 2006). The potential relevance of developmental genes in depot specific functional differences was highlighted by Brune et al., by showing that the expression of the developmental genes HOXC9 and HOXC10 is significantly higher in subcutaneous *vs*. omental human AT and correlates with body fat mass, even after adjustment for age and gender (Brune et al. 2016).

5 Regulation of Adipogenesis in Pathological Conditions

5.1 Impaired Preadipocyte Adipogenic Capacity in Obesity

Adipocyte differentiation is a complex process involving coordinated changes in gene expression, cell morphology and hormone sensitivity (Rangwala and Lazar 2000) (Fig. 7.3a). In the metabolically compromised obese state, the expression of adipogenic genes (i.e. *PPARγ2*, *FABP4*, *SREBP1c*, *FAS*) is decreased and this is associated with a hypertrophic AT pattern, increased ectopic fat deposition, insulin resistance and increased risk of developing type 2 diabetes (Dubois et al. 2006). Several studies have reported that obesity is associated with a reduction in the proportion of subcutaneous SVF cells that are committed pre-adipocytes (Permana et al. 2004; Tchoukalova et al. 2007). This may reflect exhaustion secondary to over utilisation of these precursors or an inappropriately low starting number of precursors. Interestingly, independently of the BMI, having a low SAT preadipocyte differentiation capacity is strongly associated with increased visceral obesity, visceral adipocyte hypertrophy and dysmetabolic state (Lessard et al. 2014). Moreover, SAT pre-adipocytes isolated from obese individuals show increased expression of MAP4K4, a kinase which is induced by TNFα and that inhibits PPARγ activation and consequently adipogenesis (Isakson et al. 2009). Chronic ER stress is another feature typically found in obese and insulin resistant subjects (Boden et al. 2008; Sharma et al. 2008). Recently, Koc et al., demonstrated that ER stress decreased the capacity of mature adipocytes to store lipids and diminished the adipogenic potential of preadipocytes. This suggests that exacerbated chronic ER stress typically observed in obesity could impair the differentiation of newly recruited preadipocytes and constrain the renewal of adipose tissue (Koc et al. 2015).

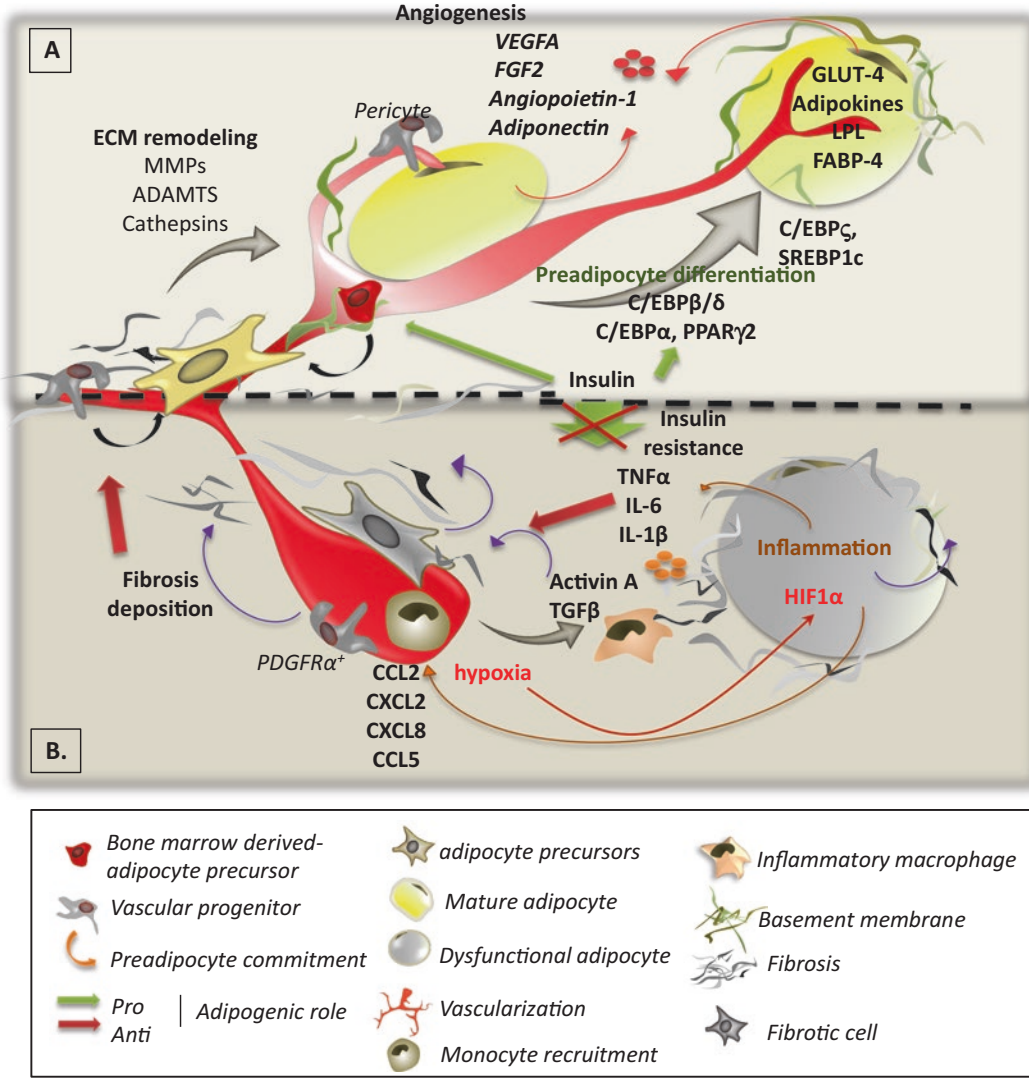

Fig. 7.3 White adipose tissue adipogenesis: role of angiogenesis, ECM, ECM remodelling and obesity. (**a**) In physiological condition adipogenesis consists in a two-step process that involves adipocyte progenitors (derived from vascular or bone marrow cells), commitment to preadipocytes (black arrows) which then differentiate into mature adipocytes, a process that involves the activation of several transcription factors, C/EBPβ and C/EBPδ at first during mitotic clonal expansion of preadipocytes and subsequently induction of C/EBPα and PPARγ2, which maintains the terminal differentiation of the adipocyte. Finally, SREBP1 is considered as the third key transcription factor for adipogenesis, which induces expression of adipocyte-specific genes such as FABP4, adiponectin, GLUT4, and lipoprotein lipase (LPL). Remodelling of the vasculature (angiogenesis) and of the extracellular matrix (ECM) are crucial for adipose tissue development. Preadipocytes are surrounded by a fibrous ECM enriched in collagen I, II and fibronectin replaced by the basement membrane surrounding mature adipocytes and composed of collagen IV and XVIII, entactin, and laminin. ECM remodelling during adipogenesis involves degradation of preadipocyte ECM by proteases (MMPs, ADAMT, and cathepsins). This liberates growth factors and matricellular proteins that are important for the synthesis of basement membrane. Angiogenesis is driven by angiogenic factors such as VEGF-A and FGF-2 (pink arrows) that are produced by (pre)adipocytes and vascular cells. The new vessel is stabilized by the production of basement membrane, and the recruitment of pericytes. (**b**) In obese WAT, adipogenesis is impaired in association with altered remodelling of the ECM and the vasculature. Hypertrophic and necrotic adipocytes, in association with hypoxia, secrete cytokines and chemokines that result in the accumulation of immune cells and tissue inflammation.

5.2 Vascular and ECM Remodelling During Expansion of Adipose Tissue

To ensure the development and expansion of AT, appropriate vascularisation is required. Concomitantly to the progression of adipogenesis, angiogenic factors, such as FGF-2, VEGF and HGF are secreted, mainly by adipose tissue progenitors, inducing a robust proportional angiogenic response. AT expansion requires the interaction between endothelial cells (ECs) and pre-adipocytes guiding cell migration via FGF- and VEGF-dependent pathways (Fig. 7.3a). The newly formed vasculature is stabilised by the production of ECM and the recruitment of pericytes (Cao 2013). Overexpression of a dominant-negative form of PPARγ or the blockade of VEGFR2 signalling impairs adipogenesis, AT expansion and angiogenesis. Conversely, activation of pro-adipogenic factors i.e. PPARγ promotes angiogenesis, EC motility and boosts the expression of VEGF, VEGF-B and angiopoietin-like factor-4 (Gealekman et al. 2008). Moreover, BMPs promote endothelial specification and subsequent venous differentiation during embryonic development (Dyer et al. 2014).

Commitment of mesenchymal precursors to different lineages is regulated by many cues in the local tissue microenvironment. This involves remodelling of both, actin cytoskeleton (Mcbeath et al. 2004) and ECM, which is constituted by a complex macromolecular network composed of structural proteins such as collagen and elastin, adhesion proteins and proteoglycans. In addition to mechanic support, ECM is also required for morphogenesis, differentiation and tissue homeostasis (Shoham and Gefen 2012) (Fig. 7.3a). ECM remodelling requires the action of enzymes such as metalloproteases (MMPs), which catalyse collagen degradation. Whereas MMP 9/10/12 do not affect adipogenesis, single allele depletion of MMP14/2 does. Moreover, knockout mice for *Mmp3/11/19*, fed a HFD shows hypertrophy of AT (Bauters et al. 2013; Mariman and Wang 2010). Several growth/angiogenic factors such as VEGF are known to be sequestered in the ECM and thus MMPs can control pre-adipocyte differentiation and microvessel maturation by regulating degradation of the ECM. TIMP-4, a natural inhibitor of MMPs, is predominantly and selectively expressed in WAT and inhibits adipogenesis by interacting with cell adhesion molecules or growth factor receptors, regulating PI-3 K, FAK, ERK signalling pathways (Mejia-Cristobal et al. 2015) and activating the NFκB cascade.

Insulin is a pro-adipogenic factor (Mariman and Wang 2010) and also contributes to ECM turnover through regulation of the expression of enzymes involved in the post-translational modification of some proteoglycans such as sulfatase-2. Moreover, insulin also acts at a post-transcriptional level to increase production of the mature form of type I collagen, collagen V fragment and C-terminal peptides of type I, II and III collagen (Wang et al. 2006). Insulin also increases the expression of prolyl-4-hydroxylase, which is involved in collagen stabilisation. Finally, *COL6A2* and *TSP1* have been identified as PPARγ target genes. Of interest, the ECM composition and its remodelling during adipogenesis differ among fat depots. For instance, expression levels of collagen IV and fibronectin are greater in VAT than in SAT, while in contrast, SAT is highly enriched for type I collagen (Mori et al. 2014).

Fig. 7.3 (continued) Cytokines (TNFα, IL-6, and IL-1β) and chemokines (CCL2, CXCL2, CXCL8, and CCL5) activate endothelial cells and promote recruitment and survival of immune cells (monocytes, mast cells, neutrophils, and lymphocytes) (orange arrows). Monocytes differentiate within WAT into inflammatory macrophages. Inflammation likely precedes fibrosis, as macrophages accumulate in obese WAT prior to fibrosis, where they promote inflammation as well as ECM synthesis via TGF-β and activin A signalling. Adipocyte progenitor (PDGFRα$^+$) and preadipocytes are proposed as the major effectors of fibrosis (purple arrows). Fibrosis impairs adipogenesis and negatively impacts adipocyte metabolism by decreasing metabolic functions such as lipolysis and adipokine secretion and by inducing an inflammatory response

5.3 Fibro-Inflammation Impairs of Adipogenesis

The inhibitory effect of inflammation on adipogenesis has been widely investigated (Gustafson et al. 2015). It is known that in obesity, infiltration and accumulation of macrophages and other immune cells in WAT results in chronic production of inflammatory factors (Fig. 7.3b). Macrophage cellular plasticity covers a spectrum of phenotypes defined by the expression of specific markers/inflammatory signatures. Predominantly, inflammatory macrophages (M1) express inflammatory cytokines (e.g. TNFα, IL-6, IL-1β) and differ from non-inflammatory macrophages (M2). Among M2 cells, some macrophages are specialised in tissue repair and collagen production (M2c) (Dalmas et al. 2011; Spencer et al. 2010). As revealed by bone marrow transplant experiments in mice, accumulation of "M1-like" macrophages in adipose tissue from obese subjects results from recruitment of blood monocytes that differentiate into macrophages within adipose parenchyma (Ghanim et al. 2004; Weisberg et al. 2003). These pro-inflammatory macrophages impair adipogenesis in WAT (Lacasa et al. 2007) of obese mice (Roberts-Toler et al. 2015; Sakamoto et al. 2013). Inflammatory factors such as TNFα produced by macrophages may also inhibit brown adipogenesis (Sakamoto et al. 2013; Valladares et al. 2001). Similarly, immune cells can impair brite/beige adipogenesis (Esteve et al. 2015). An enrichment of MSCA1+ cells has been reported in the population of native CD45−/CD34+/CD31− precursors in SAT of obese subjects. MSCA1 activity promotes triglyceride accumulation and the expression of white/brite-related genes. Local inflammation associated with obesity, in this case in SAT, may contribute to metabolic disorders through impairment of white/brite adipogenesis potential of these cells.

The first wave of macrophage accumulation in WAT at early stages of obesity represents a physiological adaptation, which is essential for healthy tissue expansion and remodelling (Wernstedt Asterholm et al. 2014). However, when adipose tissue expands and the inflammatory insult is sustained, these macrophages are more prone to become inflammatory and impact negatively on ECM homeostasis, promoting fibrosis deposition, which further impairs AT expansion and adipogenesis. The upregulation of ECM components, typically observed in AT of obese individuals and mouse models of genetic-/diet-induced obesity, is associated with metabolic dysregulation coupled to insulin resistance and liver damage (Abdennour et al. 2014; Sun et al. 2013). Several of these pro-fibrotic factors have also been reported to impair human pre-adipocyte differentiation. For instance, TGF-β and activin A are induced in obesity and negatively regulate adipogenesis (Lessard et al. 2015).

PDGFRα$^+$ adipocyte precursors are also reported to induce WAT fibrosis (Abdennour et al. 2014). PDGF is an important pro-fibrotic signal that binds the receptor tyrosine kinases PDGFRα and PDGFRβ (Iwayama et al. 2015). A WAT lineage tracing experiments using a Nestin-Cre system to label pericytes and adventicial cells revealed that despite little contribution of Nestin-Cre/Tomato$^+$ cells to WAT development in young mice, there was a significant increase in recruitment of PDGFRα$^+$ cells upon HFD challenge (Abdennour et al. 2014).

Moreover, when cultured in vitro these cells were able to differentiate into adipocytes (Abdennour et al. 2014). Further investigation is required to assess the importance of PDGFRα signalling in obese WAT fibrosis, however it is tempting to speculate that PDGFRα activation could cause cell-autonomous fibrosis by perturbation of progenitor function.

5.4 Fibrosis Negatively Impact on AT Expandability and Function

By causing ECM to become stiffer, fibrosis has a negative impact on WAT by physically limiting its expandability capacity (Abdennour et al. 2014). In obese mouse models, the onset of WAT fibrosis precedes the development of other metabolic complications such as liver steatohepatitis (Strissel et al. 2007). Several studies, focusing on mouse models depleted for ECM components,

support this hypothesis. Genetic ablation of collagens or remodelling enzymes profoundly affects adipocyte expansion and leads to improvement of whole body metabolic status. For instance, diet- and genetic-induced obese mouse models of collagen VI deficiency show increased expandability of individual adipocytes in their WAT, due to the absence of fibrotic deposits. Paradoxically, despite having larger adipocytes, they also display an improved inflammatory profile (Khan et al. 2009) and are protected from metabolic complications. This suggests that in addition to impairing adipocyte expansion, fibrosis might also impair adipocyte functionality (Abdennour et al. 2014; Pellegrinelli et al. 2014a). The same is observed in humans, where increased type VI collagen deposits are present in SAT of obese individuals in parallel with insulin resistance (Marin et al. 1992; Misra and Vikram 2003; Pasarica et al. 2009; Spencer et al. 2010).

A deleterious effect of accumulation of pericellular fibrosis in SAT is supported by recent evidence showing that if the fibrotic process is limited to VAT of obese mice, while SAT is unaffected and can grow normally, the metabolic status of the animals improves. In particular, HFD-fed mice with *Irf5* deletion in macrophages display no difference in the growth of their VAT, but increased expansion of SAT compared to wt mice. The VAT of the KO mice show accumulation of non-inflammatory macrophages (which are involved in ECM remodelling) leading to fibrosis deposition. However, in this case these changes were associated with improved insulin sensitivity both in VAT and SAT of Irf5 deficient mice (Dalmas et al. 2011).

Given that both mass and activity of BAT decrease with excess nutrients and fibro-inflammation, the presence of a fibrotic and therefore not functional BAT may exacerbate the development of obesity and associated metabolic dysfunction. To this end, recent evidence suggests that inflammation and fibrosis impact negatively on BAT function highlighting the role of some molecular candidates involved in vasculature (e.g. VEGF) and ECM turnover, such as TGF-β, endotrophin and MAGP1 (Bagchi et al. 2013; Craft et al. 2014; Sun et al. 2014).

6 Adipose Tissue Reactive Lipid Species Profile in Obesity

Deleterious metabolic consequences due to lipotoxic conditions are not only determined by the amount of fat stored but also by the quality of lipid species accumulated in a tissue. The deposition of determined bioactive lipid species such as ceramides (Cer), glycosphingolipids and DAGs has been reported to contribute to adipose tissue dysfunction in the context of obesity and associated comorbidities (Carobbio et al. 2011).

6.1 Ceramides

While the lipotoxic effect of specific species of ceramides in liver and muscle is well determined (Chavez and Summers 2012), the role of specific lipotoxic species in adipose tissue dysfunction in obesity state was less well defined until recently. Ceramides levels increase in adipose tissue of mice on HFD, concomitantly with the development of insulin resistance (Shah et al. 2008; Turner et al. 2013). For instance, the Gosejacob et al., reported that in a murine model of ceramide synthase 5 (CerS5) depletion, a reduction in the level of endogenous $C_{16:0}$-ceramide led to decreased weight gain, improved glucose homeostasis and WAT inflammatory state (Gosejacob et al. 2016).Moreover, Vandanmagsar et al., showed that increased Cer levels activate Nlrp3 inflammosome and adipose tissue macrophages in obesity (Vandanmagsar et al. 2011). Conversely, the adipose-tissue specific overexpression of acid ceramidase, the enzyme responsible for the degradation of ceramides, induced a reduction in ceramides levels in the different AT depots and an improvement of the glucose metabolism of the transgenic mice, when fed a HFD (Xia et al. 2015).

6.2 Ceramides Profile and Effect in Different AT Depots

Several studies reported an increase in ceramides/per adipocyte levels in SAT of obese men

and woman *vs*. lean healthy controls (Blachnio-Zabielska et al. 2012). Similar results were obtained in diet-induced obese mouse models (Shah et al. 2008). Moreover, the contents of specific subtype of sphingolipids i.e. SPA, S1P and ceramides i.e. C14-Cer, C16-Cer, C18:1-Cer,C18-Cer and C24:1-Cer in the subcutaneous adipose tissue were greater in obese diabetic subjects as compared to their lean non-diabetic counterparts. In the obese non-diabetic group the content of SPA, C14-Cer, C24:1-Cer C18:1-Cer and C24-Cer was higher than in the lean non-diabetic group. Moreover, content of S1P, C16-Cer, C18:1-Cer and C18-Cer was markedly higher in the obese diabetic group compared to the obese non-diabetic group. A positive correlation between total ceramides and HOMA-IR in SAT and in particular between C16-Cer and HOMA-IR was observed (Blachnio-Zabielska et al. 2012). In vitro studies also revealed that ceramides impair insulin stimulated GLUT4 expression and glucose uptake in brown adipocytes (Long and Pekala 1996). Data from brown adipocytes suggest that the de novo ceramides biosynthesis mediates the effect of TNF-α on insulin action in these cells (Aerts et al. 2007).

6.3 Dihydroceramides

Whereas the lipotoxic effect of ceramides accumulation in obesity has been widely recognised, the deleterious outcome of increased levels of dihydroceramides (DhCer), the ceramide precursors, has only recently been investigated. Dihydroceramides have been previously considered inactive lipid species (Ahn and Schroeder 2010). However recent studies (Kraveka et al. 2007; Siddique et al. 2013) highlighted their relevance as modulators of cell cycle, apoptosis, autophagy or oxidative stress, all of them processes that compromise AT development and function. In this line, Barbarroja et al., demonstrated both in vitro and in vivo that defects in *DEGS1*, desaturase regulating the dehydroceramides/ceramides ratio, in adipocytes, lead to an accumulation of DhCer in adipocytes that compromises AT function and expansion by inhibiting adipogenesis, promoting cell death and oxidative stress. The same study also showed decreased expression of *DEGS1* expression in adipose tissue of diet- and genetic-induced obese mouse models and in morbidly obese humans (Barbarroja et al. 2015). Other reports indicate that DhCer rather than Cer amounts correlate positively with BMI and waist circumference in cohorts of overweight individuals (Mamtani et al. 2014; Weir et al. 2013). Altogether, these results indicate an association between DEGS1 function and fat mass.

6.4 Glycosphingolipids

Glycosphingolipids such as gangliosides are metabolites derived from ceramides generated through an enzymatic reaction catalysed by the enzyme glucosylceramide synthase (Chaurasia and Summers 2015). Tagami et al., demonstrated that the insulin resistance induced in adipocytes after treatment with TNFα was accompanied by an increase in GM3 synthase expression associated with increased GM3 ganglioside levels. Of note, pharmacological depletion of GM3 in adipocytes prevented the TNFα-induced defect in insulin-dependent tyrosine phosphorylation of IRS-1 and counteracted the TNFα-induced defect in insulin-dependent serine phosphorylation of IRS-1, restoring insulin-sensitive glucose transport. Moreover, treatment of fat cells with exogenous GM3 had an effect on insulin signalling similar to TNFα. Moreover, in AT of rodent models of obesity i.e. Zucker fa/fa rats and ob/ob mice, the levels of GM3 synthase were significantly higher with respect to the lean controls (Tagami et al. 2002). In line with that, Wentworth et al. reported an increase in the amount of the ceramide derivatives G_{M3} gangliosides in visceral adipose tissue of obese IR woman, which is secondary to inflammation and it is suggested to contribute to insulin resistance (Wentworth et al. 2016). Mice lacking GM3 ganglioside show enhanced insulin sensitivity (Yamashita et al. 2003). Again, in vivo pharmacological inhibition of glucosylceramide synthase in ob/ob and high

fat diet fed mice, led to a decrease in glucosylceramide levels in different tissues, in circulating glucose and improved oral glucose tolerance and insulin sensitivity (Aerts et al. 2007; Van Eijk et al. 2009).

6.5 Diacylglycerols (DAGs)

While the implication of diacylglycerol in the pathogenesis of lipotoxicity and insulin resistance in peripheral organs such as muscle and liver is well determined (Amati 2012), less is known about the lipotoxic effects of DAGs in adipose tissue itself in obese states. Blachnio-Zabielska carried out the analysis of DAG species present in subcutaneous fat tissue of obese diabetic, obese non-diabetic and lean subjects. They were able to show that the content of C18:1/C18:2, C16:0/18:2, C16:0/16:0, C18:0/18:1, C16:0/18:1 ($p < 0.01$) and C18:1/18:1 increased in the obese diabetic group as compared to lean subjects. The ratios of C16:0/18:2, C18:1/18:0, C16:0/18:1 and C18:1/18:1 were higher in the obese non-diabetic as compared to the lean non-diabetic group. There were also differences in C16:0/16:0 and C18:1/18:2 content between both obese groups. The higher content of the compounds was noticed in the obese diabetic group. As expected, total DAG content increased in both obese groups as compared to the lean control group. This was the first time that a comprehensive profile of DAG species in adipose tissue in obese state has been described (Blachnio-Zabielska et al. 2012). The determination of specific relevance of the different DAG subtypes with respect to adipose tissue dysfunction need further investigation.

6.6 SAT and VAT FA Desaturation and Elongation in Obesity

Obesity is also associated with other qualitative alterations in lipid composition, which seems to be adipose tissue depot specific. We have shown that adipose tissue fatty acids chain length and mono-unsaturation increase with obesity and insulin resistance. In particular, mouse models with increasing obesity and insulin resistance show augmented Δ9 desaturated FAs (SCD1 ratio) and FAs with 18-carbons (Elovl6 ratio) in subcutaneous WAT. Conversely, data from mouse models discordant for obesity and insulin resistance i.e. AKT2 KO and Adiponectin ob/ob mice, suggested that Elovl6 ratio in SAT was associated with insulin sensitivity, whereas SCD1 ratio correlated with fat mass. In morbidly obese humans, a greater Elovl6 and SCD1 ratio was found in VAT compared to SAT (Yew Tan et al. 2015). Moreover, by profiling specific lipid species in AT of mouse models of genetic-induced obesity and insulin resistance, we showed an increase in unsaturation levels and SCD1 activity specifically in the membrane PL fraction (Carobbio et al. 2013a). This result strengthens our observation in weight-discordant monozygotic twin pairs showing that the obese sibling displayed reduced specific saturated FAs and augmented proportions of specific unsaturated FAs, such as palmitoleate, alongside an increase in indexes reflecting augmented activity of desaturation enzymes and FA chain length, in membrane PL (Pietilainen et al. 2011). These changes in lipid composition seem to contribute to the maintenance of adipocyte plasma membrane homeostasis and biophysical properties in the context of adipose tissue expansion.

Garaulet et al., also reported that in overweight and obese human subjects, AT content in n-3 and n-6 fatty acids is proportional to a reduction in adipocyte size and specific depot localization. In contrast, adipose tissue and dietary SFAs significantly correlated with an increase in fat cell size and number. No significant associations were found between n-9 acids content and adipocyte size. However, n-9 adipose tissue fatty acids content was inversely associated with fat cells number suggesting that this type of fatty acid could limit hyperplasia in obese populations. All in all, these data indicate that depending on their specific characteristics with respect of adipocytes size, number and anatomical location, different adipose tissue depots display unique depot-specific FAs composition (Garaulet et al. 2006).

7 Therapeutic Antilipotoxic Strategies Aiming at Increasing/Restoring WAT Expandability and BAT Activity/WAT Browning

When considering the specific characteristics of the different adipose tissue depots, we suggest the following strategies of therapeutic value to ameliorate/counteract the pathological effects of obesity and associated metabolic complications. Because visceral adiposity clearly represents a significant risk factor for the development of the MetS, improving the storage capacity of SAT to accumulate excess of nutrients and simultaneously limiting the storage capacity of VAT may help to decrease the deleterious effect of lipid overload on AT and whole body homeostasis (Despres and Lemieux 2006). This could be achieved by targeting the AT fibro-inflammatory environment or by increasing the recruitment capability of WAT precursors and, as a consequence, WAT's expandability potential (Fig. 7.4).

Another approach is targeting the thermogenic capacity of AT. An increase in BAT mass/activity and SAT browning/beige adipocyte recruitment is expected to maximise the energy-dissipating potential of thermogenic brown and beige fat (Fig. 7.4). In our opinion, it will be only possible to accomplish that with a better understanding of

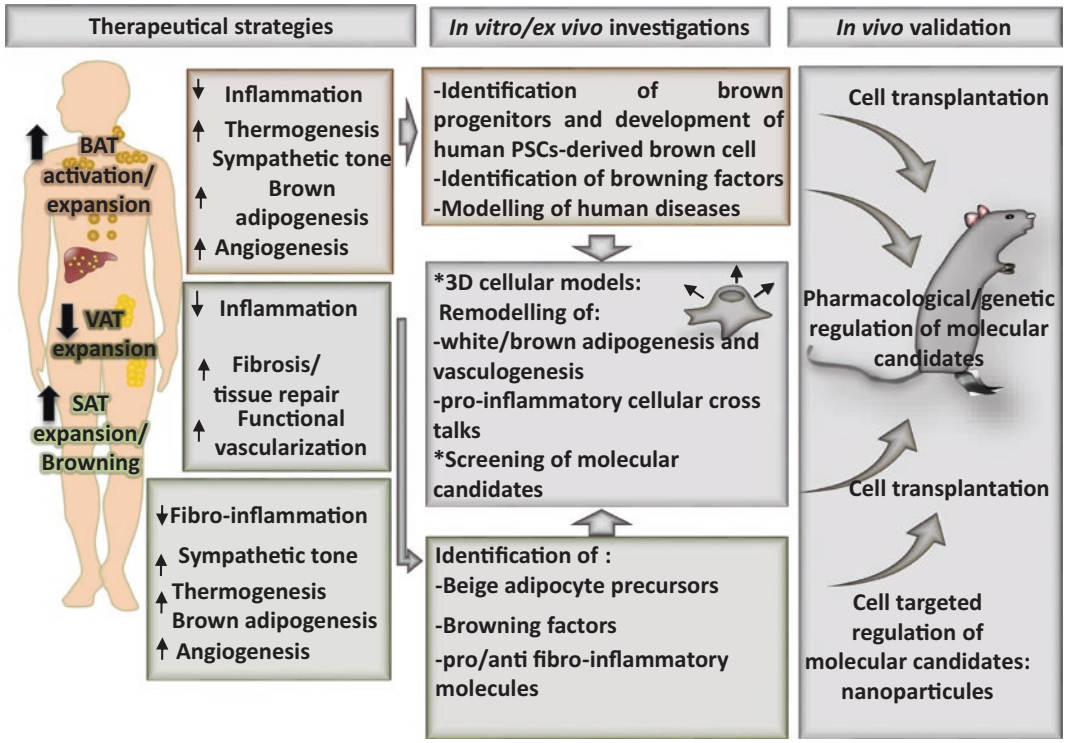

Fig. 7.4 Potential therapeutic strategies targeting WAT/BAT tissue plasticity/remodelling and response to sympathetic tone, to promote (1) healthy SAT expansion and browning, (2) limited VAT expansion and lipotoxic action and (3) BAT activation and recruitment of APs. Sophisticated technologies including innovative in vitro models of human stem cell derived adipocytes and three-dimensional culture both aiming to mimic pathophysiological features of WAT/BAT adipogenesis/cellular cross-talks, could be of great help in the identification of new molecular and cellular candidates. Using a range of new/innovative technologies/tools allowing a more targeted and translational experimentation, these candidates could be then validates in vivo, representing new potential targets to prevent obesity related disorders

its developmental/adult origins, as well as molecular and physiological features and plasticity, of adipocytes forming different AT depots in humans during the onset of obesity and related metabolic complications. This will provide a rationale for translational strategies to improve WAT expandability/functionality and brown/beige cell recruitment and activation, moving from rodent models to a clinical context and later on to a successful outcome.

8 Innovative Techniques/Models to Address Challenges in the Study of Obesity and Associated Metabolic Dysfunction

Genetically modified and nutritionally challenged animal models as well as in vitro cellular models i.e. primary cultures or immortalised cell lines have been classical research models that have provided a great service to metabolic research. However, the complexity and heterogeneity of the adipose tissue, its likely interspecies peculiarities, and its increasingly complex cross talk with other organs indicates that more sophisticated technologies are required to investigate obesity and related pathophysiological consequences. During the last decade a range of new/innovative technologies/tools have been developed that allow more targeted and translational experimentation.

8.1 3D Culture Systems

Mammalian cell culture has served as an invaluable tool in cell biology for several decades. Monolayers of adherent cells grown on flat and rigid 2D substrates, have become the principal and conventional cell culture systems. 2D cell culture studies have played a pivotal role in promoting and deepening our understanding in developmental biology, tissue morphogenesis, disease mechanisms, and many more. Simultaneously, however, a multitude of inadequacies associated with 2D culture systems have also emerged, especially with respect to the inability of these systems to emulate in vivo conditions and their limited physiological relevance. As highlighted in this chapter, in the body, nearly all cells in tissues reside in ECM in a complex 3D architecture and interact with neighbouring cells through biochemical and mechanical cues. Cell-cell and cell-ECM interactions establish a 3D communication network that maintains the specificity and homeostasis of the tissue (Kleinman et al. 2003). 3D culture systems are now being used more frequently to investigate adipose tissue homeostasis and lipotoxic effects (Fig. 7.4). Using a 3D hydrogel system Pellegrinelli et al., investigated the contribution of VAT-endothelial cells (ECs) to adipocyte dysfunction related to inflammation. The 3D setting employed in this study allowed the maintenance of unilocular mature adipocyte function. Co-culture experiments demonstrated that VAT-ECs provoked a decrease in lipolytic activity, adipokines secretion and insulin sensitivity of adipocytes from obese subjects as well as increased production of inflammatory molecules (Pellegrinelli et al. 2014b). The same group used the same 3D culture approach to study the interaction between adipocytes and decellularised material isolated from AT of obese subjects (dMAT) (Pellegrinelli et al. 2014a) and VAT adipocyte from obese patients and human myotubes (Pellegrinelli et al. 2015). In all cases, they were able to recapitulate the situation occurring in vivo in obese subjects. Differently, Choi et al., tested the effect of hyperinsulinemia on the lipolytic function of a 3D co-culture of adipocytes and endothelial cells embedded in a porous silk fibroin scaffold and found that high concentration of insulin promoted lipid accumulation and decreased lipolysis *vs.* normal insulin conditions. Conversely adipocyte monolayers did not exhibit any response. Thus the ability of a 3D system to elicit physiological responses to hyperinsulinemia in co-culture demonstrate that the 3D culture system represents the future for AT engineering and investigation (Choi et al. 2010).

Unser et al. developed a "Brown-Fat-in-Microstrands" 3D system by microfluidic synthesis of alginate hydrogel Microstrands that encapsulated cells and induced direct differentia-

tion of mouse ES cells into brown adipocytes. The cells within the micro strands responded to β-adrenergic stimulation, indicating their functionality. The successful generation of "Brown-Fat-in-Microstrands" from mouse pluripotent stem cells opens up the possibility of applying the technology to human PSCs creating a model recapitulating the native tissue both biochemically and biomechanically and allowing the study of human BAT adipogenesis and its implication in obesity and metabolic disorders (Unser et al. 2016).

8.2 Human PSC and Disease Models

The previously described human adult and foetal derived BAT and WAT cell culture systems have limited proliferative capacity, heterogeneous and progressively impaired differentiation and cannot be used to reliably produce large numbers of pure brown and white adipocytes. To surmount these issues, several groups have utilized in vitro differentiation of human PSCs to generate models of human brown and white adipogenesis (Fig. 7.4). These pluripotent stem cells present distinct advantages for in vitro modelling of adipocyte function and dysfunction: they can self-renew and thus represent a virtually inexhaustible supply of starting material; it is possible to derive patient specific disease models of known genotypes with iPS reprogramming technology; pluripotent cells can be simultaneously differentiated into several metabolically relevant lineages (e.g. brown and white adipocytes, hepatocytes, skeletal muscle, neurons); they can be genetically engineered routinely to introduce various types of mutations or make reporter cell lines. These applications have benefited from a growing practical knowledge of both the in vitro differentiation and genome engineering of pluripotent human stem cells.

8.3 White and Brown Adipocytes Cellular Models Differentiated from Human PSCs

In the past few years an increasing number of groups has managed to differentiate human PSCs into white and brown adipocytes with different degrees of success and using different protocols. There are two main approaches: (a) genetic manipulation i.e. overexpression/down regulation of key factors shown to respectively stimulate or inhibit the differentiation, following embryoid bodies (EB) formation and (b) treatment of the cells with combination of small molecules identified as promoters of differentiation. A combination of both approaches can also be used. Taura et al., managed to generate white adipocyte from PSCs by applying retinoic acid between day 2 and 5 of EBs formation. Once replated, the EBs started to form outgrowths of different cell types and when adipogenic induction was used the authors observed the formation of white adipocytes, although at low efficiency (Taura et al. 2009). After engraftment in nude mice, these adipocytes were shown to be able to survive and function (Noguchi et al. 2013). A few years earlier, Xiong et al., used a similar protocol i.e. in this case, treating the EBs with rosiglitazone during day 2–6 of their formation and were able to induce white adipocyte differentiation from EBs outgrowth (Xiong et al. 2005). Ahfeldt et al. (2012) developed a different methodology i.e. a transgene expression based approach to obtain highly differentiated and functional brown and white adipocytes from human PSCs. After generation of MPCs by EBs formation, they forced the expression adipogenic transcription factors via inducible Tet system lentiviral constructs to induce adipocyte terminal differentiation. Expression of PPARγ2 alone results in very efficient conversion (>85%) to white adipocytes while the combination of PPARγ2 with C/EBPβ and PRDM16 (non-essential) results in homogeneous and efficient brown adipocyte differentiation. Both cell types were fully functional in vitro and the programmed brown adipocytes showed full functionality in vivo when transplanted into genetically immunocompromised mice (Ahfeldt et al. 2012). Moreover, Moshen-Kanson et al., were able to form brown like adipocyte from human PSCs-derived white adipocyte by ectopically overexpressing PAX3 (Mohsen-Kanson et al. 2014).

Nishio et al. (2012) developed an alternative approach to reprogram human PSCs into func-

tional brown adipocytes both in vitro and in vivo with an efficiency of >90% by utilizing a combination of small molecules. They employed a haemopoietic cocktail (HC) composed of KIT ligand (KITGL), fms-related tyrosine kinase 3 ligand (FLT3LG), interleukin 6 (IL-6) and VEGF along with the previously reported BAT inducer Bmp7 (Tseng et al. 2008). The differentiated BAT cells exclusively contained multilocular lipid droplets and qPCR confirmed the induction of BAT-specific genes such as UCP1 and PRDM16. The functionality of the cells was assessed by measuring their respiration, oxygen consumption rates and glycerol release when challenged with βadrenergic stimuli. Transplantation of the reprogrammed brown adipocytes in mice improved fasting TG, blood glucose levels and glucose tolerance *vs*. mice transplanted with immature PSCs, proving that these in vitro matured cells can have therapeutic effects in vivo. These studies are ground breaking for opening a new approach for studying brown and white adipocyte formation and function.

8.4 Assessment of Cellular Phenotypes and Development of Screening Platform using Adipocytes Differentiated from Engineered Human PSCs

The human PSC derived adipocyte can also be used to assess and study cellular phenotype caused by new identified pathological mutations in genes believed to play a role in adipose tissue homeostasis or other key metabolic tissues (Fig. 7.4). For instance, an allelic serie of isogenic PS cell lines for the AKT2 gene which included the wt gene, a null mutation and a dominant patient derived point mutation was constructed in human PSCs by Ding et al. A single $AKT2^{E17K}$ allele mutation revealed opposing phenotypic effects on glucose uptake, triglyceride content and adipokine secretion in white adipocytes, as well as having effects on glucose production in hepatocytes. Investigations of the same kind, have been carried out for mutation affecting PLIN1 and SORT1 genes which also influence WAT function. Similar studies in PSCs derived brown adipocytes should become a powerful method for both interrogating the function of various metabolically relevant genes and testing the effects of putative human mutations on BAT function in a highly sensitive and well controlled cellular system (Ding et al. 2013). The same group used these human PSC derived adipocytes as a screening platform to identify small molecules able to promote white to brown metabolic conversion in human adipocyte. In particular they identified two inhibitors of JAK activity with no precedent in adipose tissue biology that stably conferred brown metabolic activity to white adipocyte (Moisan et al. 2015).

In summary, we think that the availability of human PSCs as tool to investigate and elucidate molecular mechanisms related to human pathologies such as obesity and metabolic comorbidities, has been demonstrated to be very useful and innovative. This recent research approach allows the identification in vitro of potential targets that can then be studied in vivo. This approach contributes quite substantially to the implementation of the principles of the 3R i.e. the refinement of the experiments and the replacement and reduction of the use of animal models.

8.5 Nanoparticles as a System for Target Delivery of Treatments to Adipose Tissue

Until recently to treat the lipotoxic and deleterious metabolic effects of obesity, the main approaches considered have been the use of nutritional and caloric restriction strategies as well as unspecific delivery methods (i.e. orally or by injection) of drugs/treatments. However, nowadays, more and more sophisticated technologies have been employed to test the possibility for cell-type specific delivery of drugs and other small molecules to counteract obesity and associated metabolic dysfunction in order to provide a safer and more efficient method of treatment. For instance, Di Mascolo et al., used 200 nm PLGA/PVA nanospheres formulated for the systemic delivery of rosiglitazone specifi-

cally to macrophages to modulate inflammation, in order to avoid the well-known side effects of this drug when administered orally (Di Mascolo et al. 2013) (Fig. 7.4). Similar specific targeting of inflammatory macrophage can be obtained using hybrid lipid–latex (LiLa) nanoparticles bearing phagocytic signals (Bagalkot et al. 2015). Moreover, Rocca et al., since overweight correlated with increased oxidative stress, investigated the antioxidant effects of cerium oxide nanoparticles (nanoceria) as a potential pharmaceutical approach for the treatment of obesity, which is correlated to increased oxidative stress. Nanoceria were tested both in vitro and in vivo; they were proven to decrease the expression of genes involved in adipogenesis, and hindered the triglycerides accumulation in 3T3-L1 pre-adipocytes. In vivo, when injected intraperitonally in Wistar rats, nanoceria did not show appreciable toxic effects but, instead contributed efficiently to reducing the weight gain and in lowering the plasma levels of insulin, leptin, glucose and triglycerides (Rocca et al. 2015).

Short chain fatty acids have been linked to a reduction in appetite, body weight and adiposity. Acetate is the main SCFA found in the circulation and is therefore the prime candidate to induce significant metabolic modulation in peripheral tissues. In order to assess the peripheral action of acetate, Sahuri-Arisoylu et al., utilized a novel nanoparticle delivery method, whereby acetate is passively targeted to the periphery. Using this method, they investigated the effects of acetate on liver lipid accumulation, inflammation and mitochondrial metabolism. Their findings suggest that the positive effects of acetate on liver lipid accumulation are a result of mitochondrial modifications in both the liver and SAT, inducing browning of SAT and an overall improvement in metabolism and body composition, without changes in calorie intake or physical activity (Sahuri-Arisoylu et al. 2016). In summary, the challenge of specifically targeting the adipose tissue poses important technological challenges but we feel this challenge may be overcome in part by new nanoparticle targeted delivery systems.

9 Conclusions

The concepts of defective adipose tissue expandability/functionality and lipotoxicity represent in our opinion a valid intellectual framework to understand how obesity is linked to metabolic complications. Furthermore, this concept provides a rationale for the use of adipose tissue based therapeutic strategies to uncouple obesity from its metabolic complications as a palliative strategy, at least until the issue of the control of body weight is resolved satisfactorily. Given that the metabolic complications of obesity typically affect very diverse organs and cell types, each with their own signalling and metabolic particularities, most current therapeutic approaches include measures to resolve each one of the specific problems identified in the context of the MetS. However, we feel that targeting the adipose tissue to improve its storage capacity and functionality and/or promoting lipid oxidation through brown fat expansion and activation, may represent a suitable strategy to ameliorate multiple manifestations of the MetS by reversing lipid induced metabolic stress.

To improve the function of the white adipose tissue it is essential to recognise the heterogeneous origin and functional characteristics of the anatomical depots as well as their contribution to metabolic disease. Similarly, the knowledge about human brown and beige cells is in its infancy in part due to the scarcity and availability of cellular models for these studies. However, in order to progress with the development of therapeutic approaches to take advantage of its antilipotoxic properties it would be necessary to elucidate human mechanisms of BAT development and activation. To this end, the availability of human PSC-derived unlimited biological material will be essential.

A remaining issue to address is the specific contribution of lipotoxicity at the level of adipose tissue to its own dysfunction. This is an area which has been eclipsed by the predominant focus on lipotoxicity in other target organs such as liver and muscle. However, improvement of adipose tissue functionality may require antilipotoxic measures directed specifically towards

WAT. We believe that therapeutic strategies targeting directly adipose tissues and promoting antilipotoxic effects in situ, by specialised delivery systems, may provide a global solution to the metabolic syndrome, besides other effects that could emerge from improvement in its endocrine functions.

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What Is Lipotoxicity?

8

Ayse Basak Engin

Abstract

Enlarged fat cells in obese adipose tissue diminish capacity to store fat and are resistant to the anti-lipolytic effect of insulin. Insulin resistance (IR)-associated S-nitrosylation of insulin-signaling proteins increases in obesity. In accordance with the inhibition of insulin-mediated anti-lipolytic action, plasma free fatty acid (FFA) levels increase. Additionally, endoplasmic reticulum stress stimuli induce lipolysis by activating cyclic adenosine monophosphate/Protein kinase A (cAMP/PKA) and extracellular signal-regulated kinase ½ (ERK1/2) signaling in adipocytes. Failure of packaging of excess lipid into lipid droplets causes chronic elevation of circulating fatty acids, which can reach to toxic levels within non-adipose tissues. Deleterious effects of lipid accumulation in non-adipose tissues are known as lipotoxicity. In fact, triglycerides may also serve a storage function for long-chain non-esterified fatty acids and their products such as ceramides and diacylglycerols (DAGs). Thus, excess DAG, ceramide and saturated fatty acids in obesity can induce chronic inflammation and have harmful effect on multiple organs and systems. In this context, chronic adipose tissue inflammation, mitochondrial dysfunction and IR have been discussed within the scope of lipotoxicity.

Keywords

Obesity • Lipotoxicity • Lipolysis • Free fatty acid (FFA) • Fatty acyl-coenzyme A (FA-CoA) • Diacylglycerol (DAG) • Ceramide • Perilipin • Triglyceride • Fatty acid translocase (FAT)/CD36 • Long-chain fatty acid (LCFA) • Plasma membrane-associated fatty acid binding protein (FABPpm) • Triacylglycerol • Insulin resistance (IR) • Mitochondrial dysfunction • Lipid droplets • Reactive oxygen species (ROS)

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1 Introduction

Obesity is not only associated with lipid accumulation in adipose tissue, but also in non-adipose tissues. Deleterious effects of lipid accumulation in non-adipose tissues are known as lipotoxicity (van Herpen and Schrauwen-Hinderling 2008). In addition to reducing fatty acid clearance from circulation, enlarged adipose tissue mass releases more free fatty acid (FFA) to circulation increase the eventual plasma FFA levels in obesity. Moreover, once plasma FFA levels are elevated, anti-lipolytic action of insulin is inhibited. In this manner the rate of FFA release into the circulation will further increase (Boden 2008; Jensen et al. 1989). The normal plasma FFA concentration is in the range of 350–500 μM. However, the majority of plasma FFAs is bound to albumin. In human skeletal muscle, value of FFA is between 1–10 nmol/g wet muscle tissues. Whole body insulin sensitivity displays a strong negative correlation with muscle fatty acyl-coenzyme A (FA-CoA) content. Exceeding the amount of FA-CoA over 2 μM is associated with a marked reduction in whole body insulin sensitivity (An et al. 2004). Elevated muscle FA-CoA interferes with mitochondrial adenosine triphosphate (ATP) synthesis by inhibiting the electron transport chain and decreasing the inner mitochondrial membrane potential. In this case reduction of FA-CoA oxidation leads to a rise in muscle FA-CoA concentration and exacerbates the mitochondrial dysfunction (Abdul-Ghani et al. 2008). Gradual increases in saturated FFAs diminish insulin-induced glycogen synthesis, glucose oxidation and lactate production. In this case decreases in both mitochondrial hyperpolarization and ATP generation impair mitochondrial functions (Hirabara et al. 2010). Although circulating fatty acids are important for the physiological regulation of a number of processes, high levels of fatty acids are deleterious (Unger 2002). Obesity causes a chronic elevation of circulating fatty acids that can reach to toxic levels within non-adipose tissues. Fatty acids may enter deleterious pathways subsequent to over-accumulation of lipids in non-adipose tissues during overnutrition (Unger 2002). When saturated long-chain fatty acids are in excess, metabolic flux favors synthesis of complex lipids like ceramides and cholesterol esters. Accumulation of these substances results in adverse effects of lipotoxicity such as endoplasmic reticulum stress, inflammation, and insulin resistance (IR) (Summers 2006; van Herpen and Schrauwen-Hinderling 2008). Atherosclerosis, cardiomyopathy retinopathy, nephropathy, neuropathy and endothelial dysfunction are considered as consequences of the elevated circulating fatty acids that reach to toxic levels (Symons and Abel 2013). In this chapter, common mechanisms of obesity-related lipid accumulation in non-adipose tissues have been discussed within the scope of lipotoxicity (Ye 2013).

2 Lipolysis

Obesity is characterized by excessive adipose tissue deposition and increased FFA release that exceeds metabolic demands (Koutsari and Jensen 2006). When the excess lipids are driven into alternative non-oxidative pathways, the storage capacity of adipose tissue has been overcome. A lipid "spill over" may occur from adipose to non-adipose tissues due to over-accumulation of unoxidized long-chain fatty acids (Kusminski et al. 2009). Actually adipose lipolysis is an important process that controls circulating FFA concentrations. Triacylglycerol hydrolysis in adipocytes produces glycerol and FFAs (Londos et al. 1999a). According to the barrier/translocation hypothesis, perilipin family protein constitutes a physical barrier to hormone-sensitive lipase (HSL). Upon perilipin phosphorylation and downregulation, lipid surface accessibility for HSL is enhanced (Londos et al. 1999b). In adipocytes, this is achieved by sequential action of adipose triglyceride lipase (ATGL), HSL, and monoglyceride lipase (Nielsen et al. 2014). Lipolysis is stimulated by various hormones and effectors. In this event, the lipid droplet-associating proteins or perilipin family proteins are polyphosphorylated by protein kinase A (PKA) and phosphorylation is necessary for translocation of HSL to the lipid droplet and enhanced lipoly-

sis (Tansey et al. 2004). In fact PKA-dependent perilipin phosphorylation facilitates perilipin interaction with lipid droplet-associated HSL (Miyoshi et al. 2006). Dexamethasone induces phosphorylation and down-regulation of perilipin that modulates lipolysis. Additionally dexamethasone up-regulates mRNA and protein levels of HSL and adipose triglyceride lipase; these effects are in parallel to increased lipolysis (Xu et al. 2009). A decreased catecholamine response affects alpha- and beta-adrenoceptor sensitivity in adipose tissue, reducing lipolysis and increasing fat stores in obesity (Zouhal et al. 2013). Thus catabolic hormone, adrenaline translocates both phospholipase C-related catalytically inactive protein (PRIP) and its binding partner protein phosphatase 1 and protein phosphatase 2A (PP2A) from the cytosol to lipid droplets. PRIP promotes the translocation of phosphatases to lipid droplets to trigger the dephosphorylation of HSL and perilipin, thus reducing PKA-mediated lipolysis (Okumura et al. 2014). Neuropeptide Y (NPY) promotes proliferation of adipocyte precursor cells and contributes to the pathogenesis of obesity. Although NPY have no effect on basal lipolysis, it potentiates a beta-adrenergic receptor agonist, isoproterenol stimulated lipolysis (Li et al. 2012). TNF-alpha increases lipolysis in differentiated human adipocytes by activation of mitogen-activated protein kinase kinase (also known as MEK, MAPKK), extracellular signal-related kinase (ERK), and elevation of intracellular cyclic adenosine monophosphate (cAMP) approximately 1.7-fold. TNF-alpha induces perilipin hyperphosphorylation by activating PKA (Zhang et al. 2002). Furthermore, TNF-alpha activates the three mammalian mitogen-activated protein kinase (MAPK), p44/42, c-Jun NH2-terminal kinase (JNK), and p38 but only p44/42 and JNK involve in the regulation of lipolysis (Ryden et al. 2002). As mentioned above, cAMP/PKA along with extracellular signal-regulated kinase-1/2 (ERK1/2) is the major early lipolytic signal. Endoplasmic reticulum stress stimuli induce lipolysis by activating cAMP/PKA and ERK1/2 signaling in adipocytes. This lipolytic activation is probably an adaptive response that regulates energy homeostasis. Because of the persistent acceleration of FFA efflux from adipocytes to the bloodstream and various tissues, endoplasmic reticulum stress impairs insulin sensitivity by contributing to lipotoxicity (Deng et al. 2012). Triacylglycerol-rich lipid droplets of adipocytes provide a major energy storage depot for the body. These lipid droplets are coated with one or more of five members of the perilipin family of proteins: adipophilin, perilipin-3 (formerly called TIP47), OXPAT/myocardial lipid droplet protein (MLDP), S3–12, and perilipin. They prevent triglyceride hydrolysis by lipases. Perilipin is the most abundant protein on the surfaces of lipid droplets, and the major substrate for cAMP-dependent protein kinase. In times of energy deficit, perilipin is phosphorylated by PKA and facilitates maximal lipolysis by HSL and adipose triglyceride lipase (Brasaemle 2007). Endoplasmic reticulum stress did not alter the level of perilipin proteins but increased the phosphorylation (Deng et al. 2012). The formation and elevation of reactive lipid moieties and FFA in non-adipose tissues promote cellular dysfunction (lipotoxicity) and programmed cell-death (lipoapoptosis) (Kusminski et al. 2009) (Fig. 8.1).

3 Fatty acid Transport

Cellular long-chain fatty acid (LCFA) uptake involves both passive diffusion and protein-mediated transport which includes fatty acid translocase (FAT)/CD36, plasma membrane-associated fatty acid binding protein (FABPpm), and fatty acid transport protein (FATP). Furthermore, it appears that FAT/CD36, as a key fatty acid transporter, regulates the LCFA utilization in heart and skeletal muscle both under normal conditions as well as in obesity and IR (Koonen et al. 2005). Increased cytosolic FAT/CD36 expression also results in a concomitant increase in mitochondrial FAT/CD36 content in muscle cells. Thus translocation of FAT/CD36 to the mitochondria enhances the mitochondrial fatty acid oxidation capacity. This means that FAT/CD36 influences both LCFA transport across the plasma membrane and also LCFA

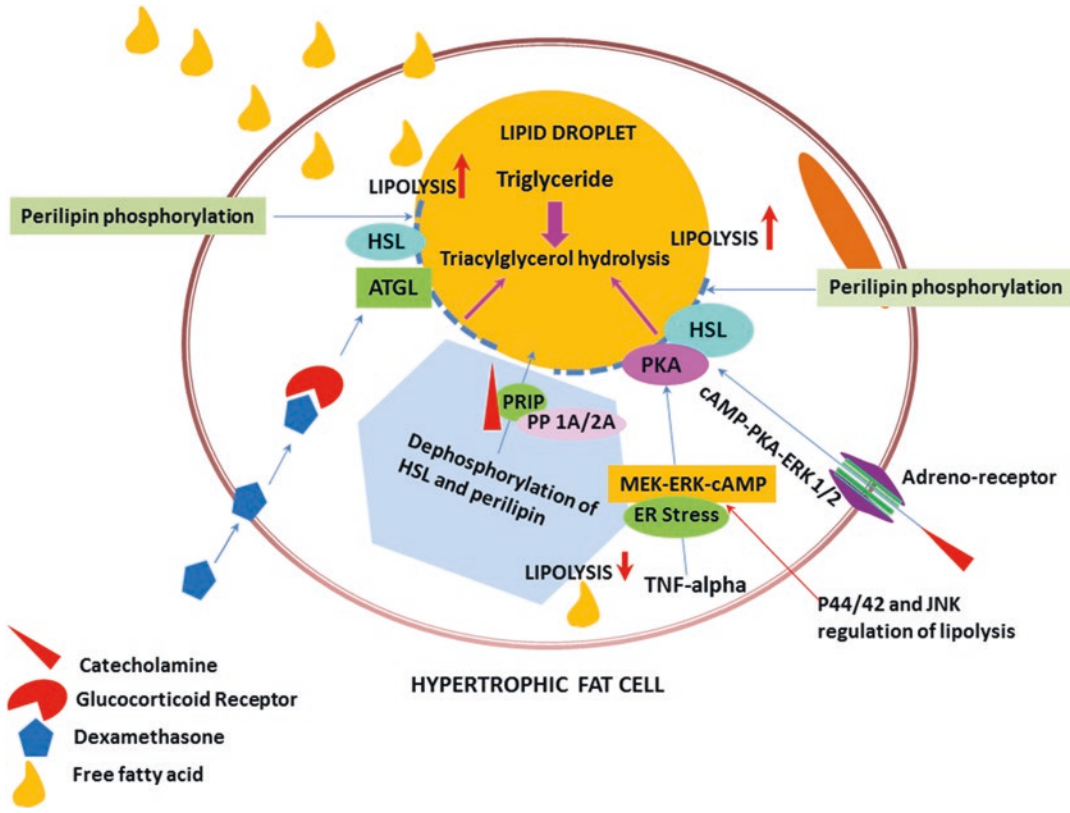

Fig. 8.1 Schematic description of basic steps of lipolysis. Lipolysis is stimulated by various hormones and effectors. The lipid droplet-associating perilipin family proteins are polyphosphorylated by protein kinase A. Phosphorylation is necessary for translocation of hormone-sensitive lipase to the lipid droplet and enhances lipolysis (*HSL* Hormone-sensitive lipase; *ATGL* Adipose triglyceride lipase; *PKA* Protein kinase A; *PRIP* Phospholipase C-related catalytically inactive protein; *PP1A/2A* Protein phosphatase 1A and protein phosphatase 2A; *JNK* c-Jun N-terminal kinase; *MEK* Mitogen-activated protein kinase kinase; *ERK ½* Extracellular signal-related kinase; *cAMP* 3',5'-cyclic adenosine monophosphate; *TNF-alpha* Tumor necrosis factor-alpha; *ER* Endoplasmic reticulum)

transport into mitochondria (Campbell et al. 2004). When saturated and unsaturated LCFAs are presented to the mitochondria for beta-oxidation and to the endoplasmic reticulum for lipid synthesis, only LCFA are used in the synthesis of ceramide. Increase in this metabolite causes lipotoxicity (Brookheart et al. 2009; Listenberger et al. 2001). Another putative fatty acid transporter, FABPpm overexpression increases the rates of LCFA transport across the sarcolemma. This effect is independent of any changes in FAT/CD36. Nevertheless, the overexpression of FABPpm alone is not sufficient to induce a parallel increment in palmitate transport and metabolism (Clarke et al. 2004). While FABPpm contributes to increasing sarcolemmal LCFA transport, it can not participate in the LCFA transport into the mitochondria (Holloway et al. 2007). On the other hand, insulin regulates protein expression of FAT/CD36, but not FABPpm, via the phosphatidylinositol 3-kinase (PI3K)/Akt (protein kinase B) insulin-signaling pathway. Eventually the insulin-induced increase in FAT/CD36 protein in a time- and dose-dependent manner results in an increased rate of LCFA transport. Meanwhile, an increase in plasmalemmal FAT/CD36 occurs (Chabowski et al. 2004). However, high levels of saturated FFA-

induced mitochondrial dysfunction are associated with disruption of PI3K/Akt insulin-signaling pathway (Hirabara et al. 2010). Both FAT and FABPpm are present on the muscle membrane and are important in regulating the uptake of fatty acids into skeletal muscle. In fact, the LCFA transport proteins are also located in several subcellular domains (Bonen et al. 2000). The peroxisome proliferator-activated receptor-gamma (PPAR-gamma) regulates the adipogenesis and insulin responsiveness. FABP4 triggers the proteasomal degradation of PPAR-gamma. Therefore, higher FABP4 in human visceral fat is an important factor in the development of obesity-related morbidities (Garin-Shkolnik et al. 2014). High saturated fatty acids and trans fatty acids intake favors a proinflammatory status that contributes to development of IR. While excess fatty acids induce hepatic IR, they also impair insulin clearance in obese non-diabetic humans (Carpentier et al. 2000). Therefore, IR and oxidative stress play an important role in development and progression of nonalcoholic fatty liver disease (Angulo 2007). FATPs are a family of six integral membrane proteins with an extracellular/luminal N-terminal and C-terminal domain with fatty acyl-CoA synthetase activity and therefore FATP proteins have the ability to trap fatty acids intracellularly (Watkins 2008).

Fatty acids may exert their effects directly by binding to cell surface receptors or to intracellular transcription factors. Monounsaturated fatty acids are more potent PPAR ligands than saturated fatty acids. Monounsaturated fatty acids activate the nuclear transcription factors PPAR-alpha and PPAR-gamma which respectively promote lipid detoxification via fatty acid oxidation and safe fatty acid storage into triglycerides (Nolan and Larter 2009). Channeling of non-esterified free fatty acids (NEFA) towards storage in the form of neutral lipids in lipid droplets protects the cell against palmitate-induced endoplasmic reticulum stress (Bosma et al. 2014). PPAR-gamma senses incoming non-esterified LCFAs and induces the pathways to store LCFAs as triglycerides (Nakamura et al. 2014). Triglycerides serve a storage function for long-chain NEFA and their products such as ceramides and DAGs. Their toxicity is originated from failure of esterification or breakdown of the triglycerides (Listenberger et al. 2003). PPAR-gamma expression is increased in humans with metabolic syndrome. Enhanced expression of several PPAR-regulated genes mediates fatty acid uptake/oxidation and triacylglycerol synthesis. Fatty acid oxidation and triacylglycerol droplet size are increased (Son et al. 2010).

Obesity-related reduction in skeletal muscle fatty acid oxidation is attributable to the reduced mitochondrial content of fatty acids, not to mitochondrial dysfunction. Thus, the mitochondrial FAT/CD36 content of lean muscle is not different than that of the obese muscle. Eventually FAT/CD36 significantly predicts the ability of mitochondria to oxidize fatty acids, independent of body mass index (BMI) status (Holloway et al. 2009). However, trafficking of fatty acid transporters between the intracellular compartments and the plasma membrane is altered in insulin-resistant skeletal muscle. In obesity FAT/CD36 permanently relocates to plasma membrane, hereby contributes to IR by increasing influx of fatty acids into muscle cells (Chabowski et al. 2007). Insulin-resistant individuals have a reduced rate of fat oxidation compared with insulin-sensitive individuals. Decreased mitochondrial fat oxidative capacity leads to an increase in intracellular fat content (Kelley and Simoneau 1994).

In physiological conditions, non-adipose tissues contain very few triglycerides. Ectopic lipid accumulation may occur in the setting of high concentration of serum triglycerides and long-chain NEFAs which are also referred to as FFAs. Reasonably, ectopic fatty acid accumulation results from disequilibrium between FFAs uptake from the environment and consumption through the mitochondrial oxidation. In this situation, the more specific disturbance due to lipid overload in non-adipose tissues is DAG and/or Acyl-CoA-related interference with insulin signaling appear. (Schaffer 2003; van Herpen and Schrauwen-Hinderling 2008). Actually intracellular utilization of LCFAs is subdivided into three steps; initial uptake across the plasma membrane, activation by esterification with coenzyme A, and

subsequent metabolism. Long chain acyl-CoA synthetases (ACSLs) not only activate fatty acids for intracellular metabolism but are also involved in the regulation of uptake. Multiple different long chain ACSLs are expressed simultaneously in the same cell type but differ in their subcellular localization. ACSLs localize to either the endoplasmic reticulum or to mitochondria and can regulate the extent of fatty acid uptake (Digel et al. 2009).

Palmitic acid mainly occurs as its ester in triglycerides and it is most common fatty acids found in meats, cheeses, butter, and dairy products. According to the World Health Organization, evidence is convincing that consumption of palmitic acid increases risk of developing cardiovascular diseases, placing it in the same evidence category as trans fatty acids ("WHO_TRS_916.pdf" n.d.). Palmitate-induced inhibition of carnitine palmitoyltransferase I, subsequent accumulation of ceramide, and inhibition of electron transport complex III cause cytochrome c release and apoptosis (Sparagna et al. 2001). The most important biological function of L-carnitine is to transport fatty acids into the mitochondria. However, carnitine insufficiency impairs entry of fatty acids into the mitochondria and consequently disturbs lipid oxidation (Reuter and Evans 2012). It is proposed that carnitine transport system at the contact sites between the outer and inner mitochondrial membranes comprises the long-chain acyl-CoA synthase and porin components. The mitochondrial carnitine system is also necessary in beta-oxidation of LCFAs. The malonyl-CoA sensitive carnitine palmitoyltransferase I/malonyl-CoA system regulates fatty acid oxidation depending on the tissue's energy demand (Kerner and Hoppel 2000). Furthermore, Listenberger et al. proposed that saturated fatty acid, palmitate-induced apoptosis occurs through the generation of reactive oxygen species (ROS) (Listenberger et al. 2001). Thus, palmitate overload rapidly increases the saturation of phosphatidylcholine (PC) and triacylglycerol in endoplasmic reticulum membranes. Markedly dilated rough endoplasmic reticulum due to increased accumulation of PC and TAG are associated with oxidative stress. These alterations initiate the flux of calcium from the endoplasmic reticulum stores to the mitochondria. The loss of mitochondrial membrane potential ultimately leads to palmitate-induced cell death (Borradaile et al. 2006). During over-nutrition, higher plasma leptin concentration stimulates phosphorylation of signal transducer and activator of transcription 3 (STAT3) in Janus kinase (JAK)/STAT pathway. Subsequently phosphorylated STAT3 enters the nucleus and regulates transcriptional activity of its target genes including peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1alpha), carnitine palmitoyltransferase I (CPT-1) and acyl-CoA oxidase (ACO). While PGC-1alpha involves in mitochondrial biogenesis, CPT-1 and ACO are responsible for the fatty acid oxidation. However, STAT3 reduces the expression of lipogenic enzymes; acetyl coenzyme A (CoA) carboxylase (ACC) and fatty acid synthase (FAS) (Unger 2003a). The adenosine monophosphate (AMP)-activated protein kinase (AMPK) is stimulated by ATP depletion and it blocks energy consuming processes. Moreover, higher plasma leptin selectively stimulates phosphorylation and activation of the alpha2 catalytic subunit of AMPK. Simultaneously leptin suppresses the activity of ACC, thereby stimulates the oxidation of fatty acid (Minokoshi et al. 2002). Obesity is associated with hypoadiponectinemia. Adiponectin regulates fatty acid utilization via AMPK-dependent mechanisms that enhance mitochondrial fatty acylCoA import (generation of malonyl-CoA by ACC). Through induction of CD36 translocation, fatty acid uptake increases. Eventually, adiponectin accelerates complete oxidation of fatty acids. Thereupon, accumulation of toxic lipid intermediates is prevented in a large scale (Fang et al. 2010).

ACC synthesizes malonyl-CoA (MCA) which is an inhibitor of fatty acid oxidation in mitochondria. Phosphorylation or inactivation of ACC controls MCA activity (Winder and Hardie 1996). MCA is a powerful inhibitor of CPT-1-mediated fatty acid oxidation (McGarry 2002). Lack of the leptin effect increases the ACC activity and produces more MCA. Virtually more fatty acid and triglycerides are synthesized however, they are less oxidized (Unger 2003a). Leptin

infusion markedly decreases the expression of key enzymes of the de novo fatty acid synthesis such as ACC, fatty acid synthase, and stearoyl-CoA desaturase-1. In this case, leptin treatment regulates adipose triglyceride lipase/hormone sensitive lipase/diacylglycerol (DAG) transferase 1 expression and alters fatty acid-triacylglyceride cycling in adipose tissue. Consequently, lipolysis and fatty acid oxidation are increased (Gallardo et al. 2007). In obesity-prone animals, carnitine palmitoyltransferase 1B expression is lower in intra-abdominal fat, however stearoyl-CoA desaturase 1 expression is higher in subcutaneous fat. Increased fat accumulation in obesity may be caused by impaired oxidative capacity due to decreased carnitine palmitoyltransferase 1B levels in the white adipose tissue (Ratner et al. 2015). Overexpression of human carnitine palmitoyltransferase-1A significantly reduces the content of intracellular NEFAs and attenuates fatty acid-evoked IR and inflammation via suppression of JNK. These changes in enzyme levels are accompanied by an increase in fatty acid uptake and a decrease in fatty acid release (Gao et al. 2011). However Schenk and Horowitz suggested that the increased rate of whole body fatty acid oxidation is correlated with an increase in the CD36-associated carnitine palmitoyltransferase-1, but not with carnitine palmitoyltransferase-1 alone (Schenk and Horowitz 2006).

4 Lipotoxicity and Mitochondrial Dysfunction

The fatty acid-overload effects mitochondria in a three-step fashion. At first, the production of ROS occurs in the early phase of fatty acid accumulation. In the second step, increase in mitochondrial proton conductance or the uncoupling of the oxidative phosphorylation is evident. At the last step, fatty acids can provoke the permeabilization of the outer mitochondrial membrane (Listenberger and Schaffer 2002; Rial et al. 2010; Schönfeld and Wojtczak 2008). Once mitochondrial membrane permeabilization (MMP) has been induced, the release of catabolic hydrolases and activators from mitochondria is facilitated. These catabolic enzymes as well as the cessation of the bioenergetic and redox functions of mitochondria finally lead to cell death (Crompton 1999; Kroemer et al. 2007). Really, fatty acids cause oxidative stress and alterations in mitochondrial structure and function. Thus, the uncoupling of the oxidative phosphorylation is one of the most recognized deleterious effects of fatty acid. The fatty acid interaction with the carriers leads to membrane depolarization and/or the conversion of the carrier into a pore (Rial et al. 2010).

Long-chain saturated FFAs induce apoptosis in a dose-dependent manner. Both saturated and unsaturated exogenous long-chain FFAs are directed to the mitochondria for beta-oxidation and to the endoplasmic reticulum for complex lipid synthesis. However only long-chain saturated fatty acyl CoAs serve as substrates for de novo ceramide synthesis. Ceramide is a lipid second messenger involved in initiation of apoptosis (Brookheart et al. 2009). Excessive amount of lipid metabolites like DAG, ceramide and saturated fatty acids in obesity can induce chronic inflammation and have harmful effect on multiple organs and systems through driving these metabolites into alternative non-oxidative pathway. FFAs and their metabolites take part in the structure of membrane and intracellular signaling or ATP generation. However excessive fat accumulation in non-adipose tissues, including the pancreas, heart, liver, kidney and blood vessel wall results in mitochondrial dysfunction (Brookheart et al. 2009).

Fatty acids that are taken up into heart and skeletal muscle are primarily oxidized or stored as triacylglycerols. As long as the fatty acid uptake and metabolism remains appropriately balanced, metabolic dysregulation does not occur. If there is a defect in mitochondrial oxidative phosphorylation, IR is associated with an increase in intramyocellular lipid content. This increase is attributed to the mitochondrial dysfunction (Möhlig et al. 2004). In this case, secondary to diminished fat oxidation, IR develops. The high rates of fatty acid catabolism in insulin-resistant muscles are attributed to incomplete fat oxidation, in which a large proportion of fatty

acids enter into the mitochondria but are only partially degraded. Unchanged rates of fatty acid oxidation can be accompanied by high rates of incomplete beta-oxidation, which encourages intramitochondrial accumulation of acyl-CoAs and leads to subsequent mitochondrial failure (Koves et al. 2008). Intracellular pH drops in fatty acid exposed cells. This decrease in intracellular pH accompanies with the fatty acids' transfer across fat cell membranes following induction of lipolysis. After un-ionized, hydrophobic fatty acids diffuses across the cell membrane, they are re-ionized (Civelek et al. 1996). Excess FFAs also increase DAG, which activates nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX2) through phosphokinase C (PKC)-dependent pathways (Inoguchi et al. 2000). In high fat-fed murine hearts, suppression of autophagosome clearance and the activation of NOX are observed simultaneously. Actually, autophagy is a crucial catabolic process involved in maintaining energy and organelle homeostasis through degradation and recycling of organelles such as mitochondria or endoplasmic reticulum. Palmitate-induced NOX2 activation is dependent on the activation of classical PKC. In this respect, diminished autophagic turnover is a novel mechanism linking lipotoxicity with a PKCbeta-NOX2-mediated impairment in pH-dependent lysosomal enzyme activity (Jaishy et al. 2015; Quan et al. 2012). NOX2-derived ROS may promote FFA-induced dysfunction of pancreatic beta-cell through JNK pathway (Yuan et al. 2010). Indeed, FFAs can increase the formation of autophagosomes. Actually inhibition of autophagic degradation is accompanied by induction of autophagosome formation (Las et al. 2011). Beta-cells exposed to fatty acids show accumulation of abnormal autophagosomes. In this case, suppression of lysosomal gene expression contributes to the impairment of autophagic turnover (Las and Shirihai 2010). Suppression of autophagy enhances fatty acid-induced apoptosis. While unsaturated fatty acid promotes the formation of triglyceride-enriched lipid droplets and induces autophagy, saturated fatty acid is poorly converted into triglyceride-enriched lipid droplets and induces lipoapoptosis (Mei et al. 2011). It has been confirmed that autophagy deficiency in beta-cells could be a factor in the progression from obesity to diabetes due to an inappropriate response to obesity-induced endoplasmic reticulum stress (Quan et al. 2013).

Accumulation of fatty acids in the peripheral tissues alters the composition of membrane phospholipids due to development of inflammation, oxidative stress, lipid peroxidation. The higher toxicity of saturated or trans fatty acids seems to be the consequence of a blockade in triglyceride synthesis (Zámbó et al. 2013). Inappropriate accumulation of excess lipid in non-adipose tissues is associated with a chronic inflammatory response which is characterized by abnormal cytokine production, increased acute-phase reactants, and activation of inflammatory signaling pathways (Wellen and Hotamisligil 2003). As a consequence, FFA-mediated lipotoxicity causes cellular stresses and inflammation by impairing normal cell signaling that may lead to apoptotic cell death (Unger 2003b). Thus increase in circulating levels of nutritional fatty acids in obesity activate toll-like receptor 4 (TLR4) signaling in adipocytes and macrophages and induce inflammatory signaling in adipose cells or other tissues and macrophages (Shi et al. 2006). TLR4 in macrophages and Kupffer cells participates in a sensing mechanism facilitating fatty acid-induced inflammation and IR (Diehl 2002). Excess lipid accumulation and abnormal energy metabolism lead to an overburdened endoplasmic reticulum. Increase in the synthetic activity of endoplasmic reticulum disrupts the normal folding of proteins and activates the unfolded protein response that is known to induce stress response pathways (de Luca and Olefsky 2008). Triggering of the endoplasmic reticulum stress by lipotoxic concentrations of saturated FFAs, subsequently leads to induction of the apoptotic transcription factor C/EBP-homologous protein (CHOP) and increases the percentage of apoptotic cells. Co-treatment with alpha-lipoic acid abolishes saturated FFA-induced lipoapoptosis by stimulating the nuclear translocation of nuclear factor (erythroid-derived 2)-like 2 (Nrf2). Eventually Nrf2 eliminates the FFA-induced oxidative stress by activating antioxidant enzymes (Valdecantos et al. 2015). The capacity of a cell to manage oxidative stress is primarily mediated through antioxidant respon-

sive elements (AREs), which are largely under the control of the transcription factor Nrf2 (Jaiswal 2004). Under the basal condition, Nrf2-dependent transcription is repressed by a negative regulator Keap1. When cells are exposed to oxidative stress, Nrf2 escapes Keap1-mediated repression and activates ARE-dependent gene expression (Zhang 2006). It is well-known that mitochondria are the major ROS generating sources. Fatty acids accumulating in the vicinity of mitochondria are vulnerable to ROS-induced lipid peroxidation. Later, these lipid peroxides could have lipotoxic effects on mitochondrial DNA, RNA and proteins of the mitochondrial machinery and provoke mitochondrial dysfunction (Abdul-Ghani et al. 2008). If fatty-acid supply exceeds the oxidation rate, fatty acyl CoA might accumulate in the mitochondria and limit further fatty acid beta oxidation because of lack of free CoA. Acyl CoA is hydrolyzed within the mitochondria to fatty acid and free CoA. The fatty-acid anions are exported to the cytosol by uncoupling protein (UCP)3, allowing rapid fatty acid beta oxidation when the increased formation of fatty acids are protonated and flipped into mitochondria. Eventually, fatty acids accumulate up to ten-fold because of the pH gradient. They cannot be metabolized because of the lack of matrix acyl CoA synthases and may be toxic. UCP3 could lower the matrix concentration more than 1000-fold by transporting the fatty-acid anions. UCP2 and UCP3 increase the proton conductance of the mitochondrial inner membrane, but only when they are activated by products of ROS metabolism (Brand and Esteves 2005). While basal proton-leak depends on the fatty-acyl composition of the mitochondrial inner membrane and the presence of adenine nucleotide translocase, inducible proton-leak is controlled with UCPs (Divakaruni and Brand 2011). Fatty acids induce a conformational change in UCP1. Furthermore, saturated fatty acids accelerate the rate of enzymatic proteolysis of UCP1. The altered kinetics of both processes indicate that fatty acids change the conformation of UCP1, reconciling the apparent discrepancy between existing functional and ligand binding (Divakaruni et al. 2012). Free fatty acids operate as natural ‘mild uncouplers’ and prevent the transmembrane electrochemical proton potential difference from being above a threshold critical for ROS formation by complex I (Korshunov et al. 1998). On the other hand, due to their protonophoric action on the inner mitochondrial membrane, “mild uncoupling effect”, FFA strongly decreases ROS generation in the reverse mode of electron transport (Schönfeld and Wojtczak 2008). Both ROS and glutathionylation activates and deactivates UCP3-dependent increases in non-phosphorylating respiration. Increased cellular ROS levels coincide with UCP3 deglutathionylation. UCP3 deglutathionylation activates UCP3-mediated uncoupling and further decreases ROS emission. In this case Cys(25) and Cys(259) are the major glutathionylation sites on UCP3 (Mailloux et al. 2011). Moreover, mitochondrial uncoupling proteins works as carriers of fatty acid peroxide anions. UCP3 translocates fatty acid peroxide anions, which accumulate on the matrix side of the mitochondrial inner membrane, to outer leaflet and protects the mitochondria from oxidative damage. The metabolic pattern of matrix is much more complicated and important for the cell than that of the intermembrane space. Therefore, UCP-mediated transfer of fatty acid peroxides from the inner to outer leaflet has favorable biological effects (Goglia and Skulachev 2003). As mentioned above, mitochondrial uncoupling proteins are important regulator of mitochondrial ROS production. Thus, UCP3 is activated by lipid peroxides. Nevertheless, it removes anions and/or peroxides from the mitochondrial matrix, thereby specifically protects fatty acids from ROS-induced oxidative damage (Hoeks et al. 2006). At the same time, UCP3 could have a detoxification role, removing lipid peroxides from the matrix side of the inner mitochondrial membrane (Goglia and Skulachev 2003).

5 Lipid Droplets and Mitochondria

Neutral lipids, including triacylglycerols and cholesterol esters are stored in lipid droplets which are ubiquitous organelles. Lipid droplets-binding protein, perilipin, plays a critical role in

determining the characteristics of lipid-droplets (Kuramoto et al. 2012). Lipotoxicity is not only dependent on the presence of fat in non-adipose tissues, but also due to maintaining lipid homeostasis and metabolism of lipid droplets. During fasting, adipose tissue lipolysis is necessary for energy demands of non-adipose tissues (Jacob 1987). In conditions of chronic excess fatty acids which are transiently packed into lipid droplets in the form of triacylglycerols or cholesterol esters (Wang and Sztalryd 2011). Lipid excess packaging into lipid droplets can be seen as an adaptive response to fulfilling energy supply without hindering mitochondrial or cellular redox status and keeping low concentration of lipotoxic intermediates (Aon et al. 2014). In fact, adipocyte lipid droplets may play an important role in lipid homeostasis by providing the transient storage of fatty acid in the form of TGs. Thus, the formation of toxic lipid intermediates and their cellular toxicity could be prevented. In this case perilipin coating the lipid-droplets surfaces have an important function for the regulation of lipid stores (Wang and Sztalryd 2011). Lipid droplets in turn prevent excess ROS production by sequestering fatty acid from oxidation and hence suppress oxidative burden (Kuramoto et al. 2012). Perilipin 3, 4 and 5 bind to more transient pools of lipid droplets, while perilipin 1 and 2 associate with more constitutive pools of lipid droplets (Kovsan et al. 2007). Perilipin 5 may play a part in protection against cellular lipotoxicity by transiently entrapping bioactive lipids in lipid droplets (Wang and Sztalryd 2011). Perilipin 5 regulates oxidative lipid droplets hydrolysis and controls local fatty acid flux to protect mitochondria against excessive exposure to fatty acid during physiological stress (Wang et al. 2011). Mitochondria are in close physical interaction with perilipin 5-coated lipid droplets. Mitochondrial dysfunction provokes prominent lipid accumulation and tissue-specific metabolic disturbances in humans (Zehmer et al. 2009). Substantially perilipin 5 inhibits hydrolysis and stabilizes the lipid droplet. On the one hand palmitate accumulates into triglycerides and on the other hand mitochondrial utilization of palmitate is decreased. In PKA-stimulated state, inhibition of lipid droplets hydrolysis is abolished. Fatty acids are released from lipid droplets and undergone beta-oxidation in mitochondria. There are physical and metabolic links between lipid droplets and mitochondria. Consequently, perilipin 5 protects the mitochondria against fatty acids alterations by regulating lipid droplets' hydrolysis and local fatty acids' flux (Wang et al. 2011). Conversely, perilipin-null mice showed increased beta-oxidation in muscle, liver, and adipose tissue resulting from a coordinated regulation of the enzymes, UCPs-2 and -3 involved in beta-oxidation. The increased beta-oxidation can remove the extra FFAs created by the constitutive lipolysis (Saha et al. 2004).

6 Lipotoxicity and Insulin Resistance

Accumulation of intramuscular lipid due to insufficient mitochondrial fatty acid oxidation may be a causative factor in the development of IR (Hegarty et al. 2003). Insulin-resistant individuals have a reduced rate of fat oxidation compared with insulin-sensitive individuals (Kelley and Simoneau 1994), and decreased mitochondrial oxidative capacity of fat leads to an increase in intracellular fat content. Lowell and Shulman suggested that a mitochondrial defect in insulin-resistant individuals could lead to an increase in intramyocellular fat content (Lowell and Shulman 2005). Elevation of FFA concentrations in insulin-sensitive subjects causes a decrease in insulin sensitivity (Belfort et al. 2005). On the other hand developing IR with aging causes an increase in the intracellular fat content, which is associated with a 40% reduction in mitochondrial oxidative phosphorylation activity (Petersen et al. 2003). In humans dysregulated insulin action has been linked with an increased uptake of fatty acids into muscle, suggesting that an increased availability of fatty acids contributes to excess muscle lipid accumulation (Bonen et al. 2004).

Various hypotheses are proposed in order to clarify the adiposity-associated IR. The predominant paradigm used to explain this link is the portal/visceral hypothesis in which visceral depots

lead to increased FFA flux and inhibition of insulin action via "Randle's effect" in insulin-sensitive tissues (Smith and Ravussin 2002). The competition between glucose and fatty acids for their oxidation and uptake in muscle results in impairment of glucose metabolism by fatty acid oxidation. This condition is known as Randle Cycle which is also referred to as "fatty acid syndrome", involves a short-term inhibition of glucose transport and phosphorylation by fatty acids (Hue and Taegtmeyer 2009). In accordance with Randle's hypothesis fat produces proportional inhibitions of insulin-stimulated glucose uptake and of intracellular glucose utilization. Fatty acid could potentially be responsible for a large part of the peripheral IR (Boden and Chen 1995). In contrast to Randle's hypothesis increased concentrations of plasma fatty acids induce IR in human skeletal muscle through inhibition of glucose transport activity. This may be as a result of subsequent decrease in insulin receptor substrate-1 (IRS-1)-associated PI3K activity (Dresner et al. 1999). Later on McGarry et al. demonstrated a reasonable mechanism for the glucose-induced inhibition of fatty acid oxidation in which malonyl-CoA signals glucose utilization, also controls LCFA entry and oxidation in the mitochondria (McGarry et al. 1977). Recently Hue et al. proposed a mechanism, while the cytosolic accumulation of citrate inhibits 6-phosphofructo-1-kinase (PFK-1) (Garland et al. 1963); it regenerates acetyl-CoA, which turns into malonyl-CoA by ACC. Subsequently malonyl-CoA inhibits carnitine palmitoyltransferase (CPT) I and the entry of LCFA moieties into mitochondria are inhibited. In brief, malonyl-CoA prevents fatty acid oxidation and favors fatty acid esterification (Hue and Taegtmeyer 2009). This condition has been identified as glucose-fatty acid cycle. The concentrations of malonyl-CoA depend on the balance between the activities of ACC and malonyl-CoA decarboxylase (MCD) (Young et al. 2001). Inhibition of MCD increases malonyl-CoA and promotes glucose utilization and limits LCFA oxidation (Hue and Taegtmeyer 2009). Expression of ACC and MCD under the control of sterol regulatory element-binding protein (SREBP-1c and PPAR-alpha, respectively (Campbell et al. 2002; Young et al. 2001). It was shown that insulin activates the transcription factor SREBP-1c, which enhances transcription of genes required for fatty acid and triglyceride biosynthesis, most prominently ACC and fatty acid synthase (Brown and Goldstein 1997).

On the other hand, failure in the development of adequate adipose tissue mass due to ectopic storage of lipids or increased fat cell size, divert excess lipid into liver, skeletal muscle and the pancreatic insulin-secreting beta cells. Virtually ectopic fat deposition is the result of additive or synergistic effects including increased dietary intake, decreased fat oxidation and impaired adipogenesis. In this respect "acquired lipodystrophy" hypothesis creates a link between adiposity and IR (Heilbronn et al. 2004). Enlarged fat cells diminish capacity to store fat and are resistant to the antilipolytic effect of insulin. Chronically increased plasma fatty acids induce hepatic and muscle IR (DeFronzo 2004). When adipocytes exceed their storage capacity, fat begins to accumulate in non-adipose tissues which consist of specific metabolites that inhibit insulin signal transduction. IR is associated with enhanced Ser/Thr phosphorylation of IRS-1 and IRS-2, which impairs their interaction with the juxtamembrane region of the insulin receptor (Paz et al. 1997). Increase in the serine phosphorylation of IRS-1 at Ser(307) site by NEFAs could be one of the mechanisms leading to a decrease in IRS-1 tyrosine phosphorylation, PI3K activity and glucose transport (Le Marchand-Brustel et al. 2003). IRS-1 serine/threonine phosphorylation may mediate the desensitization of insulin signaling by stimulating the subcellular redistribution of IRS-1 and sensitizing IRS-1 to the action of the proteasome (Pederson et al. 2001). The impairment of insulin signaling by phosphorylation of IRS on serine and threonine residues, contributes to IR. In contrast to tyrosine phosphorylation, the multisite serine and threonine phosphorylation of IRS both positively and negatively regulates insulin signaling as well as correlates with their subcellular re-localization and/or proteasome-mediated degradation (Copps and White 2012). Ubiquitin conjugation of IRS-1 is a prerequisite for insulin-induced IRS-1 proteasome degradation. Both

tyrosyl phosphorylation of IRS-1 and PI3K activation are needed to activate the IRS-1 ubiquitin-proteasome degradation pathway. Activation of this pathway during prolonged insulin exposure underlies the molecular mechanism of IR (Zhande et al. 2002). Moreover, subcellular re-distribution of IRS-1 is regulated by the mammalian target of rapamycin (mTOR)-dependent pathway and facilitates also proteasomal degradation of IRS-1 (Takano et al. 2001). Actually, tyrosine phosphorylation of IRS-1 and its binding to PI3K are critical events in the insulin signaling cascade leading to insulin-stimulated glucose transport. Elevated plasma fatty acid concentration is associated with reduced insulin-stimulated glucose transport activity as a consequence of altered insulin signaling through PI3K (Le Marchand-Brustel et al. 2003). High concentrations of plasma FFAs are involved in the etiology of obesity-associated IR. In particular palmitic acid markedly inhibits insulin-stimulated phosphorylation of IRS-1, and Akt. In this case ubiquitination of the key insulin signaling molecules facilitates their proteasomal degradation (Hirabara et al. 2010; Ishii et al. 2015). Palmitic acid -induced IR is ameliorated by inhibiting the de novo synthesis of ceramide, inhibitor kappa B (IκB)-alpha degradation or mTOR activation (Lam et al. 2011). IR can be caused in the peripheral tissues by either activating IκB kinase alpha/NF-κB (Cai et al. 2005) or endoplasmic reticulum stress (Ozcan et al. 2004).

Lipid signaling molecules can be derived from saturated fatty acids, and they include long-chain fatty acyl CoA, DAG, phosphatidic acid, triacylglycerol and ceramides. Amongst them, DAG, triacylglycerol, and ceramides are directly associated with IR (Cooney et al. 2002; Nagle et al. 2009; Schmitz-Peiffer 2000). When adipocyte storage capacity is exceeded, lipid overflows into muscle and liver, and possibly into the beta-cells of the pancreas. Consequently, IR is exacerbated. Dysfunctional fat cells produce excessive amounts of IR-inducing and inflammation-provoking cytokines (DeFronzo 2004). With the development of a chronic inflammatory state, cytokines released from either adipocytes or from macrophages antagonize insulin action (Summers 2006). Hereby a chronic low-grade inflammation and an activation of the immune system are involved in the pathogenesis of obesity-related IR (Esser et al. 2014). Eventually, the accumulation of excess lipid and accompanied inflammation in adipose tissue and liver contribute to the development and progression of IR in peripheral tissues (Shoelson et al. 2007). As a result of imbalance between plasma FFA availability, fatty acid storage and fatty acid oxidation in the obese patient, muscle triacylglycerol-muscle oxidative capacity ratio is a marker of IR (van Loon and Goodpaster 2006). Decreased mitochondrial oxidative capacity of insulin-resistant young obese humans is independent of age (Phielix et al. 2014). Reactive lipid species such as fatty acyl CoA, DAG, and ceramides are important for the development of IR (Hajduch et al. 2001; Montell et al. 2001). Main lipid signaling molecules are derived from FFAs; DAG, which activates isozymes of the PKC family, and ceramide, which has several effectors including PKCs and a protein phosphatase (Schmitz-Peiffer 2000). Total cytosolic DAG correlates negatively with insulin sensitivity. Cytosolic DAG content is also associated with PKC activation and increases IRS-1 serine 1101 phosphorylation. Inhibition of insulin-stimulated IRS-1 tyrosine phosphorylation and Akt2 phosphorylation at serine 473 disrupts glucose transport into cells (Szendroedi et al. 2014). Eventual DAG levels are associated with reduction in both insulin-stimulated IRS-1 tyrosine phosphorylation and PI3K activity (Timmers et al. 2008). In addition to PKC, IκB and JNK can also be activated by acutely raising plasma FFA levels, which cause hepatic and peripheral IR (Boden et al. 2005; Hotamisligil 2005). Activation of PKC-theta leading to increased IRS-1 Ser307 phosphorylation. Activation of these serine/threonine kinases can interrupt insulin signaling by decreasing tyrosine phosphorylation of the IRS-1 (Yu et al. 2002). The metabolic actions of insulin are diminished by saturated fatty acids via inhibiting IRS/PI3K/Akt pathway. Eventually insulin-induced glycogen synthesis, glucose oxidation and lactate production are decreased. Both mitochondrial hyperpolarization and ATP generation decline as an evidence of mitochondrial dysfunction

(Hirabara et al. 2010). Indeed, DAG, fatty acyl CoA and ceramide associate with IR and serine phosphorylation of IRS-1 (Shulman 2000). In this case JNK-mediated phosphorylation of IRS-1 is a contributing factor during the development of IR (Sabio and Davis 2010). Only saturated FFAs cause a significant increase of mitochondrial ROS production, which correlates with concomitant mitochondrial DNA damage, mitochondrial dysfunction, JNK induction, apoptosis, and inhibition of insulin signaling. Blocking de novo synthesis of ceramide abolishes the effects of palmitate on mitochondrial ROS production, viability, and insulin signaling (Yuzefovych et al. 2010). In brief, it was suggested that lipid-induced IR is depend on the accumulation of lipid signaling molecules such as DAG and ceramide in ectopic tissues rather than inhibition of glycolysis and glucose oxidation. These lipid metabolites disrupt insulin-stimulated translocation of the GLUT4 glucose transporter (Muoio and Neufer 2012). JNK and PKC phosphorylate IRS-1, thus blunting its downstream targets PI3K and Akt. This results in down-regulation of glucose transporter (GLUT)-4 and IR. Impaired insulin sensitivity in the vascular endothelium leads to increased FFA oxidation, ROS formation, and subsequent activation of advanced glycation end products synthesis, PKC activation, protein glycosylation as well as down-regulation of prostaglandin I2 (PGI2) synthase activity. These events inhibit endothelial nitric oxide synthase (eNOS) activity thereby leading to endothelial dysfunction in obesity (Creager et al. 2003). In this case, the use of fatty acids instead of glucose for energy production enhances mitochondrial hydrogen peroxide production suggests that a localized catalase increase is needed to consume excessive mitochondrial hydrogen peroxide (Rindler et al. 2013b). Significant mitochondrial ROS formation during LCFA catabolism reflects a complex process involving multiple sites of ROS production as well as modified mitochondrial function. ROS are released not only on the matrix side of mitochondria but also on the cytosolic side of the inner membrane. ROS production is more sensitive to matrix levels of LCFA catabolic intermediates, indicating that mitochondrial export of LCFA catabolic intermediates can play a role in control of ROS levels. In addition, glutathione antioxidant system of muscle mitochondria is inhibited during LCFA catabolism (Seifert et al. 2010). The oxidized glutathione/2glutathione (GSSG/2GSH) couple is the most abundant redox couple in a cell (Schafer and Buettner 2001). NADPH is a key component in cellular antioxidation systems; and NADH-dependent ROS generation from mitochondria and NOX-dependent ROS generation are two critical mechanisms of ROS generation (Ying 2008). Palmitate strongly increases the cytosolic NAD^+/NADH ratio. FFA-induced ROS generation and apoptosis are accompanied by the decoupling of glycolysis and citric acid cycle fluxes leading to abnormal cytosolic redox states. The activation of citric acid cycle fluxes by palmitate are concomitant with reduced glycolysis and increased cytosolic NAD^+/NADH ratio (Noguchi et al. 2009).

Palmitate-induced dysregulation of mitochondrial oxidative metabolism is the primary cause of ROS accumulation and apoptosis. Unlike previously known, these metabolic alterations are independent of fatty acid beta-oxidation and precede the onset of oxidative damage or apoptosis initiation (Egnatchik et al. 2014).

As mentioned previously, the development of IR as a molecular consequence of enhanced mitochondrial hydrogen peroxide production is the response to increased reliance on fatty acids for energy production (Rindler et al. 2013a). On the other hand, stressing mitochondrial membrane potential through mitochondrial UCP5 causes a compensatory increase in mitochondrial UCP3. This leads to the depletion of the mitochondrial membrane potential and an increase in ROS production through the stressed electron transport chain. The stressed electron transport chain and ROS production induce activation of JNK1, which controls forkhead box protein O1a (FOXO1a) localization through dephosphorylating of Akt (Senapedis et al. 2011). UCP3 functions to export those fatty acids that cannot be oxidized from the mitochondrial matrix, in order to prevent deleterious fatty acid accumulation inside the matrix. In addition, UCP3 is increased

in patients with defective beta-oxidation and is reduced after restoring oxidative capacity (Schrauwen and Hesselink 2004). Inhibition of Akt phosphorylation at both Ser473 and Ser308 sites by palmitic acid occurs in a dose-dependent fashion. In this case palmitic acid not only inhibits insulin-stimulated Akt phosphorylation at Ser473, but also blocks insulin-stimulated phosphoinositide-dependent kinase-1 phosphorylation at Ser241. These evidences indicate that palmitic acid may impair the upstream insulin signaling (Wang et al. 2006). Lipid-activated signaling pathways are also likely to play an important role in interference with glucose-fatty acid cycle (Schmitz-Peiffer 2000). Saturated FFAs inhibit insulin stimulation of Akt that is a central mediator of insulin-stimulated anabolic metabolism (Chavez et al. 2003). Ceramide is the second messenger in the sphingomyelin signaling pathway. Excessive ceramide could contribute to the development of IR in peripheral tissues by two independent mechanisms. First, ceramide specifically blocks the translocation of Akt (PKB) to the plasma membrane. Second, ceramide inactivate Akt through acceleration of the enzyme dephosphorylation by activating protein phosphatase 2A (Chavez et al. 2003; Stratford et al. 2004). Ceramide also inhibits insulin-stimulated glucose transport in adipocytes. Similar reductions in hormone-stimulated translocation of the insulin-responsive GLUT4 and insulin-responsive aminopeptidase may occur (Summers et al. 1998). Ceramide and/or its derivatives, ganglioside GM3 and sphingosine, antagonize insulin signaling, induce oxidative stress, and inhibit glucose uptake and storage, and thus at least three mechanisms may initiate the molecular defects that underlie IR (Summers and Nelson 2005). Firstly, ceramide is a common molecular intermediate linking both glucocorticoids and saturated fatty acids to the induction of IR; secondly, different fatty-acid classes antagonize insulin-stimulated glucose uptake by distinct mechanisms distinguished by their dependence upon ceramide synthesis; and thirdly, inhibition of ceramide synthesis in obese rodents ameliorates IR and blocks the onset of diabetes. Consequently, inhibition of ceramide synthesis markedly improves glucose tolerance (Holland et al. 2007).

According to Morino et al. the main factor in the IR observed with obesity is the accumulation of LCFA derivatives and triacylglycerol in tissues due to impaired mitochondrial beta-oxidation (Morino et al. 2006; Ruderman et al. 1999). Accumulated fatty acids are used to synthesize DAG and ceramide. These signaling intermediates negatively regulate insulin effect. In this case increase in plasma fatty acid concentration results in an increase in intracellular fatty acyl-CoA and DAG concentrations, which results in activation of PKC-theta leading to increased IRS-1 Ser307 phosphorylation. This in turn leads to decreased IRS-1 tyrosine phosphorylation and decreased activation of IRS-1-associated PI3K activity resulting in free fatty acid-induced IR (Holland et al. 2007; Yu et al. 2002). Long-chain acyl-CoA molecules are converted to acylcarnitines by carnitine palmitoyltransferase 1 before mitochondrial import. MCD degrades malonyl-CoA, which is a potent inhibitor of carnitine palmitoyltransferase 1 (Rindler et al. 2013a).

While insulin binding induces tyrosine phosphorylation of the IRS, it also increases the activity of NOX in the plasma membrane. Increased concentration of H_2O_2 proximal to the IRS induces inositol trisphosphate (IP3) receptors and facilitates IP3-stimulated Ca^{2+} release, as well as impedes Ca^{2+} signals induced by insulin (Espinosa et al. 2009). In this case, total cytosolic DAG correlates negatively with insulin sensitivity. Acute induction of muscle IR is associated with a transient increase in total and cytosolic DAG content that is temporally associated with PKC-theta activation, increased IRS-1 serine1101 phosphorylation, and inhibition of insulin-stimulated IRS-1 tyrosine phosphorylation and Akt2 phosphorylation. In contrast, there are no associations between IR and alterations in muscle ceramide, acylcarnitine content (Szendroedi et al. 2014). Enhancing hepatic mitochondrial LCFA oxidation capacity in association with the carnitine palmitoyltransferase 1A expression can reverse IR and glucose intolerance in obese mice independent of hepatic steatosis (Monsénégo et al. 2012).

Carnitine acetyltransferase (CrAT) is a mitochondrial matrix enzyme that catalyzes the interconversion of acetyl-CoA and acetylcarnitine. Reduction in CrAT activity is accompanied by muscle accumulation of long-chain acylcarnitines (LCACs) and acyl-CoAs in obesity. Promoted mitochondrial influx of fatty acids resulted in accumulation of LCACs despite a pronounced decrease of CrAT-derived short-chain acylcarnitines. This lipid-induced antagonism of CrAT contributes to decreased mitochondrial pyruvate dehydrogenase activity and diminished glucose oxidation in the context of obesity (Seiler et al. 2014). Elevated palmitoyl carnitine concentrations inhibit electron transport chain activity and decrease the mitochondrial inner membrane potential. This deleterious action of FFA metabolites on mitochondrial substrate oxidation provides a potential link between the lipotoxicity, mitochondrial dysfunction, and IR (Abdul-Ghani et al. 2008).

Synthesis of cell membrane cholesterol and fatty acids begins with acetyl-CoA. Acetyl-CoA is converted to fatty acids and cholesterol through the 12 and 23 enzymatic steps, respectively. mRNAs encoding enzymes in these two pathways regulated by the SREBP family of membrane-bound transcription factors (Horton et al. 2002). SREBP serve as master regulators of lipid homeostasis by regulating synthesis of cholesterol, fatty acids, and triglycerides (Brown and Goldstein 2009). Newly synthesized SREBP is inserted into the membranes of the endoplasmic reticulum. During the cholesterol depletion, SREBP cleavage–activating protein (SCAP) transports the SREBP from the endoplasmic reticulum to Golgi apparatus. The NH2-terminal domain designated nuclear SREBP (nSREBP), translocates to the nucleus and binds to the promoter/enhancer regions of multiple target genes. If the cholesterol content of cells increases, SREBP cannot access to Golgi apparatus (Brown and Goldstein 1997). Golgi-to-endoplasmic reticulum transport of SCAP requires SREBP cleavage and that un-cleaved SREBP actively blocks recycling. SCAP not bound to SREBP cycled normally between the endoplasmic reticulum and Golgi, indicating that SCAP contains an endoplasmic reticulum retrieval signal (Shao and Espenshade 2014). SCAP deletion reduces lipid synthesis and prevents fatty livers despite persistent obesity, hyperinsulinemia, and hyperglycemia (Moon et al. 2012). Absence of insulin receptors selectively reduces IRS-2, but not IRS-1 phosphorylation, and the impairment of IRS-2 activation is associated with lack of insulin effects. Actually IRS-2 is more important than IRS-1 in mediating insulin action in liver (Rother et al. 1998). Despite the reduction in IRS-2, insulin continues to increase SREBP-1c, which activates the mRNAs encoding enzymes responsible for phosphorylation of glucose and its conversion to fatty acids. Glucose over-production-enhanced fatty acid synthesis interaction leads to setting up a vicious cycle (Shimomura et al. 2000). Activation of SREBP1 is highly dependent on the activity of insulin-induced genes 1 and 2 (Insig1 and Insig2), SREBP1 and Insig1 may be important for the process of human adipose tissue adaptation to excess lipid storage. The IR associated-obesity is dependent on the decreased Srebp1c mRNA expression in white adipose tissue rather than the amount of fat stored in morbidly obese individuals. Insig1 mRNA expression is decreased in the subcutaneous depot of morbidly obese individuals compared with obese patients and is further reduced in the presence of IR (Carobbio et al. 2013). The suppressor of cytokine signaling (SOCS)-3-induced IR occurs by decreasing insulin-induced IRS-1 tyrosine phosphorylation and its association with the regulatory subunit of PI3K (Emanuelli et al. 2001). Indeed, increased expression of SOCS-1 and SOCS-3 in liver and muscle of obese mice is associated with decreased tyrosine phosphorylation of IRS proteins (Ueki et al. 2004). Human SREBP-1c promoter was positively regulated by insulin and negatively regulated by STAT-3. SOCS-3-mediated attenuation of the STAT signaling pathway and resulting enhanced expression of SREBP-1c. Livers of morbidly obese individuals also exhibits enhanced expression of SOCS-3 protein and attenuated JAK/STAT signaling (Elam et al. 2010).

7 Conclusion

During prolonged nutrient excess or obesity, lipid influx can exceed the adipose tissue storage capacity, and results in accumulation of harmful lipid species at ectopic sites such as liver and muscle (Erikci Ertunc and Hotamisligil 2016). Consequently, large proportion of fatty acids enter into the mitochondria. In this case, ceramide and its derivatives antagonize insulin signaling, induce oxidative stress, and inhibit glucose uptake and storage. Finally, cessation of the bioenergetics and redox functions of mitochondria lead to cell death (Kroemer et al. 2007). For another aspect, leptin resistance is essential to permit accumulation of surplus calories into adipocytes, meanwhile, the lipo-oxidative action of leptin minimizes ectopic lipid accumulation. However, in advance stages of obesity peripheral organs also become leptin resistant and lipo-oxidative action could not protect the peripheral tissues from ectopic lipid accumulation (Unger and Scherer 2010). As noted, in this event, there is no single unifying mechanism that could be easily manipulated to protect the non-adipose tissue from detrimental effect of lipid spillover. Besides, a strategy may be to increase the capacity for lipid storage and prevent lipotoxicity, a second strategy to prevent lipotoxicity may be to increase the capacity of tissues to oxidize fatty acids (Medina-Gomez et al. 2007). The important point to be considered is; targeted proteins involved in deleterious action of FFA is simultaneously effective in many other intracellular signaling pathways (Bellini et al. 2015).

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The Pathogenesis of Obesity-Associated Adipose Tissue Inflammation

9

Atilla Engin

Abstract

Obesity is characterized by a state of chronic, low-grade inflammation. However, excessive fatty acid release may worsen adipose tissue inflammation and contributes to insulin resistance. In this case, several novel and highly active molecules are released abundantly by adipocytes like leptin, resistin, adiponectin or visfatin, as well as some more classical cytokines. Most likely cytokines that are released by inflammatory cells infiltrating obese adipose tissue are such as tumor necrosis factor-alpha (TNF-alpha), interleukin 6 (IL-6), monocyte chemoattractant protein 1 (MCP-1) (CCL-2) and IL-1. All of those molecules may act on immune cells leading to local and generalized inflammation. In this process, toll-like receptor 4 (TLR4)/phosphatidylinositol-3′-kinase (PI3K)/Protein kinase B (Akt) signaling pathway, the unfolded protein response (UPR) due to endoplasmic reticulum (ER) stress through hyperactivation of c-Jun N-terminal Kinase (JNK)-Activator Protein 1 (AP1) and inhibitor of nuclear factor kappa-B kinase beta (IKKbeta)-nuclear factor kappa B (NF-kappaB) pathways play an important role, and may also affect vascular endothelial function by modulating vascular nitric oxide and superoxide release. Additionally, systemic oxidative stress, macrophage recruitment, increase in the expression of NOD-like receptor (NLR) family protein (NLRP3) inflammasone and adipocyte death are predominant determinants in the pathogenesis of obesity-associated adipose tissue inflammation. In this chapter potential involvement of these factors that contribute to the adverse effects of obesity are reviewed.

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1 Introduction

The primary event in the sequence leading to chronic inflammation in adipose tissue is metabolic dysfunction in adipocytes. These adipocytes promote inflammation via their own cytokine and chemokine synthesis machinery. Subsequently, inflammatory process is intensified by activated adipose tissue macrophages (ATMs) (Meijer et al. 2011). White adipose tissue is the primary site for the initiation and exacerbation of obesity-associated inflammation. Ten substantial molecular mechanisms have been put forward to explain the pathogenesis of obesity-associated inflammation (Ge et al. 2014); in this respect activation of toll-like receptor 4 (TLR4) by saturated fatty acids through the TLR4/phosphatidylinositol-3′-kinase (PI3K)/Protein kinase B (Akt) signaling pathway (Lee et al. 2003), increases in intracellular protein kinase C (PKC)-theta activation-associated fatty acyl-CoA and diacylglycerol (DAG) concentration with the reduction in both insulin-stimulated insulin receptor substrate-1 (IRS-1) tyrosine phosphorylation and IRS-1 associated PI3K activity (Yu et al. 2002), lipotoxicity due to increase in fatty acid release from dysfunctional and insulin-resistant adipocytes and overstimulation of hormone-sensitive lipase (HSL) (Cusi 2012), dysregulated sphingolipid biosynthesis (Summers 2006), the unfolded protein response (UPR) due to endoplasmic reticulum (ER) stress through hyperactivation of c-Jun N-terminal Kinase (JNK)-Activator Protein 1 (AP1) and inhibitor of kB (IkB) kinase (IKK)-nuclear factor kappa B (NFkappaB) pathways (Haynes et al. 2004), systemic oxidative stress through superoxide generation from nicotinamide adenine dinucleotide phosphate (NADPH) oxidases, oxidative phosphorylation, glyceraldehyde auto-oxidation, PKC activation, and polyol and hexosamine pathways (Manna and Jain 2015), promotion of macrophage recruitment, accumulation, and persistence in white adipose tissue of obese individuals by adipocyte death (Cinti et al. 2005), increase in the expression of NOD-like receptor (NLR) family protein (NLRP3) inflammasone and caspase-1 in adipose tissue (Koenen et al. 2011), hypoxia-induced lipolysis due to activation of hypoxia Inducible factor-1alpha (HIF-1alpha) and NF-kappaB in adipocytes and macrophages (Yin et al. 2009) and dysregulation of microRNAs (miRNAs) in adipose tissue (Ge et al. 2014) are considered as currently identified predominant determinants of obesity induced-chronic inflammation.

2 Adipocyte and Inflammation

Obesity promotes inflammation in adipose tissue. Human adipocytes express many cytokines/chemokines that are biologically functional. They are able to induce inflammation and activate CD4+ T cells independent of macrophages. As mentioned above the primary event in the sequence leading to chronic inflammation in adipose tissue is the metabolic dysfunction of adipocytes (Meijer et al. 2011). Actually adipocyte hypertrophy and local hypoxia due to adipocyte expansion are two important contributing

factors to the increased accumulation of macrophages in adipose tissue in the obese state. The secretion of a number of inflammation-related adipokines is upregulated by hypoxia. In this manner macrophages are phenotypically modified in response to increasing fat mass (Trayhurn 2013). Hypoxia-related adipocyte dysfunction will be discussed later.

The mRNA monocyte chemoattractant protein 1 (MCP-1) levels in human adipose tissue samples correlate with measures of adiposity (Christiansen et al. 2005). Indeed, MCP-1 expression is significantly higher in the adipocytes (Amano et al. 2014) and the MCP-1 receptor, chemokine (C-C motif) receptor 2 (CCR2), has been shown to be mostly expressed in macrophages in crown-like structures where most of proliferating ATMs are observed (Lumeng et al. 2008). These findings indicate that MCP-1 is released by adipocytes in crown-like structures, and could stimulate the proliferation of surrounding ATMs (Amano et al. 2014). In addition to MCP-1 mRNA, adiponectin, resistin, interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-alpha) are produced by human adipocytes and are correlated with the circulating MCP-1 and body mass index (BMI) (Christiansen et al. 2005; Halberg et al. 2008). Actually subcutaneous abdominal adipose tissue MCP-1 gene overexpression is a biomarker of an inflamed adipose organ in human obesity (Christiansen et al. 2005). Expression of MCP-1 mRNA displays a 7.2-fold increase in obese mice as compared with normal ones, leading to substantially elevated MCP-1 protein levels in adipocytes (Takahashi et al. 2003). Thereby MCP-1 expression in adipose tissue attracts the monocytes bearing chemokine receptor CCR2 and contributes to the macrophage infiltration in diet- induced obese and insulin resistance animals (Kanda et al. 2006; Linton and Fazio 2003). Nearly half of the cell population in human adipose tissue consists of preadipocytes which are the undifferentiated precursors of mature adipocytes (Guo et al. 2007). Preadipocytes and pro-inflammatory macrophages share numerous functional or antigenic properties (Charrière et al. 2003). Eventually preadipocytes have a heightened inflammatory cytokine response, in particular MCP-1 expression following acute high saturated fatty acid and monounsaturated fatty acid exposure, when compared to mature adipocytes. Furthermore, preadipocytes exert a predominant role, via MCP-1, to macrophage recruitment in adipose tissue and contribute to postprandial inflammatory responses (Dordevic et al. 2014). MCP-1 over-expression in adipose tissue is under the control of the AP2 gene promoter and exhibits insulin resistance, macrophage infiltration into adipose tissue, and increased hepatic triglyceride content (Kanda et al. 2006). The abundance of macrophage infiltration in adipose tissue shows a close relationship between adipocyte size. Influence of adipocyte size on adipocyte function may be transmitted through a paracrine pathway involving ATMs (Weisberg et al. 2003). It is thought that macrophage accumulation in visceral omental and subcutaneous fat depots of obese humans positively correlated with the diameters of the fat cells in each depot. However omental macrophage infiltration is greater in comparison to the subcutaneous white adipose tissue, despite the mean diameter of omental adipose cells is significantly smaller than that of the subcutaneous cells (Cancello et al. 2006). In addition to MCP-1, some more classical cytokines are abundantly released by adipocytes like TNF-alpha, interleukin-6 (IL-6), IL-1 as well as several novel and highly active molecules released like adiponectin, leptin, resistin or visfatin. All may lead to local and generalized inflammation in adipose tissue (Guzik et al. 2006). The negative regulation of the nuclear hormone receptor peroxisome proliferator-activated receptor-gamma (PPAR-gamma) is important in mediating the effects of inflammatory cytokines. Furthermore, PPAR-gamma is an essential transcriptional regulator of adipogenesis and is required for maintenance of mature adipocyte function (Tamori et al. 2002). Cytokines have two dramatic effects on adipocyte function; increase in lipolysis and decrease in triglyceride synthesis. Downregulation of PPAR-gamma could strongly contribute to these effects of inflammatory cytokines on

adipocytes. On the other hand, TNF-alpha can affect PPAR-gamma at multiple levels, including the transcription, translation and turnover of PPAR-gamma mRNA and protein (Guilherme et al. 2008). PPAR-gamma mRNA is rapidly degraded in adipocytes (Christianson et al. 2008). In addition to adipocytes, TNF-alpha is also produced by macrophages within adipose tissue of obese subjects. This process requires both the IKK-beta–NF-kappaB and the JNK–mitogen-activated protein kinase kinase kinase kinase-4 (MAP4K4)- AP1 signalling pathways (Guilherme et al. 2008). Indeed, free fatty acid (FFA)-mediated induction of proinflammatory cytokines in macrophages requires JNK1 activity (Solinas et al. 2007). Inflammation could also cause insulin resistance by a direct action of TNF-alpha on muscle insulin signaling, which is triggered by serine/threonine (Ser/Thr) phosphorylation of IRS proteins. On the other hand, in adipocytes, MAP4K4 contributes to the regulation of glucose metabolism as a suppressor of PPAR-gamma and adipogenesis. TNF-alpha-induced insulin-resistance on glucose uptake is rescued by MAP4K4 silencing (Bouzakri and Zierath 2007). Furthermore, the IL-6 concentration in adipose tissue is approximately 100-fold higher than that in plasma. This increase in IL-6 production after hypertrophic enlargement of the adipose cells induces adipose tissue dysfunction with impaired differentiation of the pre-adipocytes (Sopasakis et al. 2004). While IL-6 is associated with visceral adiposity, TNF-alpha rather indicates an association with total body fatness (Cartier et al. 2008). However, soluble tumour necrosis factor receptor 2 (sTNFR2) levels are more closely related to visceral adipose tissue accumulation than to the total adiposity (Cartier et al. 2010). TNF production by human adipose tissue is also regulated by weight loss in obese subjects. In addition, there is an inverse correlation between adipose TNF-alpha expression and adipose lipoprotein lipase activity (Kern et al. 1995). Hence, over-expression of TNF-alpha in subcutaneous adipose tissue of obese women is proportional to the magnitude of the fat depot. Thereby, TNF-alpha prevents further fat deposition by regulating lipoprotein lipase activity and leptin production (Bulló et al. 2002). TNF-alpha and IL-6 inhibit lipoprotein lipase, and TNF-alpha additionally stimulates HSL and induces uncoupling protein expression (Coppack 2001).

In obesity-associated adipose tissue inflammation, nuclear protein high mobility group box 1 (HMGB1) is identified as an inflammatory alarmin. Thus, HMGB1 secretion in adipose tissue is two-fold more in adipose tissue from obese compared to normo-weight individuals. Moreover, this HMGB1 release is in response to inflammatory signals of obesity rather than adipocyte death (Gunasekaran et al. 2013). Upon HMGB1 release, macrophages secrete pro-inflammatory cytokines. Thus adipose tissue HMGB1 mRNA levels correlate with the expression of inflammatory markers. Moreover, insulin resistance modifies the intracellular distribution of HMGB1 in human adipocytes. HMGB1 localizes to the cytosol in obese patients instead of nucleus (Guzmán-Ruiz et al. 2014). When it is released into the extracellular space by preadipocyte, soluble HMGB1 controls the secretion of IL-6 and MCP-1 in adipose tissues through the binding to the receptor for advanced glycation end products (RAGE) and triggers inflammatory response (Nativel et al. 2013). Excessive fatty acid release may worsen adipose tissue inflammation and contributes to insulin resistance (Morigny et al. 2016). Fat cells of omental adipose tissue obtained from morbidly obese women have at least 26-fold increase in the mRNAs for HSL, lipoprotein lipase, adipose tissue triglyceride lipase, and fatty acid translocase (FAT/CD36) when compared to the nonfat cells, whereas the mRNAs for inflammatory proteins are primarily present in the nonfat cells (Fain et al. 2008). Although the inflammatory signaling pathways primarily become activated by metabolic stresses originating from inside the adipocytes themselves, obesity also overloads the functional capacity of the ER. Obesity-related ER stress in turn leads to suppression of insulin receptor signaling through hyperactivation of JNK and subsequent Ser phosphorylation of IRS-1 (Ozcan et al. 2004).

3 Adipocyte Death and Initiation of Inflammatory Response

White adipocytes are characterized by a unique morphology with unilocular lipid droplets that occupy 95% of the cell volume and thereby determine the cell size, which ranges approximately from 20 to 200 μm. Accumulation of adipose tissue depends in part on new adipocyte formation via recruitment of progenitors/preadipocytes and their differentiation into adipocytes, as well as hypertrophy of existing adipocytes (Lee et al. 2013). Adipocyte turnover is an important event for the development of hyperplasic or hypertrophic obesity. The absolute number of new hypertrophic adipocytes generation in each year is 70% less than hyperplasic adipocytes. Subcutaneous adipose hypertrophy and hyperplasia are strongly related to the total adipocyte number in adults (Arner et al. 2010). Turnover of adipocytes in adult human white adipose tissue is approximately 10% annually. Nevertheless fat cell number does not decrease in adulthood, even following long-term weight loss (Spalding et al. 2008). However chronic nutrient excess leads to visceral adipose tissue expansion and dysfunction of the adipocytes. Additionally, changing in the supporting matrix and immune cell infiltrates contribute to adipose tissue hypoxia, adipocyte stress, and ultimately adipocyte death (Revelo et al. 2014). Cell death in white adipose tissue occurs primarily by necrosis-like cell death. Further the frequency of adipocyte death is positively correlated with increased adipocyte size in obese humans (Cinti et al. 2005). Crown-like structures are formed with the accumulation of lymphocytes, macrophages, and other immune cells around dying adipocytes (Revelo et al. 2014). In severely obese patients, high B cells/T cells ratio phenotype in crown-like structures reflects long-term excess adiposity and overnutrition (McDonnell et al. 2012). Although obesity is accompanied by a substantial increase in crown-like structures in all fat depots, visceral adipose tissue contains 3.5 fold more crown-like structures when compared with the subcutaneous fat depot. Furthermore in obese mice, crown-like structures and mast cells have a similar distribution pattern in abdominal fat depots (Altintas et al. 2011). Eventually more than 90% of all macrophages in white adipose tissue of obese subjects are localized around the dead adipocytes. In contrast to adjacent viable adipocytes, perilipin immunoreactivity could not be detected on lipid droplets of adipocytes surrounded by crown like structures macrophages. Hence free lipid droplets of dead adipocytes act as persistent sites of macrophage fusion and lipid uptake (Cinti et al. 2005). Freshly isolated mature adipocytes from different adipose depots release macrophage migration inhibitory factor (MIF) approximately at a rate of 10,000 pg/ml/24 h. MIF production is positively correlated with donor BMI and most importantly it is an obesity-dependent mediator of macrophage infiltration of adipose tissue (Skurk et al. 2005). Furthermore increased expression of MIF gene in adipocytes is associated with enlarged subcutaneous abdominal adipocytes, reduced circulatory adiponectin, and impaired insulin action (Koska et al. 2009).

Recently, Wensveen et al. defined that a phenotypically distinct population of tissue-resident natural killer (NK) cells create a critical link between obesity-induced adipose stress and visceral adipose tissue inflammation. Upregulation of ligands of the NK cell-activating receptor (NCR1) on adipocytes leads to NK cell proliferation and interferon-gamma (IFN-gamma) production. Eventually macrophage polarization and insulin resistance may develop in response to obesity-induced adipocyte stress (Wensveen et al. 2015). With the development of obesity, ATMs are polarized to an M1 inflammatory phenotype. Not only the numbers of M1 ATMs and the expression of M1 marker genes, such as TNF-alpha and MCP-1, but also the M1-to-M2 ratio were increased by high-fat diet induced obesity (Fujisaka et al. 2009; Lumeng et al. 2007). Thus, in obese subjects, when macrophages scavenge dead or dying adipocytes and free lipid droplets, they secrete IL-6 and TNF-alpha (Lumeng et al. 2007). Endocytic activities of ATMs are similar to M2 macrophages and accordingly they secrete high amounts of IL-10 and IL-1 receptor antagonist. However, basal or induced secretion

of pro-inflammatory mediators, TNF-alpha, IL-6, IL-1, MCP-1 and MIP-1alpha is even higher in ATMs than in pro-inflammatory M1 macrophages (Zeyda et al. 2007). Indeed two subsets of macrophages are identified in adipose tissue of obese subjects based on their surface expression of F4/80; F4/80(high) and F4/80(low) (Guri et al. 2008). The F4/80(high) ATMs subset exhibits an enhanced ability to produce pro-inflammatory cytokines, PPAR-gamma, CD36 and TLR4 whereas the F4/80(low) subset expresses IL-4 and produces lower concentrations of cytokines (Bassaganya-Riera et al. 2009). Obese adipose tissue shows the chronic inflammation-specific macrophage infiltration with the large numbers of CD8+ effector T cells and pro-inflammatory macrophages, whereas the numbers of regulatory and CD4+ helper T cells are decreased (Xu et al. 2003). In addition to the increased levels of CD8+ T cells and macrophages, the CD11c+ dendritic cells (DCs) phenotype, F4/80(low) also increases in the adipose tissues of diet-induced obesity. In this manner there may be a link between adipose tissues derived DCs and adipose tissue inflammation which is caused by pro-inflammatory mediators of Th17 cells. Thus adipose tissues derived DCs express higher levels of IL-6, transforming growth factor-beta (TGF-beta), IL-23 that are essential cytokines for Th17 cells proliferation or differentiation (Chen et al. 2014). Indeed during obesity DCs for human CD11c+ CD1c+ and for mouse CD11c(high)F4/80(low) accumulate in adipose tissue. In patients, the presence of CD11c+ CD1c+ DCs correlates with the BMI and with an elevation in Th17 cells. Similarly, CD11c(high)F4/80(low) DCs from obese mice induce Th17 differentiation (Bertola et al. 2012).

Actually classically activated M1 macrophages create a pro-inflammatory environment that blocks adipocyte insulin action, contributing to the development of insulin resistance (Harford et al. 2011). In fact, resident ATMs uniformly express markers of alternative activation. Alternatively activated phenotype (M2a) markers are macrophage galactose N-acetyl-galactosamine specific lectin 1 (MGL1) and IL-10 (Dupasquier et al. 2006). M2a marker, MGL1+ ATMs could promote new fat cell formation. The inhibition of these processes by M1 macrophages may promote adipocyte hypertrophy and death, which has been closely linked to ATMs accumulation (Strissel et al. 2007). Obesity does not significantly alter the localization of M2a marker MGL1+ ATMs in adipose tissue but instead superimposes a new population of M1-polarized M2a marker MGL1 − CCR2+ ATMs on these resident cells. A decrease in the quantity of M2a ATMs combined with the appearance of these "recruited ATMs" ultimately shifts the balance of M1/M2a ATMs to create a more proinflammatory environment in adipose tissue. With diet-induced obesity, M2a marker, MGL1+ ATMs remain in interstitial spaces, whereas a population of M2a marker MGL1 − CCR2+ ATMs with high M1 and low M2a gene expression is recruited to clusters surrounding necrotic adipocytes. The rate of recruitment of new macrophages to M2a marker MGL1 − ATM clusters is significantly faster than that of interstitial M2a marker MGL1+ ATMs (Lumeng et al. 2008). Eventually M1-polarized macrophages are characterized by the expression of proinflammatory mediators. This phenotype forms crown-like structures in obese adipose tissue and scavenges dead adipocytes and its potentially cytotoxic remnants. Thus, ATMs synergistically increase their own absorptive capacities (Cinti et al. 2005; Lumeng et al. 2008). Crown like structures are chronic sources of TNF-alpha. Even low frequency of adipocyte death may be sufficient to cause adipose tissue inflammation and promote insulin resistance (Strissel et al. 2007).

Furthermore, macrophage markers CD68 and CD14 are closely correlated with the total number of macrophages. Consistent with this, proinflammatory markers IL-6, MCP-1, and TNF-alpha are significantly elevated in adipose tissue from obese subjects compared with lean subjects. A change in the macrophage phenotype may occur by obesity with a preponderance of anti-inflammatory (M2) markers and a decrement of proinflammatory (M1) markers in adipose tissue. It is suggested that this event may be a protective mechanism in counteracting with the enhanced inflammation seen in the adipose tissue in association with obesity (Fjeldborg et al. 2014).

Adipocytes-derived saturated fatty acids are endogenous ligands that induce macrophage-inducible C-type lectin (Mincle) mRNA expression in macrophages partly through the TLR4/NF-kappaB pathway in the adipose tissue of obese subjects (Ichioka et al. 2011). At first, saturated fatty acids are released from hypertrophied adipocytes through the macrophage-induced adipocyte lipolysis, subsequently, these result in the activation of the TLR4/NF-kappaB pathway (Suganami et al. 2007). Actually, Mincle serves as a receptor for SAP130, a component of small, nuclear ribonucloprotein as a Mincle ligand that is released from damaged cells to sense cell death and induce proinflammatory cytokine production (Yamasaki et al. 2008). Scavenging of adipocyte debris is an important function of white ATMs in obese individuals. Hence, dead adipocytes associated crown-like structure or macrophage syncytia in the adipose tissue is a hallmark of chronic inflammation which is coincidental with increased TNF-alpha gene expression (Cinti et al. 2005; Strissel et al. 2007). Collectively, Mincle may play a role in sensing adipocyte death to induce proinflammatory cytokine production in the adipose tissue in obesity (Ichioka et al. 2011). In this respect, Mincle in macrophages is crucial for crown-like structure formation, expression of fibrosis-related genes and myofibroblast activation. Thereby, Mincle is localized to macrophages in crown-like structure, in a number which correlates with the extent of interstitial fibrosis (Tanaka et al. 2014). Additionally, a positive link is found between the mast cell number and fibrosis in white adipose tissue of obese nondiabetic patients (Divoux et al. 2012). The immature mast cells that infiltrate into adipose tissue at the nonobese stage, later gradually mature with the progression of obesity. Anti-mast cell protease 6 (anti-MCP-6) is secreted from mature mast cells and induces collagen 5 expression in obese adipose tissue, which may contribute to the adipose tissue fibrosis (Hirai et al. 2014). Obese subjects have more total fibrosis in omental white adipose tissue and have more pericellular fibrosis around adipocytes, when compared to lean subjects. Some adipocytes are engulfed in fibrosis and stained perilipin negative. This indicates that fibrosis is a response to dysfunctional or dying adipocytes. Thus, omental white adipose tissue fibrosis negatively correlates with omental adipocyte diameters and with triglyceride levels (Divoux et al. 2010). Adipocytes hypertrophy induces the expression of HIF-1alpha, and increased lysyl oxidase, which is one of the target genes of HIF-1alpha and mediates cross linking of collagens for adipose tissue fibrosis. Furthermore, high fat diet induces the expression of IL-13 and mediates the deposition of collagen in adipose tissue (Halberg et al. 2009). On the other hand, the extracellular matrix plays important roles in maintaining adequate adipose tissue function. Metalloelastase, MMP-12 is produced predominantly by ATMs and can be induced with both short- and long-term high fat diet challenges. MMP-12 activation leads to lipolysis (Martinez-Santibanez et al. 2015). In humans, MMP-12 expression correlated positively and significantly with insulin resistance, TNF-alpha expression, and the number of CD14+ CD206+ macrophages in adipose tissue. Despite its extensive expression in M2 macrophages and DCs, it is detected at low levels in M1 macrophages (Lee et al. 2014).

4 Inflammasome Activation

Lipid spill over from dysfunctional or necrotic adipocytes participates in dyslipidemia and lipotoxicity. Lipid metabolites serve as a source of endogenous danger-associated molecular patterns (DAMPs) that can be sensed by specific pattern recognition receptors (PRRs). Subsequently PRRs mediate activation of the cells of innate immune system (Lukens et al. 2011). In this context, obesity is associated with the increased ceramides, saturated fatty acids, reactive oxygen species (ROS), mitochondrial dysfunction and adenosine triphosphate (ATP) release from necrotic adipocytes. All these factors have been shown to activate the NLRP3 inflammasome in macrophages (Lukens et al. 2011). The NLRP3 inflammasome is the most fully characterized inflammasome and consists of the NLRP3 scaffold, the apoptosis-associated speck-like protein

containing a C-terminal – caspase recruitment domain (CARD) (ASC, encoded by PYCARD gene) adaptor, and caspase-1. Actually NLRP3 is activated upon exposure to pathogen-associated molecular patterns (PAMPs), DAMPs and by host-derived molecules (Schroder and Tschopp 2010) multiprotein complexes localize within the cytoplasm of the cell that are responsible for the maturation of proinflammatory cytokines and the activation of a highly inflammatory form of cell death, pyroptosis (Abais et al. 2015). Indeed, pyroptosis is a caspase-1-dependent programmed cell death, which features rapid plasma membrane rupture, DNA fragmentation, and release of proinflammatory intracellular contents (Xu et al. 2014). In obesity, hypertrophic adipocytes exhibit cholesterol crystals and accumulation of calcium and ROS, that might trigger the NLRP3 inflammasome pathway with subsequent massive activation of caspase-1, which ultimately results in adipocyte death from pyroptosis (Giordano et al. 2013). Dendritic cells [CD11c+CD11b+F4/80−] are exposed to high fat diet, vary toward a proinflammatory phenotype with increased IL-1beta secretion,Interleukin 1 receptor, type 1 (IL-1R1), TLR4, and caspase-1 expression (Reynolds et al. 2012). Ectopic triglyceride accumulation, adipocyte size, and macrophage infiltration in adipose tissue and circulating adipokine levels are dependent on the NLRP3 inflammasome activation. Thereby inflammasomes have critical functions in obesity and insulin resistance (Stienstra et al. 2011). In this regard, NLRP3 inflammasome senses lipotoxicity-associated increases in intracellular ceramide as an obesity-associated danger signal to induce caspase-1 cleavage in macrophages and adipose tissue (Vandanmagsar et al. 2011). Actually, ceramide, a product derived from long-chain saturated fatty acids inhibits insulin-stimulated activation of Akt and translocation of GLUT4 (Chavez et al. 2003). Diet containing high amount of saturated fatty acid enhances IL-1beta-mediated adipose tissue inflammation and insulin resistance. Actually high-saturated fatty acid consumers display reduced insulin sensitivity with elevated NLRP3 inflammasome (PYCARD and caspase-1 multiprotein complex) expression in adipose tissue (Finucane et al. 2015). Eventually, activation of NLRP3 inflammasome by damaged mitochondria leads to the caspase-1-dependent secretion of the proinflammatory cytokines IL-1 beta and IL-18 (Benetti et al. 2013; Gurung et al. 2015). Processing of IL-1beta and IL-18 requires cleavage by caspase-1, a cysteine protease, which is regulated by the inflammasomes. Caspase-1 and IL-1beta activity in adipose tissue is increased both in diet-induced and genetically induced obese animals. Under these conditions caspase-1 is upregulated during adipocyte differentiation and directs adipocytes toward a more insulin-resistant phenotype. In this context, the inflammasome plays an important role in regulation of adipocyte function and insulin sensitivity through caspase-1 activity (Stienstra et al. 2010). Abdominal subcutaneous adipose tissue biopsies from obese humans revealed that NLRP3 inflammasome activation and a Th1 shift in the T cell population in adipose tissue is related to insulin resistance, which may be explained by adipose tissue inflammatory processes (Goossens et al. 2012).

Furthermore, lipid metabolites including ceramide and diacylglycerol (DAG) can activate NAPDH oxidase and enhance ROS generation (Brookheart et al. 2009). The adenosine monophosphate-activated protein kinase (AMPK) has emerged as an essential mediator of fatty acid metabolism (Steinberg and Kemp 2009). AMPKalpha2 functions as a physiological suppressor of NADPH oxidase and ROS production (Wang et al. 2010). Thereby, FFA-enhanced ROS generation is negatively regulated by AMPK activation, which results in an increased beta oxidation of FFAs in mitochondria and decreases the lipid loading (Wang et al. 2010). Nevertheless, NAPDH oxidase-dependent generation of ROS plays an essential role in NLRP3 inflammasome activation (Schroder and Tschopp 2010). AMPK positively regulates autophagy by directly activating Unc-51 Like Autophagy Activating Kinase 1 (ULK1) (Egan et al. 2011). Saturated fatty acid-decreased AMPK activity leads to defective autophagy and the accumulation of mitochondrial ROS, by a deficiency in the clearance of dysfunctional mitochondria. Thus, excess saturated fatty acid accumulation leads to inflam-

masome activation and IL-1beta release through AMPK-autophagy-ROS signaling pathway (Wen et al. 2011). In fact, several autophagic genes are downregulated in obesity. These include Atg7, Becn1, Ulk1 and Atg5. Thus, activation complex 1 of the mechanistic target of rapamycin complex 1 (mammalian target of rapamycin complex 1, mTORC1) inhibits autophagy through post-translational phosphorylation of ULK1 (Sciarretta et al. 2012). Inhibition of autophagy increases the generation of ROS. In addition to ROS production and the NLRP3 inflammasome activation, FFA overload also induces ER stress, apoptosis and inflammation (Legrand-Poels et al. 2014). Furthermore, ER stress activates the NLRP3 inflammasome in human macrophages, resulting in the subsequent release of IL-1beta by human macrophages, with an activation mechanism similar to that of other known NLRP3 activators, requiring ROS generation. UPR-independent ER stress response regulates NLRP3 inflammasome activation (Menu et al. 2012).

5 Plasminogen Activator Inhibitor

Expression of enzymes involved in ceramide generation, neutral sphingomyelinase (NSMase), acid sphingomyelinase (ASMase), and serine-palmitoyl-transferase (SPT) mRNA are elevated in obese adipose tissues (Samad et al. 2006). While sphinganine ceramide significantly decreases, dihydroceramide content increases in obese humans. In these cases, Ser palmitoyltransferase and ceramidases activities (both neutral and acidic) are found elevated, whereas sphingomyelinase activities (both neutral and acidic) are reduced (Błachnio-Zabielska et al. 2012). However, high fat diet-mediated increase in ceramide is attenuated in mice lacking plasminogen activator inhibitor-1 (PAI-1). This evidence shows that PAI-1 plays a direct role in sphingolipid metabolism, which is augmented in a setting of obesity, where PAI-1 levels are elevated (Shah et al. 2008). Ceramide can induce the expression of PAI-1 and inflammatory cytokines and chemokines from adipocytes (Samad et al. 2006). The plasminogen activation system consists of urokinase plasminogen activator (uPA) and tissue plasminogen activator (tPA). Proteolitic effect of plasmin is inhibited by impeding plasminogen-plasmin cleavage. Four known protein inhibitors of uPA/tPA are defined as PAI-1, PAI-2, PAI-3 and nexin. PAI-1 is a fast-acting, highly specific inhibitor of tPA and uPA (Jankun and Skrzypczak-Jankun 2009). Obesity is characterized by elevated levels of circulating PAI-1. In fact, adipose tissue evolves into a major PAI-1 producing organ by gaining capacity during adipocyte differentiation. This is mediated by a decrease in E2F1 protein levels which is well-known transcriptional repressor of PAI-1 (Venugopal et al. 2007). However, the greater fat cell size and the adipose tissue mass has the greater contribution to circulating PAI-1 production of adipose tissue. However, visceral adipose tissue has a higher capacity to produce PAI-1 than subcutaneous adipose tissue. In addition to FFA, PAI-1 synthesis is most potently upregulated by TNF-alpha and transforming growth factor-beta (TGF-beta). Reasonably impaired fibrinolysis in obesity is probably due to an increased expression of PAI-1 in adipose tissue (Skurk and Hauner 2004). Indeed, exposure of adipocytes to TNF-alpha induces PAI-1 mRNA by increasing the rate of transcription of the PAI-1 gene. This dramatic induction of PAI-1 mRNA in response to TNF-alpha is mediated by p38, PI3K, tyrosine kinases, and the transcription factor NF-kappaB (Pandey et al. 2005). Increased FFA concentrations in blood of nondiabetic, overweight subjects markedly raise circulating PAI-1 concentrations, with a concomitant increase in the expression of the PAI-1 gene in adipose tissue. This means that adipocytes also stimulate PAI-1 expression in macrophages and potentiates the increase in PAI-1 messenger RNA expression in response to FFAs (Kishore et al. 2010). PAI-1 inhibition results in enhanced adipocyte differentiation and is also associated with significantly upregulated PPAR-gamma, CCAAT enhancer-binding protein-alpha (C/EBPalpha), and adipocyte-selective fatty acid-binding protein (aP2) expression in differentiated adipocytes. Conversely, PAI-1 overexpression in differentiated adipocytes inhibits

adipocyte differentiation and is accompanied by decreases in PPARgamma, C/EBPalpha, and aP2 levels (Liang et al. 2006).

6 Activation of Toll-like Receptor 4 by Saturated Fatty Acids

Nutritional fatty acids, whose circulating levels are often increased in obesity, activate TLR4 signaling in adipocytes and macrophages and that the capacity of fatty acids to induce inflammatory signaling in adipose cells or tissue and macrophages (Shi et al. 2006). Fatty acid levels are elevated in obesity and induce inflammatory pathways by an unknown mechanism. Low grade inflammation leads to the development of insulin and leptin resistance. Recent studies show that these effects could be mediated through the activation of TLR (Fresno et al. 2011).

TLR4-induced ER stress may be an obligatory step mediating the saturated fatty acids -mediated endothelial dysfunction (Kim et al. 2015b). TLR4-deficiency protects the mice against high-fat diet-induced ER stress. The mRNA levels of NF-kappaB regulated TNF-alpha, IL-1beta and IL-6 are not changed after hig-fat-diet. Similarly, phospho-IKB-alpha (Ser 32) is not changed after signaling downstream of TLR4. These evidences show that TLR4 is essential for the development of high-fat diet induced ER stress (Pierre et al. 2013). In fact, fatty acids derived from lipolysis of hypertrophic adipocyte are taken up by macrophages and stored as triacylglycerol droplets (Caspar-Bauguil et al. 2015). A paracrine loop involving FFA and TNF-alpha between adipocytes and macrophages establishes a vicious cycle that aggravates inflammatory changes in the adipose tissue (Suganami et al. 2005). Endogenous fatty acids, which are released from adipocytes via the beta3-adrenergic stimulation, activate the TLR4/NF-kappaB pathway. In this respect, large quantities of saturated fatty acids release from hypertrophied adipocytes via the macrophage-induced adipocyte lipolysis. These ligands, induce the inflammatory changes in both adipocytes and macrophages through TLR4/NF-kappaB activation (Suganami et al. 2007). Actually, saturated fatty acids released via adipocyte lipolysis stimulate TLR4 signaling. Activating transcription factor 3 (ATF3) is a member of the ATF/cyclic adenosine monophosphate (cAMP) response element-binding protein (CREB) and its stimulation acts as a transcriptional repressor of saturated fatty acids/TLR4 signaling in macrophages of obese adipose tissue (Suganami et al. 2009). TNF receptor-associated factor 6 (TRAF6) is stimulated in response to fatty acid-induced inflammation. Following activation of TLR4 by saturated fatty acid, TRAF6 is upregulated. Ultimately, TRAF6 induces proinflammatory cytokine production (Tian et al. 2015). Generally, miRNAs prevent protein synthesis through degrading mRNAs and inhibit their translation. In this context, miRNAs have critical roles in TLR4 pathway (Friedman et al. 2009). During TLR4 activation by saturated fatty acids, miRNA-194 expression is downregulated. In contrast, overexpression of miRNA-194 directly decreases TRAF6 expression and attenuates the release of TNF-alpha in saturated fatty acid-activated THP-1 monocytes (Tian et al. 2015). miRNA-146 also directly decreases IL-1 receptor-associated kinase 1 (IRAK1) and TRAF6 expression and attenuates the release of proinflammatory cytokines through the inactivation of NF-kappaB P65 in hypoxia/reoxygenation-induced macrophages (Jiang et al. 2014). Adipocyte hypertrophy and local hypoxia are two of the most important factors that contribute to the accumulation of macrophages in the adipose tissue of obese subjects (Trayhurn 2013). During obesity, adipocytes release pro-inflammatory factors like chemokine (C-C) motif) ligand 2 (CCL2), TNF, or FFA that induce the recruitment and activation of ATMs (Olefsky and Glass 2010). Subsequently, ATMs release TNF-alpha, IL-6, IL-1beta, migration inhibitory factor and resistins. All these proinflammatory factors amplify this inflammatory circuit and leads to insulin resistance by blocking insulin action in adipocytes (Biswas and Mantovani 2012). In particular, IL-6 impairs the activities of the PI3K/Akt/glycogen synthase kinase (GSK) pathway via down-regulating miRNA-200 s. This sug-

gests that miRNA-200 s is a link between IL-6 and insulin resistance (Dou et al. 2013).

TLR4 deficiency attenuates adipose tissue inflammation in obesity but does not impede the accumulation of ATMs. Furthermore, TLR4 signaling plays a direct role in mediating adipose tissue macrophage phenotype in diet-induced obesity (Orr et al. 2012). On the other hand, the TLR and inflammasome pathways drive low-grade inflammation in obesity-related metabolic diseases. Saturated FFAs activate TLRs in macrophages and adipocytes. Thereby, an inflammatory cascade is triggered (Masters et al. 2011). Activation of TLR signaling in adipocytes causes the insulin signaling pathway inhibition, whereas upregulation of TLR4 in macrophages impairs inflammatory response to saturated FFAs and protects against insulin resistance during obesity (Nguyen et al. 2007; Olefsky and Glass 2010). Excessive fatty acid release due to increased basal lipolysis influences whole body insulin sensitivity and enhances adipose tissue inflammation. Ultimately, excessive circulating fatty acids may ectopically accumulate in insulin-sensitive tissues and impair insulin action (Morigny et al. 2016). Unfolded proteins accumulate in the ER under conditions of cellular stress induced by over-nutrition (Schröder and Kaufman 2005). The UPR functioning depends essentially on three main ER sensors involving the activation of ATF-6, protein kinase R (PKR)-like eukaryotic initiation factor 2α kinase (PERK) and inositol requiring enzyme 1 (IRE-1). All of them together act to restrict new protein synthesis and increase the production of chaperones (Lowe et al. 2012). UPR activation links to the development of insulin resistance through JNK activation, inflammation, and oxidative stress (Brasaemle 2007). ER stress is also associated with activation of NF-kappaB and that alpha subunit of translation initiation factor 2 (eIF2alpha) phosphorylation (Deng et al. 2004) While IRS-1 tyrosine phosphorylation reduces, IRS-1 Ser phosphorylation (pS) is enhanced in obese animals compared to lean ones. In these animals IRS-1 concentration remains unchanged, IKKalpha/beta pS and JNK theronine/tyrosine phosphorylation is increased (Zolotnik et al. 2012). Thus, eIF2alpha phosphorylation-dependent NF-kappaB activation associated with decreased levels of the inhibitor IKKalpha protein (Deng et al. 2004). Increased IKKbeta Ser phosphorylation and IRS-1 Ser phosphorylation, both of which independently can have deleterious effects on insulin-stimulated PI3K activation in high-fat diet (Yaspelkis et al. 2009). Saturated fatty acid-induced NF-kappaB activation is partly mediated through the TLR4/PI3K/Akt signaling pathway. Furthermore, saturated and polyunsaturated fatty acids reciprocally modulate the activation of TLR4 and its downstream signaling pathways involving MyD88/IRAK/TRAF6 and PI3K/Akt. Eventually, TLR4-mediated target gene expression and cellular responses are also differentially modulated by saturated and unsaturated fatty acids (Lee et al. 2003).

7 Adipose Tissue Inflammation and Insulin Resistance

Obesity-associated hyperinsulinemia drives adipose tissue inflammation which contributes to factors that suppress insulin-stimulated de novo lipogenesis and systemic insulin sensitivity (Pedersen et al. 2015). Adipokines and chemokines are key mediators that play crucial roles in crosstalk between adipocytes and macrophages in the adipose tissue inflammation (Bai and Sun 2015). TLR4 is activated with the saturated fatty acids, which are released from hypertrophied adipocytes. In this case, macrophage-induced adipocyte lipolysis stimulates NF-kappaB signaling and expression of TNF-alpha and IL-6. Eventually, both adipocyte- and macrophage-induced inflammatory changes through NF-kappaB activation provokes insulin resistance in obesity (Song et al. 2006; Suganami et al. 2007). M1 ATMs exacerbate local inflammation by producing TNF-alpha, IL-6 and IL-1beta. These cytokines subsequently promote insulin resistance. Furthermore, macrophage migration inhibitory factor is enhanced during obesity. This chemokine is directly associated with the degree of peripheral insulin resistance (Finucane et al. 2012), whereas its deficiency

partially protects from high-fat diet induced insulin resistance by attenuating macrophage infiltration (Finucane et al. 2014). Infiltration of cytotoxic T-cells into obese adipose tissue precedes macrophage accumulation. T-cell-derived cytokines promote the recruitment and activation of M1 macrophages. Activated M1 macrophages augment adipose tissue inflammation and insulin resistance (Harford et al. 2011). Moreover, tissue-resident immune cells play a major role in the regulation of obesity-induced inflammation. Besides the adipose tissue macrophages and other pro-inflammatory cells including neutrophils, Th1 CD4+ T cells, CD8+ T cells, B cells, DCs, and mast cells, however, adipose tissue also contains anti-inflammatory cells that counteract to the pro-inflammatory immune cells (Lee and Lee 2014). Compared with adipose tissue from lean subjects, adipose tissue from obese subjects contained increased areas of fibrosis, which correlated inversely with insulin sensitivity. The majority of macrophages are associated with fibrosis. Macrophages in crownlike structures are predominantly M1, but those in fibrotic areas are M2 phenotype with a lower level of IL-1 expression and a higher ratio of IL-10 to IL-12. Thus, adipose tissues of insulin-resistant obese humans demonstrate increased fibrosis with M2 macrophage abundance (Spencer et al. 2010). Human omental adipose tissue fibrosis in severe obesity is consistent with a higher degree of insulin resistance and also contains M2 macrophages. (Guglielmi et al. 2015). Particularly, protein inhibitor of activated signal transducer and activator of transcription 1 (STAT1) (PIAS1) functions in the innate immune system and is a key regulator of the inflammation cascade. PIAS1 inhibits the macrophage infiltration in adipose tissue, thus suppressing amplification of the inflammation cascade, which in turn improved insulin sensitivity (Liu et al. 2015). Adipose tissue from insulin-resistant obese have three-fold to ten-fold increases in numbers of CD4+ T cells that produce interleukin IL-22 and IL-17 compared with metabolically normal insulin-sensitive obese subjects (Fabbrini et al. 2013). Obese human adipose tissue-derived stem cells also induce Th17 promotion and monocyte activation. This proinflammatory environment inhibits insulin response of adipocyte (Eljaafari et al. 2015).

Collectively, there are a number of potential biochemical mechanism for the contribution of proinflammatory signaling to insulin resistance (Wellen and Hotamisligil 2005). In particular, JNKs are activated within numerous metabolically important cells and mediate obesity-associated disruptions in metabolic homeostasis in obesity (Pal et al. 2016). Indeed, FFAs can activate macrophages and bone marrow-derived DCs via TLR2-, TLR4- and JNK-dependent inflammatory pathways. Metabolic pathways utilize immune signaling mechanisms to trigger the activation of CD11c+ proinflammatory cells. Consequently, FFA-induced, TLR-mediated activation of JNK proinflammatory pathways in CD11c+ immune cells promotes inflammation and subsequent cellular insulin resistance (Nguyen et al. 2007). Actually, JNK can be activated by FFAs through TNF-alpha-independent mechanisms. In obesity, activated JNK is a major contributor to FFA-induced cellular insulin resistance. Furthermore, TNF-alpha is a downstream effector of activated JNK (Nguyen et al. 2005). TNF-alpha, through activation of p38 MAPK and IKK, produces Ser phosphorylation of insulin resistance and IRS-1. Actually, its tyrosine phosphorylation is impaired by insulin. Eventually the corresponding activation of PI3K and Akt, leads to insulin resistance (de Alvaro et al. 2004). TNF-alpha mediates insulin resistance by enhancing adipocyte lipolysis through the stimulation of JNK and IKKbeta/NF-kappaB pathway which may increase Ser/Thr phosphorylation. IL-1beta also contributes to insulin resistance by impairing insulin signaling in peripheral tissues and macrophages. Macrophages from insulin-resistant obese are associated with increased production. In this case, forkhead transcription factor-1 (FOXO1) correlates with elevated levels of IL-1beta mRNA. Furthermore, FOXO1 signaling through NF-kappaB induces insulin resistance by producing proinflammatory cytokines in obesity (Su et al. 2009). Leptin-induced signaling via STAT3 rapidly activates the negative feedback regulator suppressor of cytokine signaling-3 (SOCS3), which inhibits leptin-induced signal

transduction (Wunderlich et al. 2013). Among the most prominent cytokines that are over-represented in the bloodstream of obese individuals with obesity are TNF-alpha and IL-6; however only IL-6-induced signaling is mediated via JAK and STAT3 (Heinrich et al. 2003). In chronic JAK-STAT3-SOCS3 signaling in obesity, the IL-6-bound receptor complex activates intracellular JAK2 subsequently leading to STAT3 activation, which in turn to elevating SOCS3 expression (Wunderlich et al. 2013). During the progression of obesity IL-6 may induce insulin resistance through two different signaling pathways. Firstly, IL-6 induces insulin resistance by activating STAT3 and upregulating the transcription of its target gene SOCS3. These effects are consistent with the IL-6-dependent activation of extracellular-related kinase 1/2 (ERK1/2), a Ser-Thr protein kinase involved in Ser STAT3 phosphorylation (Serrano-Marco et al. 2012). Secondly, IL-6 induces insulin resistance by impairing the activation of the PI3K/Akt/GSK pathway and glycogenesis (Dou et al. 2013).

8 Oxidative Stress

Obesity can induce systemic oxidative stress through superoxide generation from NADPH oxidases, oxidative phosphorylation, glyceraldehyde auto-oxidation, PKC activation, and polyol and hexosamine pathways. Additional factors are relevant to the initiation of oxidative stress in obesity include hyperleptinemia, low antioxidant defense, chronic inflammation, and postprandial ROS generation (Manna and Jain 2015). Adipocytes from insulin resistant obese subject exhibit increased oxidative stress and impaired antioxidant defense. Additionally, proteasome activity is impaired in adipocytes of diet-induced obese mice. In fact, proteasomal dysfunction in adipocytes results from protein oxidation and protein misfolding. Actually, this event constitutes major pathogenic mechanism in the development of insulin resistance in obesity (Díaz-Ruiz et al. 2015). When cellular antioxidant and repair pathways fail to restore protein oxidative damage, cells activate additional defense mechanisms to prevent the accumulation of oxidized proteins. Oxidized protein residues are toxic for cells and threaten cell viability. Actually, proteasomes provide a second line of defense against the free radicals and oxidants (Grune and Davies 1997). Thereby the removal of damaged proteins is extremely important for the maintenance of normal cell function. In this context, the 20S proteasome functions primarily for removal of damaged proteins (Pickering and Davies 2012). The enhanced proteolytic activity of the proteasome is important for the preservation of cell viability. Activated proteasomes are better able to degrade oxidized proteins than the standard proteasome. Direct comparison of purified 20S proteasome and immunoproteasome demonstrated that the immunoproteasome can selectively degrade oxidized proteins (Pickering et al. 2010; Seifert et al. 2010). Proteasome dysfunction mediates obesity-induced ER stress, leading to hyperactivation of JNK and insulin resistance (Otoda et al. 2013). Inflammatory pathways diminish UPR function through inducible nitric oxide synthase (iNOS)-mediated S-nitrosylation of IRE-1alpha, which contributes defective IRE-1alpha activity, impaired ER function, and prolonged ER stress in obesity (Yang et al. 2015). Peroxisomal fatty acid metabolism, cytochrome P450 microsomal reactions and the mitochondrial respiratory chain generate large amount of free radicals, which is associated with an irregular production of adipokines. Eventually high ROS production and the decrease in antioxidant capacity lead to adipose tissue inflammation (Fernández-Sánchez et al. 2011). Formation of advanced glycation endproducts/advanced-lipoxidation endproducts and its precursors, including methylglyoxal (MGO), are increased in conditions characterized with hyperglycemia, hyperlipidemia and enhanced oxidative stress. This metabolic profile is typical for obesity (Gaens et al. 2013). Glutathione S-transferase A4 (GSTA4)-dependent production of proinflammatory glutathione metabolites, glutathionyl trans-4-hydroxy-2-nonenal (GS-HNE) and its metabolite glutathionyl-1,4-dihydroxynonene (GS-DHN) are produced by adipocyte in response to oxidative stress. In this case, increase in lipid peroxida-

tion causes nonenzymatic formation of GS-HNE and GS-DHN. These metabolites have the potential to maintain obesity-induced inflammation, adipocyte dysfunction and insulin resistance (Frohnert et al. 2014). Obesity-induced oxidative stress in adipocytes as well as the presence of inflammatory changes in adipose tissue may lead to activation of tissue-resident macrophages (Frohnert and Bernlohr 2014). Upregulation of NADPH oxidase in multiple tissues occurs as a systemic response in obesity (Jiang et al. 2011). Intracellular ROS formation occurs upon stimulation of insulin in adipocytes. However, preadipocytes respond to insulin to a higher degree than that of adipocytes. Furthermore, NADPH oxidase 4 (NOX4)-mediated ROS could be released intracellularly and triggers adipogenesis in preadipocytes. Thus, long-term high fat diet results in massive obesity with adipocyte hypertrophy and hyperplasia. Subsequently, a significant decrease occurs in NOX4 expression. NOX4 is a hallmark for relative adipocyte mass and differentiation (Mouche et al. 2007). Additionally, NOX2-derived ROS destroys insulin receptor and endothelial function in dietary obesity (Du et al. 2013). Mitochondria are not only a target for ROS produced by NADPH oxidase but also a significant source of ROS in obesity, which may stimulate NADPH oxidases (Dikalov 2011). On the one hand, saturated fatty acids induce carnitine palmitoyltransferase-1 expression, which may contribute to saturated fatty acid-induced ROS overproduction. In this process, accelerated beta-oxidation causes excessive electron flux in the respiratory chain. Excess electrons supply for mitochondrial oxidative phosphorylation contributes the over-production of ROS (Nakamura et al. 2009). On the other hand, protons can reenter the mitochondrial matrix through different uncoupling proteins, affecting the control of free radicals production by mitochondria. Disorders of the mitochondrial electron transport chain, over-generation of ROS and lipoperoxides or impairments in antioxidant defenses have important causal role in obesity-related inflammation (Martínez 2006). Moreover, increased expression of NADPH oxidases associated with obesity causes dysregulated production of adiponectin, PAI-1, IL-6, and MCP-1, and reduced expression of detoxifying enzymes (Kim et al. 2006). TNF-alpha itself stimulates endothelial NOS (eNOS) activity to generate nitric oxide (NO) through a pathway involving its lipid messenger, ceramide in obesity (Bulotta et al. 2001). In this case, TNF-alpha stimulates eNOS phosphorylation at Ser 1177 via PI3K-Akt signal transduction pathway (Kawanaka et al. 2002). Furthermore, under the conditions of nutrient excess and obesity, phosphorylation of eNOS is diminished due to saturated fatty acid-mediated induction of insulin resistance (Kim et al. 2008). Eventually elevated FFAs lower NO bioavailability (Steinberg et al. 2000) by downregulating the PI3K/Akt/eNOS-dependent insulin signaling axis (Madonna and De Caterina 2009). On the other hand elevated levels of FFAs in obesity trigger macrophage activation (Li et al. 2010). Macrophages and dendritic cells express MMP12 that restrains adipose tissue expansion while promoting the expression of inflammatory mediators. In inflammatory conditions, iNOS is expressed at high levels by DCs or macrophages. The expression of MMP12 mRNA by CD14+ CD206+ macrophages varies 100-fold in human subcutaneous adipose tissue. Moreover the MMP12 is localized in crown-like structures in adipose tissue. This indicated that MMP12 regulates NO generation by macrophages during inflammation (Lee et al. 2014). NADPH oxidases and xanthine oxidase, can be activated and produce large amounts of superoxide. Ten-fold increase in superoxide generation with the simultaneous NO formation will increase peroxynitrite formation by 100-fold. Under proinflammatory conditions, simultaneous production of superoxide and NO can be strongly activated to increase production 1000-fold, which will increase the formation of peroxynitrite by a 1,000,000-fold (Pacher et al. 2007). Hence, the expression of iNOS is an important aspect of obesity-associated chronic inflammation. As shown, simultaneously increased production of ROS could limit NO bioavailability because they convert NO to peroxynitrite that in turn leads to nitrosative stress (Codoñer-Franch et al. 2011).

Innate lymphoid type 2 cells, forkhead box P3 (FOXP3)-positive regulatory T (Treg) cells,

and IL-10 counteract the low grade inflammation and insulin resistance in classical or metabolically healthy obesity (Pereira and Alvarez-Leite 2014). In fact, metabolically healthy obese individuals have effective immunoregulation to resist chronic obesity-related inflammation. These regulatory mechanisms could be important in the delayed onset of metabolic complications, even in extremely obese individuals (Pereira et al. 2014). Actually, Treg cells production is decreased in diet-induced obese mice. Meanwhile, high-fat diet also enhances apoptosis of Treg cells by diminishing their mitochondrial transmembrane potential. In this context, high-fat diet increases ROS productions and significantly decreases antioxidant enzymes expressions in the Treg cells of diet-induced obese mice (Wang et al. 2014). Basic unit of mitochondrial permeability transition pore consists of the voltage-dependent anion channel (VDAC), adenine nucleotide translocase (ANT) and the matrix-located regulatory subunit cyclophilin-D (Cyp-D). Oxidant-stimulated formation of VDAC-adenine nucleotide translocase-CypD complex can agglomerate a number of apoptotic proteins. The apoptotic pathway is triggered by the release of apoptotic proteins through the mitochondrial permeability transition pore openings. The pore complex-induced membrane rupture promotes the release of apoptosis-inducing factors during the apoptosis (Crompton 1999).

9 Hypoxia and Adipose Tissue Inflammation

In the adipose tissue, hypoxia is associated with the increased expression of inflammatory genes and decreased expression of adiponectin. In diet-induced obesity, subsequent reduction in body weight by calorie restriction is associated with an improvement of oxygenation and a reduction in inflammation (Ye et al. 2007). Adipocyte death occurs during rapid expansion of adipose tissue in hypoxic conditions. Indeed, adipose tissue hypoxia may result in disturbances in adipokine secretion, increased macrophage infiltration and inflammation in obese adipose tissue (Goossens 2008). Hypoxia develops when oxygen supply does not meet the demand of the surrounding tissues. Actually, oxygen diffusion is limited at most to 100 μm, whereas in obesity, hypertrophied adipocytes up to 140–180 μm in diameter. This excessive distance between the hypertrophic adipocytes creates a relatively hypoxic state. In brief, local tissue hypoxia and hypoperfusion of adipose tissue is common feature in obese subject. Adiponectin and PPAR-gamma mRNA expressions are reduced while PAI-1 level is increased in hypoxic adipocytes (Hosogai et al. 2007). Angiogenesis influences white adipose tissue expansion. However, the number of vessels per adipocyte and the expression level of receptor 2 of vascular endothelial growth factor (VEGF) is incompatible with the increasing number of adipocytes in obesity (Lemoine et al. 2012). In obese patients, even serum VEGF is positively associated with visceral fat content compared with lean subjects (Miyazawa-Hoshimoto et al. 2003), overweight/obese subjects have 44% lower capillary density and 58% lower VEGF. This might be due to lower PPAR-gamma 1 and higher collagen 6 mRNA expression, which correlate with adipose tissue oxygen concentration and adipose tissue inflammation in obesity (Pasarica et al. 2009). In addition, to induce the VEGF expression in adipocytes of obese subject the PI3K-Akt pathway is required for HIF-1alpha activation (He et al. 2011). Induction of HIF-1alpha in diet-induced obesity does not increase VEGF-A. Adipose tissue hypoxia serves as an early upstream initiator for adipose tissue dysfunction by inducing local fibrosis (Halberg et al. 2009). Furthermore, hypoxia stimulates the expression of the genes encoding angiopoietin-like protein 4, IL-6, leptin, MIF and PAI-1 (Wang et al. 2007). In particular MIF is a potent macrophage migration inhibitory factor and is expressed by human adipocytes (Skurk et al. 2005). Thus, increased expression of MIF mRNA in inflamed adipose tissue indicates the activation of MIF gene transcription in response to adipose tissue inflammation (Kim et al. 2015a). Consequently, elevated MIF expression enhances angiogenesis as well as macrophage recruitment (Wang et al. 2007), whereas, MIF deficiency partially protects

the organism from high-fat diet induced insulin resistance by attenuating macrophage infiltration to adipose tissue, ameliorating adipose inflammation (Finucane et al. 2014).

10 Conclusion

Lipid overload in obesity can indirectly stimulate the pro-inflammatory state. In addition to peripheral tissues, the hypothalamus is also similarly vulnerable to ectopic lipid accumulation and lipotoxicity (Lam et al. 2005). Excess lipids in the adipocyte increase the substrate load, but inefficient mitochondrial oxidative phosphorylation leads to generation of ROS that can damage mitochondrial constituents in obesity-associated inflammation (Goh et al. 2016). In this respect, suggested therapeutic measures in obesity should involve the mechanisms that have been described regarding the corrective effects on AMPK/IRS-1/PI3K and TLR4/PI3K/Akt signaling pathways, production and release of pro-inflammatory adipokines, both mitochondrial dysfunction and mTOR hyperactivation, abundantly released cytokines by adipocytes and insulin resistance.

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Microbiota and Lipotoxicity

10

Evren Doruk Engin

Abstract

Obesity and metabolic syndrome is a multisystemic disorder, that is characterized by excess caloric intake and spillover lipotoxicity caused by ectopic lipid accumulation in non-adipose tissues. Low grade chronic inflammation and insulin resistance are the hallmarks of the disorder, which further aggravate the condition. Gut microbiota constitutes an indispensible part of human superorganism's energy harvesting apparatus. The dynamic composition of microbiota changes with age, life style and host metabolic background. The wealth of genetic repertoire provided by these microorganism enables to extend host's substrate processing and harvesting capability. Some of these compounds including short chain fatty acids and indole act as signalling molecules on mammalian cells and modulate their behaviour. Nonetheless, this symbiotic style of interaction is restrained by immune system. The role of chronic low grade inflammation in metabolic syndrome is well established. Treg cells are the key players that sense and reshape the composition of microbiota. In this regard, any disturbance in Treg functionality may aggravate the inflammation and shift the symbiotic balance towards dysbiosis, which is characterized by autoimmunity and insulin resistance. Thus, immune system is responsible for the modulation of host and microbiota metabolisms and Treg cells act as a bridge in between.

Keywords

Gut microbiota • Metabolic syndrome • Insulin resistance • Treg cells

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1 Introduction

Evolution has provided us with an efficient survival system, which enables the organism to deposit surplus lipids in specialized cells called adipocytes. The fuel homeostasis of the organism depends on the ability of these cells to prevent the overwhelming of the limited lipid storage capacity of nonadipocytes, which otherwise would cause destructive lipid overload—lipotoxicity. Ultimately, this caloric backup is meant to be spent at times of starvation (Unger et al. 2010).

Despite the ceaseless complaints of modern man of the tremendous pace of life, industrialization has yet brought over-automation to our lives, to let us sit down silently and have some sort of machine to do the laborous work. In accompaniment to this physically inactive lifestyle, the shift from family kitchen to commercially prepared food has become the hallmark of the modern times. The change in eating habits not only expanded our caloric intake, but also introduced highly processed food to our diets. Obviously, this discrepancy leads to a spillover and ectopic accumulation of lipids, causing lipotoxicity, a condition that is deleterious for nonadipose organs (Mazidi et al. 2016; Zimmet et al. 2001).

Adipocytes are not just lipid laden sacks or formless mass of connective tissue, instead they exploit critical metabolic and hormonal roles. Both lipid metabolism and feeding behaviour is orchestrated by the complementary activities of adipocytes and hypothalamus. Adipocyte hormone leptin plays a key role via limiting the caloric inflow and enhancing fatty acid oxidation (Unger et al. 2010).

Arguably, perpetual excess caloric intake disrupts this delicate balance, triggering an array of consequences which culminate in a pathological condition called metabolic syndrome. Substantial damage ensues in nonadipocytic target organs by the virtue of ectopic lipid accumulation. Pancreatic islets, liver, heart, skeletal muscle and kidneys become primarily affected by this insult, imparting in the resulting clinical picture accordingly (Guida and Venema 2015). The whole picture is accompanied by a low grade inflammation and emerging insulin resistance. These factors aggravate metabolic syndrome, which in turn, puts the patient in a viscious cycle. Therefore, obesity and its consequences present as a multisystemic disorder, which affect both longevity and the quality of life of the patient. Worst of all, this insidious, yet relentless multisystemic disease has become epidemic, especially among high-sugar, high-fat Western-style diet consumers (Zimmet et al. 2001).

2 The Superorganism

While traditionally, the disturbance of the balance between caloric input and expenditure have been emphasized as being the main contributors to obesity and metabolic syndrome, the role of microbial flora had long been overlooked. Although the presence of indigenous microbiota has been identified more than three centuries ago, the symbiotic rather than merely physical nature of this coexistence has relatively recently been appreciated. As we gain deeper understanding of the intricate interactions between the microbiome and the host, the term “human superorganism” gets fulfilled (Relman 2012).

From an evolutionary perspective, the macroevolution of eucaryotes has leaded the emergence of animals, as super-active and effective consumers, which has to absorb the resouces from autotroph plant cells, the primary producers. However, animals are cytoplasm specialists, the consumers of the soft parts of plants, thus, are not equiped with mechanisms to deal with the protective cell wall component by themselves. Coping with this rugged cell wall material requires the a special set of decomposing enzymes which are readily supplied by cell-wall specialist microorganisms (Abe and Higashi 1991). The extent of metagenomes of animal species, as superorganisms, are deeply affected by their feeding behaviours. The partititoning of mammalian fecal microbial diversity reflects the complexity of carbohydrate substrates accessed. Herbivore microbiotas contain as much as 14 bacterial phyla, along with specially evolved gut morphology to accommodate extensive fermentation process. Accordingly, omnivores harbor 12 and carnivores only 6 bacterial phyla (Ley et al. 2008).

Average human "superorganism" gut is inhabited by 1×10^{13} to 1×10^{14} microorganisms which roughly spans 1000 species of bacteria, yeast and parasites. Together with other body sites, a typical microbiome surpasses the human cells by a factor of 10. Collectively, this biodiversity encompasses 2 orders of magnitude more genes than the human host. Along with the tremendous metabolic repertoire granted by this gene pool, gut microbiota constitutes a substantial biomass of 2 kg, which actively renews itself every 3–4 days. Essentially, the distal intestine can be regarded as an anaerobic bioreactor. In this respect, gut microbiota is comparable to a regular organ in the body, both in terms of biomass and metabolic function (Mazidi et al. 2016). With its up to 40 m^2 surface area, gut is the most important interface to exterior. Maintanence of the metabolic and immunological homeostasis is the most important function of highly specialized absorptive, secretory and enteroendocrine cells of the gut in concert with the microbiota (Helander and Fändriks 2014). Collectively, assistance in defence against pathogens, maturation of immunity and degredation of non-digestible polysaccharides has been implied for gut microbiota. Notably, the vascular development in small intestinal villi has been found dependent on the interaction of gut microbiota with Paneth cells. These cells are placed in the crypt bases as key cellular component of the innate immune system. In response to microbial products, they secrete an array of peptide products, ranging from antimicrobial peptides to factors affecting the stem cell niche towards angiogenesis. Fully developed microvasculature further enhances the absorbtive capacity of the intestine (Stappenbeck et al. 2002).

In mammalians, the members of this microbial mass exhibit an uneven distribution over the length of the gastrointestinal tract. Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, Verrucomicrobia, Cyanobacteria, Fusobacteria, Spirochaetes and TM7 make the main components of mammalian gut microbiota (Table 10.1). Cell mass and diversity increases from duodenum to colon (Brown et al. 2013). The metabolic role of protozoa—which mostly belong to the class *Kinetofragmnophorea*—in the herbivorous digestive tracts has been well established. Along with bacterial biomass, these mostly anaerobic protozoa participate in fermentation of plant polysaccharides to volatile fatty acids, lactic acid, carbon dioxide and hydrogen (Dehority 1986). However, there is still a controversy concerning "normal" protozoal flora members in humans. Essentially, Blastocystis is one of the well studied organisms in the "grey zone", as it could neither be assigned as a pathogen or a commensal. Blastocystis has been shown to colonize healty individuals, without any evidence of symptomatic carrier status. Furthermore, Audebert et al. has recently shown that Blastocystis colonization is associated with healty gut microbiota (Audebert et al. 2016). On the other hand, dragonflies infected with noninvasive gregarine parasites have been shown to manifest the markers of metabolic syndrome—inappropriate hemolymph carbohydrate response to insulin, thoracic fat accumulation, chronic p38 MAP kinase involved immune and stress response activation (Schilder and Marden 2006). This might be a promising experimental model for parasite induced metabolic syndrome.

Table 10.1 The composition of microbial species along mammalian gastrointestinal tract (Brown et al. 2013)

Gut segment	Organisms
Duodenum	*Streptococcus*, *Lactococcus*, *Staphyllococcus*
Jejunum	*Lactobacillus*, *Streptococcus*, *Enterococcus*, Yeast species
Ileum	Segmented filamentous bacteria, Enterobacteriaceae, *Bacteroides*, *Clostridium*
Colon	*Bacteroides*, *Clostridium*, Lachnospiraceae, Proteobacteria, Actinobacteria, Prevotellaceae, TM7, Fusobacteria, Verrucomicrobium

3 The Superorganism in Action

Dysbiosis indicates a microbial imbalance or maladaptation on or inside the body and has been associated with a number of pathological conditions like inflammatory bowel disease, obesity and cancer. In an effort to explain the breakdown

in the balance between "protective" versus "harmful" intestinal bacteria, numerous theories have been considered. However, the exact mechanisms that transform our "mutualistic" symbionts remains to be explored (Tamboli et al. 2004).

Gordon group has demonstrated the role of gut microbiota in the regulation of fat storage in adipocytes. They used germ free mice which maintained their lean phenotype, even under Western-style diet, until they were colonized with cecal microbiota from regular "conventionally" fed animals. As soon as the gut microbiota is restored, significant increase in body fat ensues, accompanied by relative insulin resistance in these "conventionalized" animals. Gut microbiota conveys the enzymes required to breakdown complex carbohydrate bearing dietary fibres, which otherwise are non-digestible by human intestinal cells. Reasonably, the enhanced release of these lipogenic substrates make them available for absorption (Bäckhed et al. 2007). The microbiota encoded genes for a large number of glycoside hydrolyses are required to convert these complex fibres made up of xylan, pectin and arabinose containing carbohydrates. Actually, germ free mice require at least 30% more calorie intake to maintain their body weight (Hsiao et al. 2008). This immense contribution to the energy harvesting and metabolic capacity of the gut, places the microbiome at a pivotal position in the regulation of energy metabolism. Furthermore, processing and harvesting of dietary polysaccharides into monosaccharides, increased insulin levels and induce the translocation of two transcription factors; carbohydrate response element binding protein (ChREBP) and sterol response element binding protein 1 (SREBP-1) to nucleus in liver cells. Acetyl-CoA carboxylase (Acc1) and fatty acid synthase (Fas) are the two lipogenic enzymes which are under control of these transcription factors (Su et al. 2015). The triglyceride cargo of liver generated lipoproteins is carried away to the pheripheral tissues, taken up by the virtue of lipoprotein lipase (LPL) and stored in the adipocytes. Fasting induced adipose factor (FIAF) is one of the potent inhibitors of LPL. FIAF also promotes degradation of triglycerides into fatty acids, which are in turn metabolized. The presence—and possibly the composition—of gut microbiota have been shown to inhibit the action of FIAF, therefore, enhance triglyceride accumulation in adipocytes (Bäckhed et al. 2004). The mode of action of FIAF is evolutionarily conserved. As in germ free mice, FIAF suppression and subsequent increase in body fat also occurs in conventionalized gnobiotic zebrafish (Rawls et al. 2004).

Notably, as measured by means of oxygen consumption, the metabolic rate in conventionally fed or conventionalized animals has been found higher than germ free mice—a discrepancy, that is attributable both to the metabolic activity of the microbiota itself and the changes induced in the host energy metabolism (Bäckhed et al. 2004).

Ileal enteroendocrine L cells devise another important corner stone in the elaborate interaction between gut microbiota and the host. L cells, act as chemical and immunological sensors that respond to nutrients, microbial products and cytokines and in turn, produce an array of peptide hormones, including peptide YY, glucagon like peptide-1 (GLP-1) and glucagon like peptide-2 (GLP-2). The later two are processed from proglucagon polypeptide and secreted in equimolar quantites, yet with opposing effects. GLP-1 induces insulin secretion from pancreatic beta cells, slows down gastric emptying, decreases triglyceride rich apoB48 lipoproteins. By the virtue of these effects, GLP-1 has been considered neuroprotective, cardioprotective and antiinflammatory (Hsieh et al. 2010). Nonetheless, GLP-2 has been associated with enhanced intestinal lipid absorbtion, production and secretion of triglyceride rich apoB48 containing chylomicrons in CD36 scavenger receptor mediated manner. Accordingly, the activities of GLP-2 has been linked with postprandial hyperlipidemia, metabolic dyslipidemia, insulin resistance and eventually, atherosclerosis (Hsieh et al. 2009). The favorable effects of GLP-1 on metabolic syndrome have appealed considerable therapeutic interest. Dipeptidyl peptidase-4 (DPP4) is responsible for removal of GLP-1 and another incretin hormone, gastric inhibitory polypeptide

(GIP). Sitagliptin proves beneficial by its inhibitory effect on DPP4. Recombinant GLP-1 derivative GLP-1R agonist peptides have also been proven useful. Extendin-4 but not extendin(9–39) improved insulin sensitivity (Knudsen et al. 2007).

4 Sensing the Quorum

The bacterial fermentation products of dietary carbohydrates consist of butyrate, propionate and acetate. These short chain fatty acids have been depicted for activating G protein coupled receptors on enteroendocrine L cells' plasma membranes, where they stimulate GLP-1 secretion (Cani et al. 2013). Another key molecule that provide trans-kingdom communication is the abundant tryptophan metabolite, indole. Bacteria from genera Escherichia, Bacteriodes and Clostridium bear tryptophanase which metabolize tryptophan into indole, pyruvate and ammonia. Indole has lately been realized as a quorum sensing pheromone (Arora et al. 2015; Lee and Lee 2010; Mueller et al. 2009). The effects of indole on the permeability of mammalian cell membranes had been attributed to its ability to interact with lipid bilayer (Bean et al. 1968; Gaede et al. 2005). However, indole have recently been shown to inhibit voltage gated potassium channels on enteroendocrine L cells, where it modulates the action potential waveform by enhancing Ca^{2+} influx. In turn, indole stimulates GLP-1 release to the portal circulation (Chimerel et al. 2014). Apart from indole, vast number of quorum sensing pheromone molecules remain to be explored, which serve to microbiota—host intercommunication.

5 Immune System: The Daemon of Symbiosis

The maintainence of symbiotic nature of gut microbiota depends on perfectiy balanced defence mechanisms. The intact epithelial layer that covers more than 30 m^2 of surface area is composed of various types of highly specialized cells (Helander and Fändriks 2014). While enterocytes consititute the most abundant cell type, the mucin producer goblet cells and antimicrobial peptide secreter Paneth cells contribute to the integrity of the mucosal barrier (Ismail and Hooper 2005). The epithelial barrier is further fortified by the presence of gut—associated lymphoid tissue (GALT), which is diffusely inhabit submucosa. With its enormous microbial load, gut mucosa is a site for challenge with potential pathogens. Together with the mucosa layer, GALT continously samples antigens that pass from lumen and mount an appropriate response (Oviedo-Boyso et al. 2014). Immune system provides protection against pathogens by the virtue of its ability to discriminate between "self" and "non-self". The innate immune system achieves this by means of receptors that recognize microbe associated molecular patters (MAMPs). Successful recognition of these patterns by innate immunity is the prerequisite in order to proceed to adaptive humoral and cellular responses, accordingly (Jiang and Chess 2009). However, the prosperity of the symbiosis in gut largely depends on the balance between "protective" versus "harmful" lumen microorganisms (Tamboli et al. 2004). Therefore, in the case of gut microbiota, the immune system is not only obligated to discriminate between self and non-self, but also, has to be tolerant against symbiont non-selves.

Lipopolysaccharide, peptidoglycan, lipoteichoid acid, lipoarabinomannan, lipopeptides, lipoglycas, lipomannans, glycosylphophatidylinositol, CpG DNA and dsRNA are important extracellular and intracellular MAMPs. Toll-like receptors (TLRs) and nod-like receptors (NLRs) are two important groups among a broad range of pattern recognition receptors (PRRs). An array of TLRs have been identified that specifically recognize individual MAMPs (Fig. 10.1) (Abreu et al. 2005; Kawai and Akira 2007; Oviedo-Boyso et al. 2014). Most members of TLR family rely on myeloid differentiation primary response gene 88 (MyD88) dependent signalling, whereas, TLR3 and 4 uses TIR-containing adapter inducing IFN-β (TRIF) pathway. Consequently, nuclear translocation of NFκB, AP-1 and interferon regulatory factor 3 (IRF3) leads to the

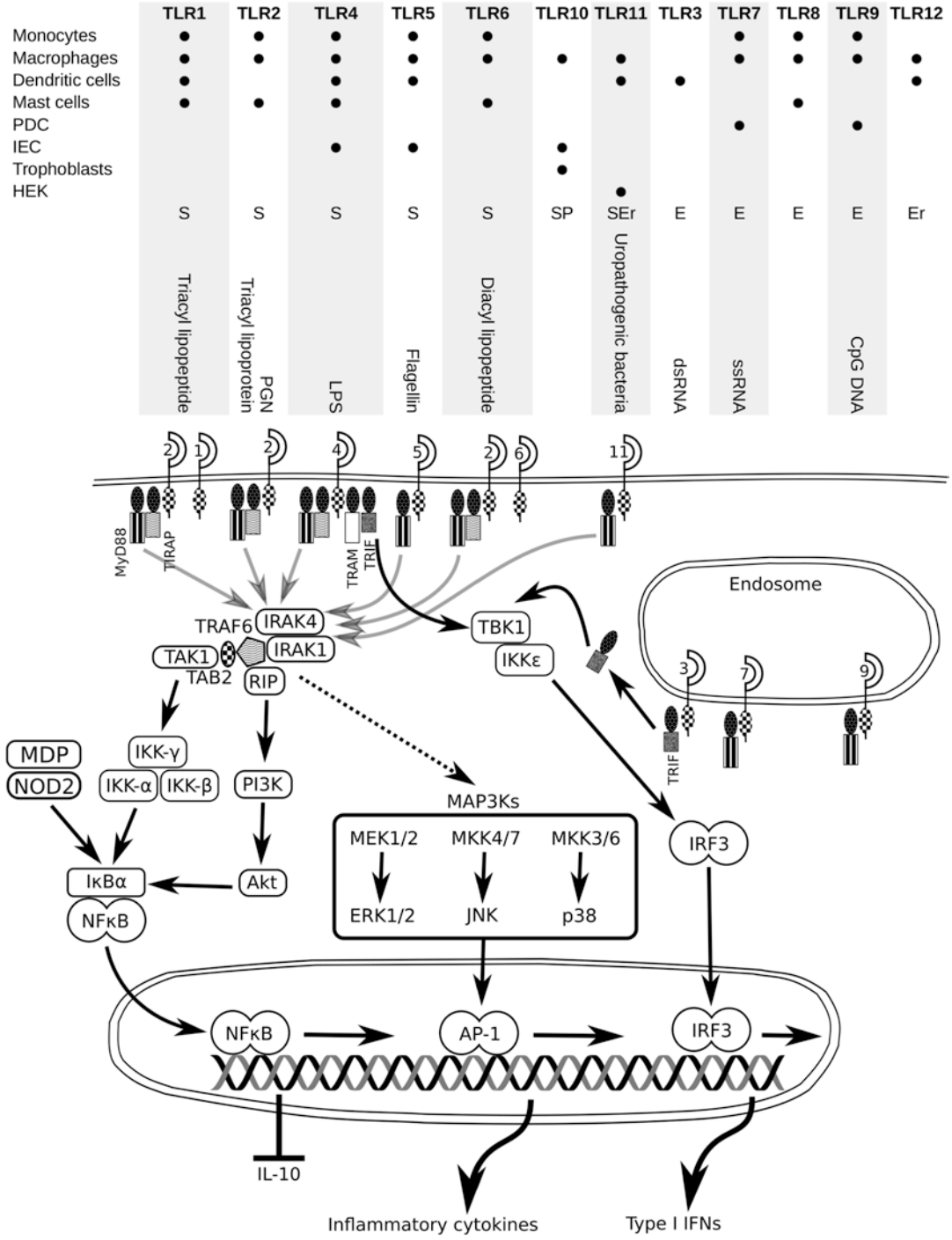


Fig. 10.1 Toll-like receptors, nod-like receptor NOD2 expressing cells, signalling pathway. *IRAK* IL-1 receptor-associated kinase; *TRAF* TNF receptor associated factor; *MAP3K* Mitogen activated protein kinase kinase kinase; *IκB* Inhibitory kappa B; *IKK* IκB kinase; *TAK* TGFβ-activated kinase; *TAB* TAK1 binding protein; *TBK* TANK-binding kinase; *PI3K* phosphoinositide 3-kinase, *Akt* Protein kinase B (Abreu et al. 2005; Cario 2005; Kawai and Akira 2007; Oviedo-Boyso et al. 2014)

expression of inflammatory cytokine and type I interferon genes (Oviedo-Boyso et al. 2014). NLRs are responsible for intracellular detection of a similar array of MAMPs (Cario 2005).

The LPS induced loss of glycemic control in sepsis has long been anticipated (Steven et al. 2015). However, the link between inflammation and metabolic disorders has been coined out by Hotamisligil et al. (Hotamisligil et al. 1993). Its now well established that, the inflammatory and metabolic responses evoked by obesity, induces low grade local inflammation, defective insulin signalling and disrupted metabolic homeostasis (Hotamisligil 2006). The extent of the surface area makes gut the most important interface with the external environment. Thus, gut is a major player in metabolic and immune homeostasis. Its mainly adaptive immune systems function to limit the responses, via tolerance mechanisms, in order not to shift symbiosis towards dysbiosis in the gut (Tamboli et al. 2004).

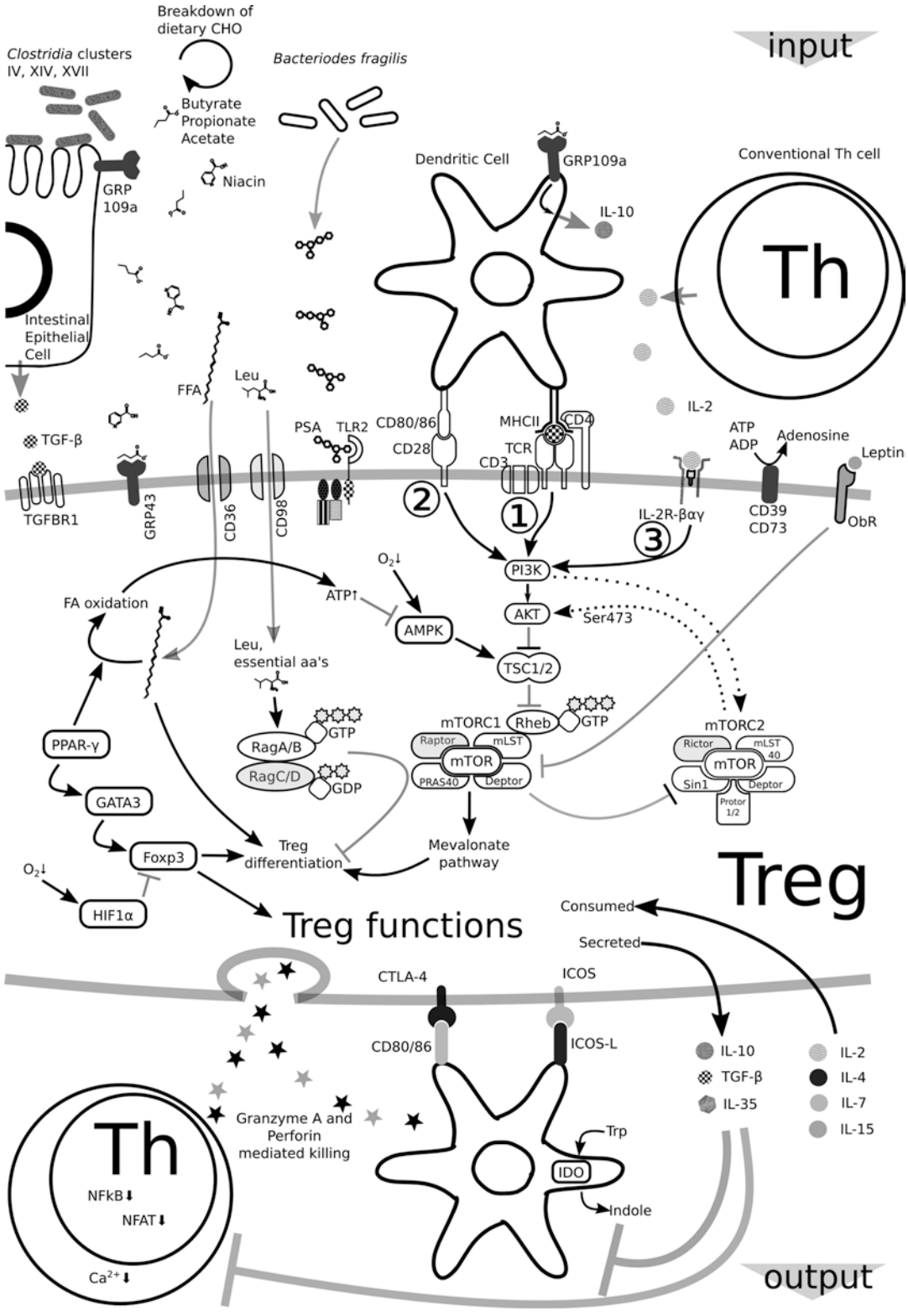
The idea of regulatory T cells is around since seventies. Yet, the role of $Foxp3^{+}CD25^{+}CD4^{+}$ Treg cells in the regulation of immune system has recently been anticipated (Sakaguchi et al. 2007). The activation and differentiation of naive Th cells to effector Th1, Th17 and Th2 subclasses require cognate antigen stimulation, accompanied by an instructive cytokine milieu. Treg cells can either be of thymic origin or can be generated from naive gut associated lymphoid tissue (GALT) Th cells, by the virtue of multiple signals integrated from the microenvironment. Thus, nutrient molecules, vitamins and hormones along with microbiota derived factors are deterministic on Treg generation and homeostasis (Howie et al. 2014; Zeng and Chi 2015). Intuitively, malnutrition has been associated with nutrition immunity link. Likewise, it has been realised that, dysregulation of the immune system causes insulin resistance and diabetes (Mathis 2013). By balancing host and microbial metabolism, Treg cells act as a bidirectional bridge between immune system and metabolic fitness.

As in the conventional T cells, TCR-CD4-CD3-MHCII complex formation (signal 1), CD28-CD80/86 costimulation (signal 2) and CD25-α chain bearing high affinity IL-2R activation (signal 3) are prerequisites for Treg activation. Unlike conventional T cells, Treg's also strictly require TGF-β signal (Fig. 10.2).

The serine/threonine kinase mTOR (Mechanistic target of rapamycin), is the principle integrator of nutrient sensing. Upon complexing of mTOR with Raptor or Rictor, mTORC1 (complex 1) or mTORC2 (complex 2) is formed respectively, as an early event in T cell activation. The intensity, duration and type of mTOR signalling determines the fate of the naive Th cell. Initially low level of mTORC1 and sustained low level of PI3K/Akt activation is strictly required for Treg differentiation. This signalling profile is consistent with lipid oxidation in aerobic conditions (Han et al. 2014). The regulator of fat metabolism, PPAR-γ (Peroxisome proliferator activated receptor gamma), increases CD36 scavenger receptor expression, in order to enhance lipid uptake fatty acid oxidation. The hallmark of Treg cells, Foxp3 (Forkhead Box P3) is also activated by PPAR-γ (Zeng and Chi 2015). The timely formation of mTORC1, activates mevalonate pathway and along with the expression of Treg effector molecules, CTLA-4 (Cytotoxic T-lymphocyte associated protein 4) and ICOS (Inducible T-cell COStimulator). G-protein coupled receptors GRP41, GRP43 and GRP109a, sense short chain fatty acids (butyric, propionic and acetic acid) and niacin. Treg GRP43 helps to sustain high levels of fatty acid oxidation (Fig. 10.2) (Cani et al. 2013).

CTLA-4 and ICOS bind to CD80/86 and ICOS-L on dentritic cells respectively, to induce IDO (Indolamine 2,3-deoxygenase). IDO mediated transformation of tryptophan to indole, causes depletion of this amino to further contribute immunological tolerance (Fallarino et al. 2003). Treg made granzyme A and perforin is directed to kill reactive dentritic and T cell populations (Schmidt et al. 2012). Treg CD39/CD73 mediated ATP hydrolytic activity causes energy depletion, further enhacing the suppressive function (Fig. 10.2) (Cani et al. 2013).

On the other hand, certain essential amino acids like leucin, subside Treg cellularity in mTORC1 dependent manner (Cani et al. 2013). Increase in body adipocyte mass is positively


input
Breakdown of dietary CHO
Clostridia clusters IV, XIV, XVII
Bacteriodes fragilis
Butyrate
Propionate
Acetate
Niacin
GRP 109a
Intestinal Epithelial Cell
Dendritic Cell
GRP109a
IL-10
Conventional Th cell
Th
FFA
Leu
TGF-β
PSA
TLR2
CD80/86
CD28
MHCII
CD4
TCR
CD3
IL-2
ATP
ADP
Adenosine
Leptin
TGFBR1
GRP43
CD36
CD98
IL-2R-βαγ
CD39
CD73
ObR
PI3K
AKT
Ser473
O2↓
ATP↑
AMPK
FA oxidation
Leu, essential aa's
TSC1/2
RagA/B
GTP
RagC/D
GDP
mTORC1
Rheb
GTP
Raptor
mLST
mTOR
PRAS40
Deptor
mTORC2
Rictor
mLST 40
mTOR
Sin1
Deptor
Protor 1/2
PPAR-γ
GATA3
Foxp3
Treg differentiation
Mevalonate pathway
Treg
O2↓
HIF1α
Treg functions
Consumed
Secreted
CTLA-4
ICOS
CD80/86
ICOS-L
IL-10
TGF-β
IL-35
IL-2
IL-4
IL-7
IL-15
Granzyme A and Perforin mediated killing
Trp
IDO
Indole
Th
NFkB⬇
NFAT⬇
Ca2+⬇
output

correlated to serum leptin levels (Al Maskari and Alnaqdy 2006). Acting through mTORC1, leptin limits Treg generation (Zeng and Chi 2015). The increased obesity induced hyperinsulinemia triggers PI3K/Akt activation, which in turn induces glucose intake and preferential use of this substrate (Fig. 10.2) (Han et al. 2014).

6 Dynamic Microbiota

The association between gut microbiota composition and calorie intake—harvesting process appears to be bidirectional. Ley and co-workers compared the gut microbial composition of obese—leptin deficient ob/ob C57BL/6J mice and their lean ob/+ and +/+ siblings. The leptin deficient ob/ob mice consumed almost 50% more chow diet and gained approixmately 80% more weight compared to the lean individuals. Typically, distal intestine of mice accommodate Firmicutes and Bacteriodetes as dominant flora members. However, massive parallel sequencing and UniFrac analysis of 16s rRNA sequences genes in fecal samples taken from the three groups revealed significant decrease in the abundance of Bacteriodetes in favor of Firmicutes, in ob/ob mice (Ley et al. 2005). Alterations or intentional manipulation of gut microbiota community structure may have importance in regulating energy balance. Likewise, the use of non-absorbable gram-positive active antimicrobials as growth enhancer agents may have a similar impact on gut microbiota. Using these agents in animal feedstock has become a common practice—in one report, it has been estimated that nearly 90% of antimicrobial in use in USA was for non-therapuetic agricultural purposes (Allen and Stanton 2014; Gustafson and Bowen 1997).

Most of the time, the terms microbiome and microbiota are used interchangeably. The former refers to the collective genomes of organisms residing in an environment, while the later refers the organisms themselves. While studying microbiota via culture based techniques may be a tedious task, microbiome studies are gaining popularity, with the advent of novel appoaches and technologies in DNA sequencing. Briefly, whole genome shotgun sequencing strategy involves preparing a DNA library of potentially overlapping segments of a genome. As a prerequisite of DNA sequencing chemistries, each member of the library has to be sequenced individually. Later on, these individual small sequence reads are sorted by matching their overlapping parts, in such a way that they form large continous segments of DNA, called scaffolds. Thereafter, scaffolds are are placed end to end to form a complete genomic sequence. This computationally intensive process is called "assembly" and have succesfully been used to study genomes of single organisms (Staden 1979). Venter et al. and Tyson et al. have extended this approach to analyze the taxonomic composition, gene diversity and abundance of mixed microbial communities in environmental samples (Tyson et al. 2004; Venter et al. 2004). In the pionering study of Gill et al., purified DNA from fecal samples of two healty individuals had been used to generate both random DNA and 16S rDNA amplicon

Fig. 10.2 The metabolic and immunologic inputs which drive Treg generation and functions. *TGF-β* Transforming growth factor β; *TGFBR1* TGF-β receptor 1; *GRP* G protein-coupled receptors; *GRP43* Short chain fatty acid receptor ffar2; CD36; *PSA* Polysaccharide A; *ObR* Leptin receptor; *FA* Fatty acid; *AMPK* AMP activated protein kinase; *TSC1/2* Tuberous sclerosis protein 1/2; *Rheb* RAS homologue enriched in brain; *mTOR* Mechanistic target of rapamycin; *Raptor* Regulatory-associated protein of mTOR; *mLST* mTOR associated protein LST8 homologue; *PRAS40* Proline rich Akt substrate of 40 kDa; *Deptor* DEP domain-containing mTOR-interacting protein; *Rictor* Rapamycin insensitive companion of mTOR; *Sin1* Stress activated MAP kinase interacting protein 1; *Protor* Protein observed with Rictor-1; *RAG* RAS related GTP-binding protein; *PPAR-γ* Peroxisome proliferator activated receptor gamma; *GATA3* (DNA sequence) GATA binding protein 3; *Foxp3* Forkhead Box P3; *HIF1* Hypoxia induced factor; *CTLA-4* Cytotoxic T-lymphocyte associated protein 4; *ICOS* Inducible T-cell COStimulator; *NFAT* Nuclear factor of activated T cells; *IDO* Indolamine 2,3-deoxygenase (Ermann and Fathman 2003; Gedaly 2015; Howie et al. 2014; Jiang and Chess 2009; Jobin 2014; Meijer et al. 2010; Worthington 2015)

libraries which were in turn, sequenced by using Sanger methodology. Overall, nearly 60% of 70 k high quality sequence reads had been assembled for each individual. Enrichment of the assembled data by using pathway database of Kyoto Encyclopedia of Genes and Genomes (KEGG) and Cluster of Orthologous Groups database had been performed to explore the metabolic repertoire of the human distal gut microbiome. The analysis of 16s rDNA sequences obtained from amplicon and random DNA libraries revealed nearly 300 phylotypes present in the gut microbiota (Gill et al. 2006). Unfortunately, the workflow of whole genome shutgun sequencing via Sanger methodology is costly and labor intensive. First of all, libraries are traditionally prepared by ligating DNA segments into low capacity cloning vectors, which are then, introduced to host bacteria. This step enables the amplification and purification of the individual members of the library by using microbiological producedures, followed by cycle-sequencing.

As expected, analysis of a limited number of individuals and low depth of coverage are the constraints for this sort of pipeline. Thus, liberal use of this strategy had only been possible with the advent of massively parallel next generation DNA sequencing technologies (Mardis 2008). Next generation DNA sequencing platforms make use of microfluidics to automatize and closely couple DNA library preparation and sequencing steps. This huge step forward in DNA sequencing enabled even small sized groups to perform voluminous sequencing projects.

The two initiatives, MetaHIT (Metagenomics of the Human Intestinal Tract) project and The Human Microbiome Project, revealed the wealth of microbial gene repertoire that we carry in our bodies. Taking the conceivable heterogeneity among individuals into account, microbiota itself might be considered as a greater source of genetic and metabolic diversity, compared to pool of human genetic variability ("Human Microbiome Project," 2016; Yatsunenko et al. 2012). As a part of MetaHIT project, fecal samples from over 500 individuals residing in United States, Venezuella and Malawi have been examined in terms of microbial diversity. Massive parallel sequencing of variable region 4 of rRNA genes was performed to characterize and compare the fecal microbial communities of children and adult subjects from these three geographically distinct regions. According to UniFrac analysis, children and adults exhibit distinct microbial diversity. In the first 3 years of life after birth, the phylogenetic composition of bacterial communities evolve to resemble the adult form (Lozupone and Knight 2005; Yatsunenko et al. 2012). Moreover, children show significantly greater interpersonal variation compared to adults, in all three geographical regions. In addition to unique age related microbiome patterns, geography and cultural traditions also seem to have deep influence on the gut microbiome. Principle coordinate analysis (PCoA) of UniFrac distance matrices seperates the US and non-US individuals as well as Venezuellian and Malawians, according to gut microbiome diversity. Lastly, although bacterial diversity increases with age in all three populations, US adults reside the least diverse microbiome (Yatsunenko et al. 2012). The age dependency of gut microbial composition has already been stressed in a former study by Kurokawa et al. The comperative metagenomic analysis of fecal samples has taken taxonomic and gene compositional snaphots of 13 healty individuals of various ages. Compared to adults, infants exhibited simpler gut microbiota with greater inter-individual variation. As expected, simpler taxonomic composition correspond to a slimmer gene repertoire—infant-type microbiomes overexpress only 136 gene families, compared to 237 in adult-type counterparts (Kurokawa et al. 2007). In contrast, Yatsunenko et al. have examined 110 KEGG EC profiles, but could not detect any EC number that is unique either to adults and or babies (Yatsunenko et al. 2012). Although Sanger DNA sequencing technology provides longer and more relaible reads, next generation sequencing technologies are able to generate far better depth of coverage at a fraction of cost—rare DNA species can readily be discovered. Most probably, Kurokawa et al. might have missed least abundant microbial gene copies, as they have used Sanger DNA sequencing pipeline. Moreover, available bioinformatics resources are

ever expanding—which is crucial for annotation and enrichment of the raw sequencing data.

Acquiring and sustaining a "healthy" microbiome at the very beginning of life may have profound impact in the lifetime health of an individual. Until recently, it was not quiet clear, at what point of our lives we become "superorganisms". Due to conceivable ethical concerns, numerous studies have overlooked the in utero state and explored the microbial composition of feces which primarily reflect the acquisition and diversity of microbiota in term and preterm neonates. Intestinal colonization profile of both term and low birth weight prematures include Enterobacteriales, Pseudomonas, Staphylococcus and Enterococcus spp. However, the establishment of bifidobacteria delays in low birth weight infants, compared to term counterparts (LaTuga et al. 2011; Sakata et al. 1985). However, the diversity of species in the fecal samples of preterm infants were far limited compared to term infants (Magne et al. 2006).

Reasonably, the gastrointestinal tract of a neonate was presumed to be sterile until birth. However, the widely accepted paradigm of a sterile in utero life has recently been replaced by recognition of the presence of fetal gut microflora which has been demonstrated both by metagenomic analysis and cultivation techniques. In one study, consecutive analysis of spontaneously released meconium and fecal samples from preterm babies during the first 3 weeks of their lives disclosed distinct profiles of microbiota. Meconium was rich in *Firmicutes*, including *Bacilli*, whereas, *Proteobacteria* dominate fecal samples. In the first week of birth, eventually, obligate anaerobes become predominating species (Moles et al. 2013).

7 Conclusion

The advent of highly efficient systems biology tools, such as massively parallel DNA- and RNA-Seq has revolutionized the way and amount of data that we collect from biological systems. The "snapshots" taken by using these methods and functional studies in model organisms have revealed that, metabolic syndrome presents as a multi-systemic disorder, in which gut microbiota is intricately involved in the pathogenesis.

It appears that, we acquire—a rather simpler form of—gut microbiota in fetal life. From birth to adulthood, we carry, evolve and reshape this huge metabolically active microbial mass along with us, according to our partly genetically determined metabolic repertoire, environment and life style. Many factors are deterministic over the composition of the microbiota, from eating behaviours to immunological status of the individual. Nonetheless, the interaction is exclusively bidirectional. In the fully symbiotic end of the spectrum, gut microbial mass, provides us with the enzymes required for digestion and absorption of certain dietary components. On the dysbiotic end, gut microbiota aggravates the inappropriate immunological responses and metabolic syndrome.

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Endoplasmic Reticulum Stress and Obesity

11

Erkan Yilmaz

Abstract

In recent years, the world has seen an alarming increase in obesity and closely associated with insulin resistance which is a state of low-grade inflammation, the latter characterized by elevated levels of proinflammatory cytokines in blood and tissues. A shift in energy balance alters systemic metabolic regulation and the important role that chronic inflammation, endoplasmic reticulum (ER) dysfunction, and activation of the unfolded protein response (UPR) play in this process.

Why obesity is so closely associated with insulin resistance and inflammation is not understood well. This suggests that there are probably other causes for obesity-related insulin resistance and inflammation. One of these appears to be endoplasmic reticulum (ER) stress.

The ER is a vast membranous network responsible for the trafficking of a wide range of proteins and plays a central role in integrating multiple metabolic signals critical in cellular homeostasis. Conditions that may trigger unfolded protein response activation include increased protein synthesis, the presence of mutant or misfolded proteins, inhibition of protein glycosylation, imbalance of ER calcium levels, glucose and energy deprivation, hypoxia, pathogens or pathogen-associated components and toxins. Thus, characterizing the mechanisms contributing to obesity and identifying potential targets for its prevention and treatment will have a great impact on the control of associated conditions, particularly T2D.

Keywords

Autophagy • Endoplasmic reticulum stress • Lipotoxicity • Obesity • Type 2 diabetes

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1 Introduction

Obesity has closely associated with many diseases including type 2 diabetes, atherosclerosis, hypertension, cancer, and vascular diseases including heart attacks and stroke (Haslam and James 2005; van Meer et al. 2008). In modern times increased food intake, energy consumption and less physical activity resulted high prevalence of obesity. According to epidemiologic studies still increasing all over the world. Identifying cellular mechanism alterations which affected from obesity may give potential targets for prevention and treatment. Taking under the control of obesity associated conditions particularly type 2 diabetes (T2D) is very important. The close relationship between obesity and insulin resistance is not understood very well and probably there are many causes for obesity related insulin resistance. One of these appears to be endoplasmic reticulum stress (ER Stress).

The elucidation of mechanisms that contribute to the development of obesity and uncovering potential targets are very important for its treatment. So, its possible expect prevention of associated conditions like type 2 diabetes. Indeed, recently in a study I was involved, we have shown that obesity may lead to ER Stress in metabolically regulated? tissues like adipose and liver. Genetically obese (ob/ob) and high fat diet fed mice, ER stress markers and JNK activity are significantly increased compared to lean animals (Özcan et al. 2004). The relationship between ER stress and insulin resistance was supported with cultured cells which XBP1 overexpressing and deficient cells.

XBP1-deficient cells exhibit markedly increased sensitivity to ER stress and succumb to insulin resistance, at least in part, through IRE1a dependent activation of JNK and serine phosphorylation or degradation of IRS1. In contrast, cells experimentally equipped with higher levels of activated XBP1s become refractory to ER stress and exhibit protection against insulin resistance. XBP1 haploinsufficiency in mice replicates the findings in cultured cells, as these animals succumb to ER stress and develop hyperinsulinemia, hyperglycemia, and impaired glucose and insulin tolerance compared to wild-type animals. XBP1 haploinsufficiency results in a small but significant increase in body weight, and in the liver and adipose tissues of these mice there is increased phosphorylation of PERK and IRE1a and also increased JNK activity coupled with a loss of insulin sensitivity.

Some other studies got similar results and confirmed that ER stress has a role in obesity dependent insulin resistance and type 2 diabetes. Deficiency of the ER chaperone protein ORP150 (oxygen-regulated protein 150), either whole body or liver specific, resulted impaired glucose tolerance and insulin receptor signaling. So, ORP150 has a role in metabolic homeostasis as a protective manner. Conversely, increased expression of ORP150 leads to significantly improved glucose tolerance and insulin receptor signalling (Ozawa et al. 2005; Nakatani et al. 2005).

Other connection between ER stress and metabolism is ER chaperone Grp78. Its been shown that Grp78 has an important role on metabolism, increased insulin sensitivity and decreased hepatic steatosis observed in Grp78 overexpressing obese mice (Kammoun et al. 2009). On the other hand, missing one allele of Grp78 resulted compensatory increased other ER chaperones, so overall enhanced ER folding capacity and metabolic homeostasis has been protected.

Another model has proven that ER stress related with obesity and metabolism. eIF2a is an important factor for protein translation, phosphorylation of Ser51 has inhibitory effect on protein translation. Ser51 mutant form of eIF2a carrying mice developed obesity and prone to type 2 diabetes. It has dysfunctional endoplasmic reticulum phenotype. So, this model is good example for ER stress related metabolism as well (Scheuner et al. 2001).

2 UPR and ER Stress

The ER is a principal site of protein synthesis, maturation and, together with the Golgi apparatus, the transportation and release of correctly folded proteins. Addition to synthesis role ER is also integrating cellular signals adopt the cell to the environment, so it has a crucial role managing cellular homeostasis. ER has evolved adaptive

folding capacity called unfolded protein response, in adverse conditions changing cellular homeostasis and protects the cell (Gregor and Hotamisligil 2007). Unfolded protein response activated by different conditions such as misfolded or mutant proteins, imbalance of calcium levels, energy and glucose deprivation, hypoxia, inhibition of protein glycosylation, toxins, pathogens. Previously intracellular signals has been shown to related with unfolded protein response, inflammation and obesity. Increased activity of mammalian target of rapamycin (mTOR) characteristic of obesity. Another important factor is JNK, has been shown increased activation during the ER stress. In obesity conditions increased energy availability and synthetic demand challenges ER and integrated with stress signaling so may lead ER stress. Increased serine phosphorylation of insulin receptor substrate-1 is observed in both insulin resistance and tunicamycin induced ER stress conditions. Given that unfolded protein response and insulin resistance closely integrated (Ozcan et al. 2006).

Transcription factor XBP-1 is an important factor for UPR. Even in heterozygous XBP-1 null mouse model on the high fat diet strongly regulate insulin receptor signaling and results in insulin resistance and type 2 diabetes. On the other hand, transgenic mice protected from diet induced insulin resistance. Altered ER folding capacity and cellular stress responses may have a direct effect on metabolic activities. In addition to genetic models induction by chemical chaperones like 4-phenylbutyric acid (4-PBA) and Tauroursodeoxycholic Acid (TUDCA) strongly sensitize insulin receptor signaling and rescue animals from insulin resistant phenotype. Chemical chaperones is also enhanced peripheral (adipose tissue and muscle) glucose uptake and hepatic glucose production rates shown by hyperinsulinemic euglycemic clamp studies (Ozcan et al. 2006).

In contrast, enhancing ER folding capacity by transgenic strategies protects mice against diet-induced insulin resistance. Recently, we showed that in addition to genetic models, modulation of ER function by chemical chaperones phenyl butyric acid and taurine-conjugated ursodeoxycholic acid provide strong insulin sensitizing effects and normalize hyperglycemia in animal models. These treatments lead to markedly enhanced insulin receptor signaling in peripheral tissues and restored proper insulin action. Hyperinsulinemic eugylcemic clamp studies showed that chemical chaperones significantly impact both hepatic glucose production and peripheral glucose disposal rates with significantly increased adipose tissue and muscle glucose uptake. Moreover, both phenyl butyric acid and taurine-conjugated ursodeoxycholic acid treatments resulted in a marked reduction in fatty infiltration of liver, which is frequently observed in obesity. Taken together, these studies illustrate that ER stress is a critical mechanism underlying obesity-induced JNK activity, inflammatory and stress responses, and insulin resistance and offer potential new therapeutic opportunities against obesity, insulin resistance and type 2 diabetes.

3 ER Stress and Metabolism

Unfolded protein response under the control of three endoplasmic reticulum stress sensor proteins inositol-requiring enzyme (IRE)-1, protein kinase-like ER kinase (PERK), and activating transcription factor (ATF6). These proteins activated during different stress conditions including metabolic changes which effect on protein folding status. UPR elements (IRE1, PERK, ATF6) activate different intracellular pathways and controls vital cellular mechanisms like protein translation and sometimes overlapping pathways. UPR is one of the most important adaptive response system that sense stress and organize signaling events that reestablish ER homeostasis or induce apoptosis. So, UPR capable to command the cell that can integrates various environmental fluctuations.

The PERK arm of the UPR mediates inhibition of protein translation via phosphorylation of the a subunit of eIF2 (Harding et al. 1999; Shi et al. 1998). This leads decreased protein synthesis and reduce ER protein folding load. Downstream at the PERK, ATF4's expression and target genes related to antioxidant response and amino acid transport increased (Harding et al. 2000; Ma et al. 2002). PERK activates transcription factor C/EBP (CCAAT/enhancer binding protein) and Gadd34 (growth arrest and DNA damage-inducible

protein 34), which results eIF2a Ser51 inhibitory phosphorylation and effected on protein translation (Deng et al. 2004; Jiang et al. 2003).

IRE1 activation results in splicing of transcription factor XBP1 (X-box binding protein-1) mRNA. Spliced form of XBP1 translocates to the nucleus and regulates expression of ER chaperones and ERAD (ER associated degradation) proteins (Sidrauski and Walter 1997). IRE1a's cytoplasmic domain can interact with TRAF2 (TNF receptor–associated factor 2) to activate JNK and induce inflammatory signals as well as apoptosis (Hu et al. 2006; Özcan et al. 2004). In addition to this, IRE-1 has been suggested to activate the p38, extracellular signal-regulated kinase (ERK), and NF-κB pathways through interaction with the adaptor protein Nck (noncatalytic region of tyrosine kinase) and a protein complex composed of IKK and TRAF2 (Hetz and Glimcher 2009). Prolonged UPR signaling promote apoptosis through IRE1, proapoptotic bcl-2 associated factors Bax and Bak can also make complex with it. Another isoform of IRE1 (IRE1b) expressing in intestine suggesting that a specialized role in secretory capacity (Bertolotti et al. 2001).

The other branch of the UPR, ATF6, ATF6 family transcription factors, ATF6a especially a stronger transcription factor in response to ER stress (Yamamoto et al. 2007). ATF6 cleaved into an active form in golgi, response to ER Stress (Haze et al. 1999). Conversely, ATF6a expression decreased in the liver of obese mice other than some UPR components. Glucose homeostasis improved with adenoviral ATF6a reconstitution (Wang et al. 2009). So, it's a possibility that there are some other UPR mediated important signals regulate metabolic signals, not only chaperone production and protein processing.

Many studies have demonstrated that improper functioning of the UPR plays an important role in chronic metabolic diseases, including obesity, insulin resistance, and diabetes (Özcan et al. 2004). Obesity results in increased ER stress, particularly in the liver and adipose tissue of mice (Hotamisligil 2010). XBP1 whole body knockout mouse is not viable because embryonically lethal, however heterozygous XBP1 mice develop insulin resistance on high fat diet, even in Balb/C background. Balb/C background known as resistant to diet induced obesity and diabetes. This strong phenotype attributable to induction of enough JNK1 activity and inhibition of insulin action (Özcan et al. 2004). Another model for ER stress and insulin resistance relationship is eIF2a Ser51 mutant mice which is a non-phosphorylatable eIF2a model and become obese and insulin resistant in response to high fat diet. Many clinic studies shown that ER stress strongly correlated with human health, body mass index, insulin sensitivity after gastric bypass surgery increased insulin sensitivity and reduced ER stress has been observed (Gregor and Hotamisligil 2007; Sharma et al. 2008). Also, it has been reported that PERK knockout mice develop diabetes early days of life because of defective Beta-cell development. In humans PERK mutations result in early-onset Beta cell loss and severe diabetes (Harding et al. 2001; Zhang et al. 2002). In the context of obesity and type 2 diabetes, decreased B-cell mass is likely an event preceded by insulin resistance and metabolic dysfunction. However, peripheral insulin resistance results in a tremendous strain on β cells caused by increased demand for insulin production. Thus, increased stress on the UPR during prolonged nutritional excess and insulin resistance may play a role in the eventual transition from insulin resistance to overt diabetes, which is characterized by Beta-cell loss and dysfunction. Genetic overexpression of the ER chaperones ORP150 (oxygen-regulated protein 150) and GRP78 (glucose-regulated protein 78) also improves metabolic regulation in mice (Kammoun et al. 2009; Nakatani et al. 2005; Ozawa et al. 2005).

In the context of metabolic diseases UPR-mediated pathways that remedy of ER homeostasis are protective. But engagement of other signaling pathways or prolonged UPR signaling, might have a negative impact in metabolic homeostasis.

4 Lipotoxicity Induced ER Stress

Lipotoxicity is a phenomenon that lipid accumulation occurs in different tissues but not only adipose. Accumulated lipids may destabilize the signaling mechanisms and pathways, so can cause cellular dysfunction and cell death. Mechanisms of lipid accumulation pathways and

cell death is not understood very well. Recently its been shown that saturated fatty acids induce endoplasmic reticulum stress but not unsaturated fatty acids in the liver. Apoptosis is also observed after treatment with saturated fatty acids. ER stress induction has been related with increased ceramide concentrations, interestingly unsaturated fatty acid induction reverted ER stress phenotype formed by saturated fatty acids.

Although previous literature reinforced that ER stress was efficacious to cause b cell death and dysfunction (Harding et al. 2001; Oyadomari et al. 2002; Scheuner et al. 2001), in vitro models of lipotoxicity recently suggested relation to the more prevalent T2DM. Accordingly, ER stress was related (Karaskov et al. 2006; Laybutt et al. 2007) and subjected to apoptosis in b cells with chronic exposure to high levels of FAs. It was confirmed by further studies that, diabetes patients had elevated ER stress markers in their pancreatic islets (Huang et al. 2007; Marchetti et al. 2007). Furthermore, lipotoxic ER stress is different from the classic UPR, by means of the capacity for independent modulation of downstream pathways and its initiation way. It is interesting that, depending on whether initiated by protein overload or protein misfolding, the arms of the UPR can be differentially regulated (Shinjo et al. 2013).

Although it is not implied in T2DM, several rare human mutations cause misfolding of proinsulin ending in ER stress and B cell failure. A useful model of this is the Akita mouse (Liu et al. 2010). Despite that, a more common role for the b cell failure might be the disturbances in the ER milieu (alterations in pH, redox, or Ca2+) that encloses the overall folding capacity. Definitely, many ER stressors, such as nitric oxide, cytokines, and glucotoxicity seem to act via depressing luminal Ca2+ content (Cardozo et al. 2005; Oyadomari et al. 2002). Lipotoxic ER stress was attempted to be explained by a similar mechanism (Cunha et al. 2008; Hara et al. 2013) but it is still not evidentially precise. Variable effects of individual FAs have been reported in terms of extent, timing, and specificity (Cunha et al. 2008; Gwiazda et al. 2009; Karaskov et al. 2006) although quantifying ER Ca2+ depletion is technically arduous. A recent study postulated that, compared to cytokines and glucotoxicity, palmitate (albeit tested only at high concentrations), the sarco/endoplasmic reticulum Ca2+-ATPase (SERCA) pump inhibitor and thapsigargin were more efficacious at lowering ER Ca2+. Ca2+ depletion from the ER also occurred with the overexpression of mutant secretory proteins, suggests that this phenomenon occurs as a result likewise as a trigger of ER stress (Hara et al. 2013). Notwithstanding, diminished ER Ca2+ is essential for the initiation of ER stress in response to cytokines, glucotoxicity, and nitric oxide donors, because overexpression of SERCA2b has a role in rescuing from the effects of these agents on apoptosis. Nevertheless, a caution has not been demonstrated for lipotoxic ER stress. Finally, although SERCA2b expression in b cells is inhibited by both glucotoxicity and cytokines, no evidence exist that this consists of lipotoxicity (Cardozo et al. 2005; Cunha et al. 2008; Hara et al. 2013).

Despite that, diminished expression of SERCA2b is observed in islets of patients with T2DM together with disrupted Ca2+ handling and b cell dysfunction in db/db mice (Evans-Molina et al. 2009; Hara et al. 2013; Kono et al. 2012). Knowing that glucotoxicity accompanies the latter model rather than lipotoxicity (Kjørholt et al. 2005), SERCA2b decrement and additionally protein misfolding might contribute to the progression rather than initiation of T2DM. Consequently, the role and the mechanism of other ER stressors in ER Ca2+ depletion in b cells is better defined than lipotoxicity. Furthermore, ER Ca2+ is only an indirect measure of the actual state of protein folding. In a study this has been analyzed with an ER-localized reporter. This reporter encompassed the vesicular stomatitis virus glycoprotein tagged with GFP (VSVG-GFP). Thapsigargin was shown to induce protein misfolding but not palmitate, by using a specific antibody for the native (properly folded) conformation of VSVG-GFP (Preston et al. 2009). To better discern these issues, techniques to assay the folding state of endogenous protein cargo under lipotoxic or diabetic conditions are needed.

The importance of protein overload was demonstrated by recent studies using both lipotoxic and genetic models of b cell failure and ER stress. Evidently, it might be anticipated that, ER stress

would emerge from a compensatory increase in proinsulin biosynthesis within b cells to reverse the effect of insulin resistance. T2DM develops in a minority of insulin-resistant patients, demonstrating that this mechanism is not sufficient to initiate terminal ER stress in b cells. Furthermore, only modest ER stress was demonstrated in vitro or in vivo models of (nonmutant) overexpressed pro-insulin (Hodish et al. 2011; Pétremand et al. 2012). This suggests that accomplishing with increased pro-insulin biosynthesis is part of the compensatory response for insulin resistance and inversely, insufficiency to achieve this might contribute to diabetes susceptibility (Chan et al. 2013).

As an alternative, protein overload might result from distorted exit of protein from the ER, opposing to increased biosynthesis. In fact, B cells transfected with the temperature-sensitive VSVG-GFP reporter have been made use by several reporters. This accumulates in the ER of cells maintained at a nonpermissive temperature, but is rapidly trafficked to the Golgi after switching to 32°C. Thus, lipotoxicity has been precisely demonstrated to disrupt ER-to-Golgi protein trafficking. This defect was specific only for saturated fatty acids (Boslem et al. 2011; Preston et al. 2009; Véret et al. 2013). During glucolipotoxicity, retention of GFP-tagged proinsulin in the ER suggested disrupted export into the secretory pathway (Wikstrom et al. 2013). Whereas, the inconvenience of these studies is that they depend on fluorescent reporters, and trafficking of endogenous cargo has still not been shown. On the other hand, it was suggested that, endogenous secretory cargo containing ER-derived vesicles' budding is also inhibited in B cells chronically exposed to saturated FA which is consistent with an impairment of protein trafficking (Boslem et al. 2013). Additionally, it was shown that defective protein trafficking is enclosed as an early feature of B cell failure in animal models (Absood et al. 2013). Surprisingly, Akita mice is included in these animal models which is expected to reflect a pure model of protein misfolding. However, to block export of secretory cargo from the ER, the mutant pro-insulin acts in a trans-dominant manner. Finally, the protein overload promotes ER stress which leads to protein synthesis inhibition, loss of insulin content and eventually apoptosis (Izumi et al. 2003; Liu et al. 2010). Interestingly, in mice, which pro-insulin mutant protein is modestly expressed compared to wild type protein, are not overtly diabetic. However, depending on depletion of pro-insulin and activation of ER stress markers, they display impaired glucose tolerance. Additionally, individual b cells that have the greatest loss of insulin content also showed (native) pro-insulin accumulation in the ER and increased ER stress (Hodish et al. 2011). Importantly, in pre-diabetic db/db mouse islets, a similar pattern was observed (Absood et al. 2013). Both in many murine models of diabetes (Liu et al. 2010) and human disease (Marchetti et al. 2007), distended ER and depletion of insulin granules are morphologically observed. Impaired ER-to-Golgi protein trafficking was suggested by these studies to be an early general deficit that leads to ER stress caused by protein overload and to result in impaired pro-insulin maturation and insulin content loss before apoptosis. However, the flow of these events and the mechanisms that connect them is to be clarified. Finally, ER stress encloses protein trafficking (Preston et al. 2009; Pétremand et al. 2012); thus a response might also be amplified by protein overload triggered initially by protein misfolding.

5 Metabolite Toxicity

Another key issue, is the identity of the metabolite or mechanism by which saturated FAs compromise B cell protein folding or trafficking which is convenient for therapeutic intervention. There are several possibilities suggested by non-B cell model studies. Firstly, enhancement in either cholesterol or the ratio of phosphatidylcholine:phosphatidylethanolamine (PC:PE) in the ER membrane might disrupt SERCA activity (Fu et al. 2011). Another possibility would be a general increase in ER phospholipid saturation that might disrupt ER structure and/or secretory vesicles' budding (Ariyama et al. 2010; Pineau et al. 2009). Finally, it is evident that ER lipid saturation can activate PERK and IRE1, free of the interaction of their lumenal domains with misfolded proteins (Volmer et al. 2013). Despite that, there are argues against these mechanisms

of their role in palmitate treated b cells. In these studies neither PC:PE nor phospholipid saturation of the ER membrane was altered by genetic manipulations of lipid metabolism that relieve defective protein trafficking and ER stress. Instead a role for sphingolipids, initially ceramide (Boslem et al. 2011), was accentuated, which is coherent with observations that links this metabolite with lipotoxic apoptosis in B cells (Boslem et al. 2012; Unger 2002) and ER stress in yeast (Payet et al. 2013). Surprisingly, an increase in ER ceramide caused a selective and ER-localized decrease in both sphingomyelin and free cholesterol rather than involved in a toxic mechanism which leads to dysregulation of ER lipid rafts (Boslem et al. 2013). The latter are comprised in loading of cargo into secretory vesicles (Hayashi and Su 2010); accordingly their disruption in lipotoxic b cells could provide a new clarification for the participating protein overload and ER stress. Recently, other lipid species such as high-density lipoprotein (HDL) and sphingosine-1 phosphate have also been shown to rescue protein trafficking and b cell function (Véret et al. 2013). The role of these lipid species whether they impact on ER lipid rafts will be of interest. Even though this occurs in post-Golgi compartments, there is a possibility that diminished processing of pro-insulin might feed back to disrupt ER-to-Golgi transport, and in this way, also contribute to protein overload.

6 ER Stress and B Cell Apoptosis

In general, apoptosis has been the major focus in studies related with b cell failure downstream of ER stress. However, the distal effector mechanisms are still in conflict. CCAAT/-enhancer-binding protein homologous protein (CHOP), required for full apoptosis in b cells due to lipotoxicity, is the best studied inducer of ER stress (Oyadomari et al. 2002). One postulated mechanism is the inhibition of CHOP by antioxidant genes (Song et al. 2008). Also, by promoting dephosphorylation of eIF2a CHOP induces growth arrest and DNA damage-inducible protein (GADD34) and reverses the effects of PERK activation and derepress translation. The resultant would be an oxidative stress that might promote b cell death directly, and indirectly by further compromising protein misfolding and exacerbating ER stress (Back et al. 2009; Song et al. 2008). Involving selective modulation of eIF2a phosphorylation, these studies are based on elegant in vivo models. Crossing CHOP null mice onto the db/db background reinforced the physiological relevance for this which resulted in clear protection against b cell oxidative stress. Improvements in hyperglycemia, or lipid sparing in b cells secondary to enhanced adiposity might also explain this (Maris et al. 2012; Song et al. 2008).

Recently, a more important role for ATF4 versus CHOP is argued. The two transcription factors promote a partially overlapping set of genes. They effectuate an anabolic program of amino acid handling and protein synthesis. In addition to GADD34, further induces translational recovery (Han et al. 2013). Also, in islets of Akita mice, expression of several of these genes is increased, which corresponds to an induction of protein synthesis versus control islets. However, a cytotoxic role for ATF4 was argued according to other observations. Eukaryotic translation initiation factor 4E-binding protein 1 (eIF4EBP1) is one of the direct target genes of ATF4 which promotes b cell survival (Yamaguchi et al. 2008). When upregulated, ATF4 was postulated to participate in improved b cell function both downstream of the master gene regulator pancreatic and duodenal homeobox 1 (Pdx1) and following treatment with the insulin secretagog glucagon-like peptide-1 (GLP-1) (Sachdeva et al. 2009; Yusta et al. 2006).

The translational recovery hypothesis, described primarily using strong pharmacological stressors in vitro, or pro-insulin folding or eIF2a/PERK signaling defective mice demonstrates a more situation than occurs with T2DM or even lipotoxic models. Actually, it is obvious since CHOP transcriptional induction directly by palmitate is different from that due to pharmacological stressors (Pirot et al. 2007). Furthermore, another role for palmitate in regulating the deubiquitylation and stabilization of CHOP protein was indicated (Qi and Xia 2012). According to this lipotoxicity can effect on CHOP expression independently of upstream activation of the UPR.

However, there are more specific examples of an inconsistency between translational recovery and lipotoxicity. Particularly, although pharmacological inhibition of eIF2a dephosphorylation (represses translational recovery) protects against b cell apoptosis due to chemical ER stressors, it also worsens ER stress and cell death due to lipotoxicity (Cnop et al. 2007). Moreover, as might be expected from the translational recovery model, chronic exposure to FAs inhibits pro-insulin synthesis both in vivo and in vitro, as opposed to stimulating it (Capito et al. n.d.; Zhou and Grill 1994).

7 Apoptotic Pathways

CHOP/ATF4 are not only effectors of lipotoxic ER stress in b cells and more direct links to the apoptotic pathway have been suggested. In one scenario, early translational repression via PERK/eIF2a phosphorylation leads to loss of the rapidly turned-over protein, myeloid cell leukemia sequence 1 (Mcl-1), an antiapoptotic member of the BH3 family (Allagnat et al. 2011). In contrast, p53 upregulated modulator of apoptosis (PUMA) and DP5/Hrk which are pro-apoptotic members, are also upregulated by palmitate, downstream of PERK but independently of CHOP/ATF4 (Cunha et al. 2008). Instead, ATF3 synergizes with IRE1/JNK signaling in the case of DP5/Hrk and serves upstream of TRB3 and forkhead box O3 (FoxO3a) for PUMA. To chase the roles of these various proteins in lipotoxic ER stress, the in vivo expression of these various proteins were modulated, and using a whole-body knockout mouse for DP5/Hrk further supportive evidence was provided in vivo (Cunha et al. 2008).

It is known that IRE1 activation directly regulates degradation of many ER-associated mRNAs. Recently, a novel mechanism was declared involving depletion of the miRNA miR-17 that in turn controls stability of its own mRNA targets. One of these proteins is the thioredoxin interacting protein (TXNIP). Previously, it was implied in mediating oxidative stress due to glucotoxicity (Chen et al. 2010). TXNIP was declared to be induced by strong ER stress and its deletion rescues glucose intolerance and b cell apoptosis in the Akita mouse (Lerner et al. 2012). TXNIP potentially acts downstream of ER stress to induce oxidative stress and also inflammation (Lerner et al. 2012; Oslowski et al. 2012), and both actions have been linked to b cell failure in T2DM. Nevertheless, TXNIP is not induced in islets by chronic exposure to FAs and is not essential for lipoapoptosis (Oslowski et al. 2012). The indication that TXNIP is an important downstream mediator of pharmacological or genetic ER stress, the relevance of TXNIP to lipotoxicity, and arguably T2DM is to be defined. However, miR-17 targets mRNAs other than TXNIP. Caspase-2 is one of its targets which might link ER stress to the intrinsic apoptotic pathway via activation of the pro-apoptotic BH3 protein Bid (Upton et al. 2012). Obviously, how ER stress mediates B cell apoptosis in the setting of T2DM is to be established by more work to have a complete picture.

Ectopic accumulation of lipids, especially fatty acids (FA), is one feature of the metabolic syndrome, believed to cause insulin resistance via multiple mechanisms. In non adipose tissues, the rise in lipid content indicates direct evidence of lipotoxicity. Muscle-specific overexpression of lipoprotein lipase assays causing increased hydrolysis of circulating triglycerides leads to skeletal muscle insulin resistance (Ferreira et al. 2001), while in heart or liver, increased lipid transport leads to lipotoxic cardiomyopathy and nonalcoholic fatty liver disease, respectively (Chiu et al. 2005; Koonen et al. 2007). In addition to the effect of increased lipid flux on insulin sensitivity, multiple lipid intermediates have been shown to promote insulin resistance. In obesity, observed free fatty acids (FFA) induction in circulation induce activation of JNK, IKK, and PKC and IRS-1 Ser-307 phosphorylation (Schenk et al. 2008). The fatty acid palmitate has a specific role in promoting insulin resistance. It stimulates endoplasmic reticulum (ER) stress, cytokine production, and activates JNK (Özcan et al. 2004; Shi et al. 2006). Additionally, palmitate activates NF-κB signaling, whereas interference of this pathway inverses lipid-induced insulin resistance (Kim et al. 2001; Sinha et al. 2004). Interestingly, the detrimental effect of palmitate on skeletal muscle insulin resistance can be inverted by confusion with oleate. In this way,

its conversion changes from phospholipids and diacylglycerol (DAG) to triglycerides (Peng et al. 2011). This points out that, insulin resistance is induced by FFA through multiple mechanisms, and combinations of FA can influence insulin signaling. This highlights the important interplay of lipids with respect to dietary interventions.

The lipid metabolite DAG has also been shown to induce insulin resistance. By activating PKC-u and inducing IRS-1 Ser-307 phosphorylation, elevated muscle DAG (intramyocellular lipid) leads to muscle insulin resistance (Yu et al. 2002). Contrarily, decreasing DAG levels in skeletal muscle and liver protects mice against high-fat-diet-induced insulin resistance (Liu et al. 2007; Ahmadian et al. 2009; Samuel et al. 2010).

Sphingolipid ceramide rise in plasma levels is observed in obese and diabetic patients and is related to severe insulin resistance (Haus et al. 2009). It has been shown to induce insulin resistance via PKC and JNK activation (Westwick et al. 1995; Schenk et al. 2008). According to this, ceramide synthesis inhibition adjusts insulin resistance (Holland et al. 2007). Additionally, ceramides inhibit Akt activation by increasing the interaction of PP2A with Akt, and phosphorylation of Akt at Thr-34 by PKC z, and this results in reduced binding of PIP3 to Akt (Teruel et al. 2001; Powell et al. 2003; Blouin et al. 2010).

Alteration of membrane – lipid composition also affects insulin signaling in addition to effects on kinases. Saturated-unsaturated FA ratio increase is found in type-2 diabetic patients and is thought to decrease membrane fluidity and insulin sensitivity (Field et al. 1990; Bakan et al. 2006). Furthermore, phosphatidylcholine (PC) to phosphatidylethanolamine (PE) ratio increase in endoplasmic reticulum activates ER stress and is associated with insulin resistance (Fu et al. 2011).

A primary event in response to nutritional abundance is sequestration of excess fuel in adipocytes (Spiegelman and Flier 2001). It is known that excessive accumulation of adipose tissue is a major risk factor for metabolic disease, despite this built-in storage function. Although mechanisms convert surplus energy into fat, why does excess buildup finally lead to disease? One of the answers might be that the organ itself has to deal with permanent demand on its lipid storage and processing functions. When the critical threshold is exceeded, signs of stress begins in the adipocyte, such as; hypertrophy and associated mechanical stress, compositional changes of lipids and other nutrients, hypoxia, disruption of mitochondrial function, production of reactive oxygen species, apoptotic signaling, increased fatty acid (FA) release, altered adipokine signaling, inflammation, and ER stress (Gregor and Hotamisligil 2007; Hotamisligil 2010). These processes are linked and they influence each others' function. Now they have been linked to metabolic dysfunction and disease. As an example, excess circulating free FAs are received and deposited in other tissues which can not deal with them. The resulting "lipotoxicity" has been shown to influence glucose utilization and insulin action in liver and muscle tissues (Schaffer 2003; Unger 2002). Additionally, lipid signaling relation with increased inflammatory and stress signaling has recently been declared (Hotamisligil 2010).

Besides, in obesity, adipokines and other adipocyte-derived signals circulatory level changes have been linked to disruption of an array of pathways important for metabolic homeostasis. These pathways comprise insulin and glucose signaling in peripheral tissues, control of energy intake and expenditure by the central nervous system and systemic lipid metabolism (Cao et al. 2008; MacDougald and Burant 2007).

IRE1 protein kinase function seems to be retractable for activation and have a subtle or counter regulatory role in stabilization and destabilization of the oligomeric complexes. Despite that, PERK autophosphorylation is crucial for downstream signaling, initially by phosphorylation of the translational eukaryotic initiation factor 2 alpha. Hyperglycemia can lead to glucose toxicity and tissue damage, and this can lead to secondary inflammation in turn. Likewise, when harmful lipid mediators accumulate abnormally lipotoxicity in adipocytes, liver, and muscle in obesity, cellular stress and tissue dysfunction occur ending in inflammation (DeFronzo 2010).

It was reported in recent studies that, the expression of lipid metabolites, proinflammatory cytokines and cellular stress (including oxidative and endoplasmic reticulum (ER) stress). can be elevated by FFAs which is termed as lipotoxicity.

It occurs in many peripheral tissues including the heart, liver skeletal muscle and pancreas (Boden 2011). Several studies reported the direct effect of a high fat diet and the related FFAs and ceramides on endothelial dysfunction (Symons et al 2009; H. Zhang et al. 2012; Q.-J. Zhang et al. 2012). Finally, ER dysfunction and aberrant immune signaling are concerned as significant and combining factors affecting metabolic regulation and contributing to the emergence of metabolic disease.

8 ER Stress and Autophagy

Primary degradation machine of misfolded proteins is ubiquitination and proteasome pathway. ER associated protein degradation system (ERAD) process activated during the ER stress. Lipotoxicity has effect on activate this system. Downregulation of ubiquitination and proteasome pathway gene expressions and proteasome activity in diabetic patients and human islets after the palmitate treatment . So, lipotoxicity initiates or amplifies ER stress via ubiquitination and degrading proteins or autophagy. ER Stress can trigger autophagy in many cell types, targeting misfolded proteins and related molecular mechanisms are not understand well (Høyer-Hansen and Jäättelä 2007). Fatty acid treatment induce autophagy in islets and a chemical chaperon, PBA inhibited this effect (Choi et al. 2009). In some experimental models has increased ER stress in Beta cells such as ob/ob or Akita mice, shown elevated autophagy markers. Rapamycin treatment to Akita islets results decreased ER stress and autophagy (Bachar-Wikstrom et al. 2013; Las et al. 2011; Quan et al. 2011). Reciprocal relation in lipotoxicity and ER stress needs to further investigation.

Misfolded protein aggregates in the cytoplasm can induce ER stress via reduced proteasome activity and unfolded or misfolded proteins accumulate in the ER. Ser51 mutant eIF2a (non-phosphorylatable form of eIF2a) model has shown PERK-eIF2a signaling linked to autophagy (Cardozo et al. 2005). ER stress, starvation and viral infection signals especially depended to eIF2a. eIF2a activation in autophagy process is not a necessity to go through ER stress.

ATF4 and CHOP pathway can transcriptionally induce autophagy related genes (B'chir et al. 2013). IRE1 activation by TRAF2 and JNK resulted signal mechanisms which related with autophagy (Deegan et al. 2012). Autophagy and ER stress are part of cell protection mechanisms. UPR capable of organize survival and death signals and switch between cellular outcomes by sensitive mechanisms of UPR.

9 Chemical Chaperones

Protein folding in the ER and UPR signaling plays critical roles in the pathogenesis of metabolic diseases, obesity, type 2 diabetes and atherosclerosis. Preclinical and clinical studies demonstrate the therapeutic potential of a wild range of small molecular modulators and regulators of ER stress and the UPR for treatment of metabolic syndrome. Chemical chaperones have shown efficacy in animal and clinical studies.

Molecular chaperones recognize and bind peptides to assist and get stabilized conformations. After the suitable conformation molecular chaperones help assembly and translocation across membranes. Under the stress conditions aggregated unstable proteins may appear, molecular chaperones restrict and present these misfolded proteins to the proteasome system. Molecular chaperones able to recognize hydrophobic residues in the unfolded proteins by this way and discriminate from folded proteins. Nucleoplasmins, chaperonins and heat shock proteins are molecular chaperons. Heat shock proteins response different stimuli like infection, inflammation, starvation and hypoxia. A special class chaperones are endoplasmic reticulum resident chaperones such as calcium-dependent Hsp70 member Grp78, Hsp90 member Grp94 and calreticulin. Recent reports indicate that, modulation of molecular chaperones as promising targets in the various diseases.

Chemical chaperones are small molecules that are non-selective in their ability to stabilize mutant proteins and facilitate their proper folding, slightly reminiscent of the chaperoning function of the many intracellular molecular chaperones. Most chemical chaperones are

usually osmotically active such that they equilibrate cellular osmotic pressure. These osmolytes are compatible with protein function and can act as 'chemical chaperones' by increasing the stability of native proteins and assisting refolding of unfolded polypeptides. Chemical chaperones belong to mainly three classes of osmolytes including carbohydrates (glycerol, sorbitol, inositol), amino acids and derivatives (glycine, taurine, alanine, proline) and methylamines (betaine, trimethylamine N-oxide) (Engin and Hotamisligil 2010; Ishida and Nagata 2009).

However, the majority of these chemical chaperones require high concentrations for effective folding of mutant proteins that can cause nonspecific effects and toxicity, hence making them unsuitable for in vivo applications, especially for chronic indications. Currently, two chemical chaperones, namely, 4-phenylbutyric acid (PBA) and tauroursodeoxycholic acid (TUDCA) are approved by US Food and Drug Administration (FDA) for use in humans. While PBA has been approved for clinical use in urea-cycle disorders in humans, TUDCA has been used as a liver-protecting agent in human cholestatic liver diseases, which is currently under clinical testing. PBA is a low-molecular weight fatty acid that has been found to have chaperone-like activities.

PBA could act to stabilize and, hence, prevent mutant protein to be directed to degradation pathway in the ER and facilitate its translocation to the cell surface. In addition, PBA and TUDCA have been shown to exert their chaperonic activity within the central nervous system (Inden et al. 2007; Solá et al. 2003).

Administration of these drugs needs to management of conditions such as obesity and type 2 diabetes may require long term dosing. Long term clinical trial trials also necessary to observe potential side effects. And the adaptation of UPR signaling especially IRE1-XBP1 and PERK-eIF2a pathways in many organs and tissues needs to be determined.

10 Conclusion

ER stress is a critical mechanism in obesity induced insulin resistance. The balance and stability of the endoplasmic reticulum is crucial for the cell. Accumulated misfolded proteins and/or decreased folding capacity reveals ER stress. Mitigation of the ER stress is an important therapeutic strategy for related diseases. Known chemical chaperones at the time and additional molecules that needed for increased folding capacity and function of unfolded proteins may alleviate ER stress. New strategies like targeting UPR components with small molecules maybe a promising strategy for their antidiabetic effects. Inadequate protein processing begun to understood that an important factor in pathogenesis of obesity and insulin resistance. Modulation of stress response offers new potential therapeutic opportunities against insulin resistance, type 2 diabetes and wide spectrum of pathologies.

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Insulin Resistance, Obesity and Lipotoxicity

12

Dilek Yazıcı and Havva Sezer

Abstract

Lipotoxicity, originally used to describe the destructive effects of excess fat accumulation on glucose metabolism, causes functional impairments in several metabolic pathways, both in adipose tissue and peripheral organs, like liver, heart, pancreas and muscle. Lipotoxicity has roles in insulin resistance and pancreatic beta cell dysfunction. Increased circulating levels of lipids and the metabolic alterations in fatty acid utilization and intracellular signaling, have been related to insulin resistance in muscle and liver. Different pathways, like novel protein kinase c pathways and the JNK-1 pathway are involved as the mechanisms of how lipotoxicity leads to insulin resistance in nonadipose tissue organs, such as liver and muscle. Mitochondrial dysfunction plays a role in the pathogenesis of insulin resistance. Endoplasmic reticulum stress, through mainly increased oxidative stress, also plays important role in the etiology of insulin resistance, especially seen in non-alcoholic fatty liver disease. Visceral adiposity and insulin resistance both increase the cardiometabolic risk and lipotoxicity seems to play a crucial role in the pathophysiology of these associations.

Keywords

Lipotoxicity • Insulin resistance • Type 2 diabetes mellitus • Obesity • Fatty acids • Diacylglycerol • Ceramides • Cytokines • Endoplasmic reticulum stress

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1 Introduction

Type 2 diabetes and obesity are the most common metabolic diseases. The primary cause of type 2 diabetes is unknown, it is clear that insulin resistance plays an early role in its pathogenesis, followed by defects in insulin secretion by

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pancreatic beta cells (Lowell and Shulman 2005). Insulin resistance is associated with a metabolic and cardiovascular cluster of disordes (dyslipidemia, hypertension, visceral obesity, glucose intolerance, endothelial dysfunction), which are independent risk factors for cardiovascular disease (CVD) (Defronzo 2010). The molecular causes of insulin resistance, impaired insulin signalling through the phosphoinositol-3 kinase pathway with intact signalling through the mitogen-activated protein kinase pathway, are responsible for the impairment in insulin-stimulated glucose metabolism (Defronzo 2010). Insulin resistant state develops one to two decades before the onset of the disease (Lillioja et al. 1988, 1993; Defronzo et al. 1992). It is the best predictor for later development of the disease (Warram et al. 1990). Reducing insulin resistance prevents the development of diabetes (Azen et al. 1998).

Skeletal muscle and liver are the two key insulin-responsive organs responsible for maintaining normal glucose homeostasis (Lowell and Shulman 2005). To dephicer insulin resistance, it is important to understand the cellular mechanisms responsible for insulin resistance in these organs (Lowell and Shulman 2005).

2 Normal Insulin Signalling

For the biological effects insulin, it must first bind to specific cell surface receptors (Taniguchi et al. 2006). This activates second messengers which initiate a phosphorylation-dephosphorylation cascade that stimulates glucose transport (GLUT-4), glucose phosphorylation (hexokinase II), glycogen synthase (which controls glycogen synthesis) and both phosphofructokinase and pyruvate dehydrogenase (which regulate glycolysis and glucose oxidation) (White et al. 1988). In the muscle, insulin binding to its receptor (White et al. 1988; Taniguchi et al. 2006) leads to tyrosine phosphorylation of insulin receptor substrate-1 (IRS-1), which mediates insulin's effect on glucose metabolism. In the liver, IRS-2 phosphorylation mediates the actions of insulin. IRS-1 activates phosphatidylinositol (PI)-3 kinase (Sun et al. 1992), which catalyses 3′ phosphorylation of PI, PI-4 phosphate and PI-4,5 diphosphate, and augments glucose transport and glycogen synthase (Ruderman et al. 1990; Brady et al. 1997; Dent et al. 1990).

Insulin signalling also plays a critical role in activating nitric oxide synthase, which regulates nitric oxide production (Steinberg et al. 1994; Zeng et al. 2000; Montagnani et al. 2001). Nitric oxide is a potent vasodilator and anti-atherogenic agent (Steinberg et al. 1994). Nitric oxide deficiency activates multiple pathways involved in atherogenesis (Brunner et al. 2005; Naruse et al. 1994). Thus, a defect in insulin signalling, not only impairs glucose utilisation, but causes hypertension and accelerated atherosclerosis. Insulin is a potent growth factor, whose growth-promoting effects are mediated by the mitogen-activated protein (MAP) kinase pathway (Wang et al. 2004). This pathway plays an important role in atherogenesis. Insulin resistance in the PI-3 kinase (metabolic) pathway with intact MAP kinase signalling activates multiple inflammatory pathways, including inhibitor kB (IkB)/nuclear factor kB (NFkB) and c-Jun N-terminal kinase (JNK), which also cause insulin resistance. Sustained physiological hyperinsulinemia activates multiple genes involved in inflammation (Yuan et al. 2001; Hirosumi et al. 2002; Coletta et al. 2008) (Fig. 12.1).

3 Insulin Resistance and Atherosclerosis

This pathogenic sequence establishes the molecular basis linking insulin resistance, inflammation and accelerated atherosclerosis in people with type 2 diabetes mellitus and also explains why insulin resistance is a strong predictor of cardiovascular disease (CVD) (Golden et al. 2002). In diabetic and obese patients, continued MAP kinase pathway stimulation causes vascular smooth muscle proliferation, increased collagen formation and excessive production of growth factors/inflammatory cytokines, contributing to accelerated atherosclerosis (Cusi et al. 2000). Type 2 diabetes and obesity are characterised by

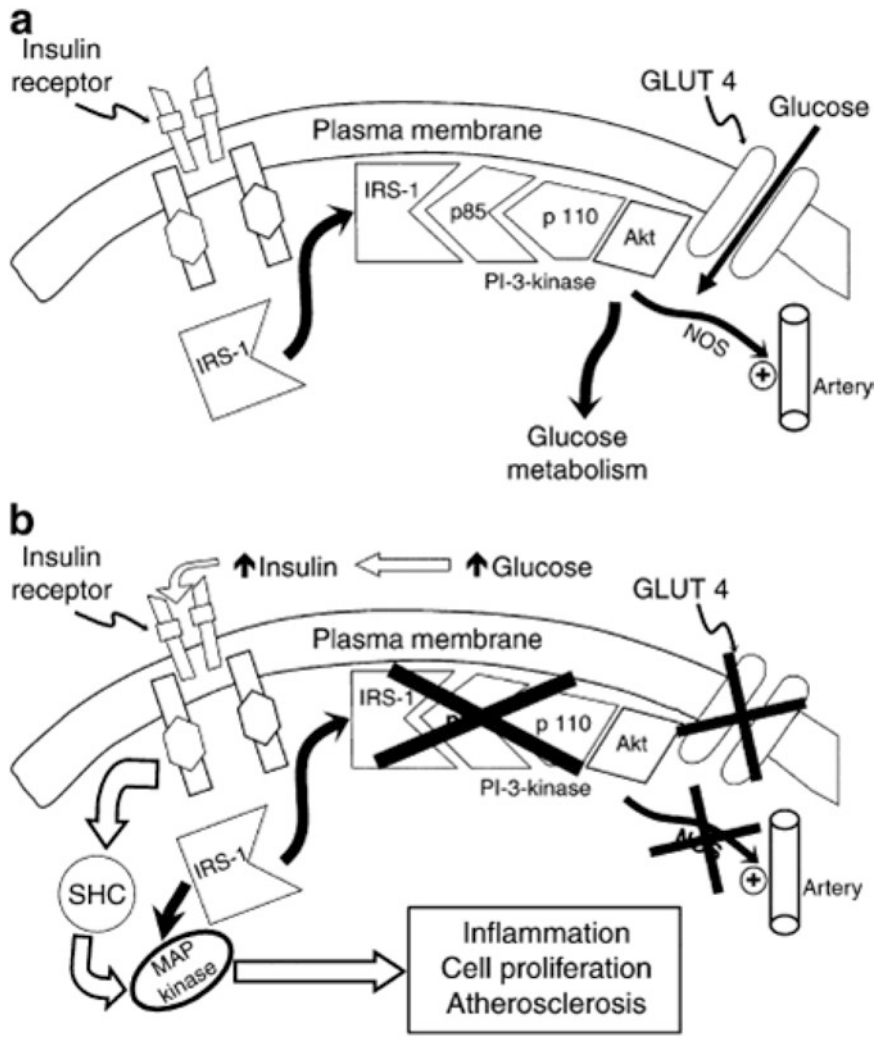

Fig. 12.1 (**a**) Insulin signal transduction system in individuals with normal glucose tolerance. *NOS* nitric oxide synthase. (**b**) Insulin signalling is impaired at the level of IRS-1 in type 2 diabetic patients leading to decreased glucose transport/phosphorylation/metabolism and impaired nitric oxide synthase activation/endothelial function. Insulin signalling through the MAP kinase pathway is normally sensitive to insulin. The compensatory hyperinsulinaemia (due to insulin resistance in the IRS-1/PI-3 kinase pathway) results in excessive stimulation of this pathway, which is involved in inflammation, cell proliferation and atherogenesis. *SHC* Src homology collagen (Defronzo 2010). Defronzo, R. A. 2010. Insulin resistance, lipotoxicity, type 2 diabetes and atherosclerosis: the missing links. The Claude Bernard Lecture 2009. Diabetologia, 53, 1270–87

low-grade chronic inflammation, which could contribute to accelerated atherosclerosis (Evans et al. 2002; Garg et al. 2003; Pickup and Crook 1998). Increased activity of ıkb/NFkb provides a molecular mechanism responsible for inflammation and insulin resistance in type 2 diabetes mellitus (Duckworth et al. 2009). The free NFkB translocates to the nucleus, where it binds to target genes, stimulating inflammatory mediators (TNFa, IL-1B, IL-6, PKC) involved in atherogenesis (Evans et al. 2002; Barnes and Karin 1997; Yamamoto and Gaynor 2004). These cytokines cause serine phosphorylation of IRS-1, inhibiting insulin signalling and causing insulin resistance (De Alvaro et al. 2004) (Fig. 12.2).

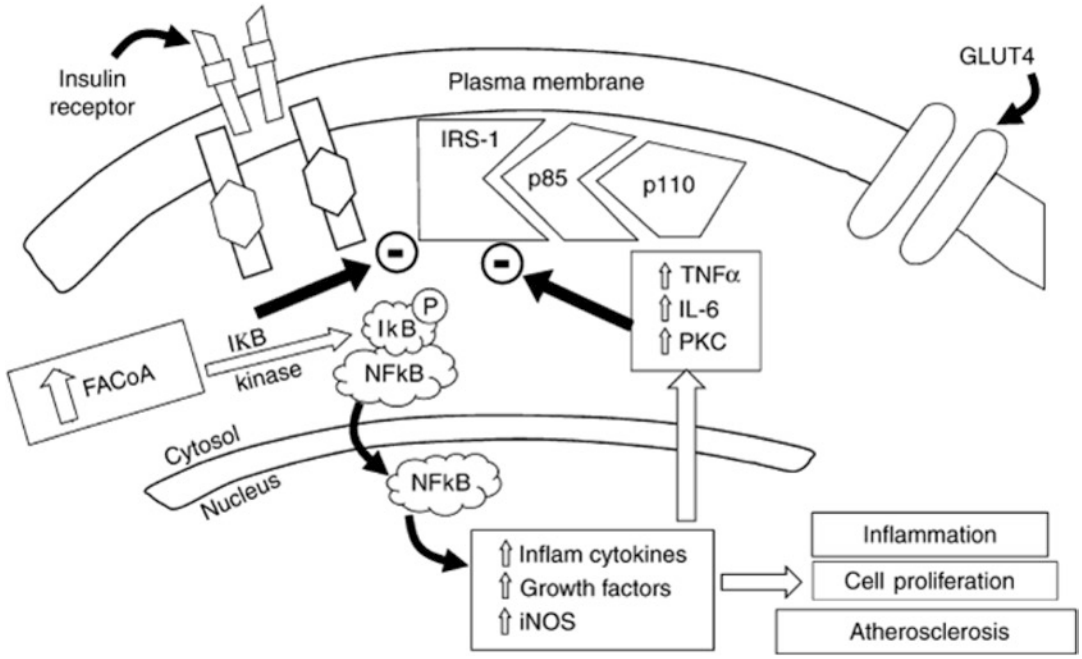

Fig. 12.2 Relationship between intracellular fatty acyl CoA levels, IkB/NFkB and the insulin signal transduction pathway (Defronzo 2010). *iNOS* inducible nitric oxide synthase. Defronzo, R. A. 2010. Insulin resistance, lipotoxicity, type 2 diabetes and atherosclerosis: the missing links. The Claude Bernard Lecture 2009. Diabetologia, 53, 1270–87

Adipose tissue produces adipocytokines, which travel to distant sites (muscle, liver, arterial tissue) where they exert deleterious effects on metabolism and vascular function. Adipose tissue of obese and type 2 diabetic individuals is infiltrated by mononuclear cells and is in a state of chronic inflammation (Henry et al. 1993; Wellen and Hotamisligil 2003). The adipocytes and infiltrated macrophages secrete proinflammatory/prothrombotic cytokines (TNFa, resistin, IL-6, plasminogen activator inhibitor-1, angiotensinogen) that promote atherogenesis and cause insulin resistance (Bays et al. 2004). Adipocytes also produce adiponectin, a potent insulin-sensitising, anti-atherogenic cytokine (Bays et al. 2004).

4 Lipotoxicity

Functional impairments associated with increased circulating levels of lipids and their induced metabolic alterations in fatty acid (FA) utilization and intracellular signaling, have been broadly termed lipotoxicity (Wende et al. 2012). Lipotoxicity is the deleterious effect of tissue fat accumulation on glucose metabolism (Unger 2003). Non essential fatty acid (NEFA) elevation in type 2 diabetes causes severe muscle/liver insulin resistance and inhibits insulin secretion (Kashyap et al. 2003, 2004; Richardson et al. 2005; Dresner et al. 1999). Elevated plasma NEFA impair glucose oxidation/glycogen synthesis and decrease glucose transport/phosphorylation (Kashyap et al. 2004; Dresner et al. 1999). Intramyocellular and intrahepatic fat accumulation are closely associated with organ-specific insulin resistance. Intracellular toxic metabolites of triacylglycerol (TAG) and NEFA metabolism [long-chain fatty acyl COAs (LCFA-CoA), diacylglycerol, ceramides] cause severe insulin resistance by impairing insulin signalling and multiple intracellular steps of glucose metabolism (Kashyap et al. 2004; Belfort et al. 2005; Griffin et al. 1999). Over 40 years ago, Randle et al. demonstrated that fatty acids caused insulin resistance in a rat muscle in vitro. According to them, increased oxidation of muscle fatty acids

would produce increased levels of intracellular acetyl COA and citrate, which would inhibit two enzymes involved in glucose utilization, pyruvate dehydrogenase and phosphofructokinase (Randle et al. 1963). Inhibition of the glycolytic pathway at these steps would increase intracellular glucose and glucose-6-phosphate concentrations and reduce insulin stimulated glucose uptake (Randle et al. 1963). Increased plasma NEFA/intramyocellular levels of toxic lipid metabolites (LCFA-CoA's, diacylglycerol, ceramides) play a role in the pathogenesis of muscle/liver insulin resistance (Defronzo 2010).

Although Randle's hypothesis may hold true for acute settings, it does not explain insulin resistance in chronic disease states, which can be attributed to reductions in both insulin-stimulated muscle glycogen synthesis and glucose oxidation (Shulman et al. 1990). Subsequent in vivo measurements have clearly demonstrated that lipid-induced insulin resistance in skeletal muscle can be attributed to impaired insulin signaling and decreased insulin-stimulated glucose transport, and not to decreased glycolysis, as Randle hypothesized (Cline et al. 1999; Dresner et al. 1999; Griffin et al. 1999). Molecular studies have suggested that insulin resistance could be attributed to impaired GLUT4 translocation, largely due to defects in insulin signaling (Ciaraldi et al. 1995; Garvey et al. 1998).

5 Lipid Moities that Cause Insulin Resistance

The kind of the free fatty acids, instead of the quantitity of fatty acids, are effective in lipotoxicity. Dietary fatty acids can be classified as saturated fatty acids, monounsaturated fatty acid and polyunsaturated fatty acids (PUFA). PUFA's can further be classified as n-3 and n-6 PUFA's according to the position of the first bond. Most findings are consistent with the fact that the saturated FA's are related to obesity, insulin resistance and cardiovascular disease. A diet rich in mono- and polyunsaturated FA's are inversely related with the presence of metabolic syndrome (Gillingham et al. 2011; Robinson and Mazurak 2013).

The lipid moieties are also classified as circulating lipids (e.g., endotoxins and prostaglandins) and intracellular lipid intermediates [such as diacylglycerols, ceramides, and phosphatdylinositol triphosphate (PIP3)]. Both the circulating and intracellular ones provide some specific degree of signaling, that is localised to certain regions of the cell. Certain lipid moieties seem to play a more active role in insulin resistance.

Fatty acids are rapidly esterified with coenzyme A to fatty acyl-CoAs upon entry into the cell. When these are transferred to a glycerol backbone, mono-, di-, and triacylglycerols are formed. They can also be esterified with sphingosine to form ceramides. Some of the intracellular lipid intermediates (e.g., diacylglycerol and ceramides) function as second messengers in key signaling pathways. The intracellular lipid intermediates are thought to be responsible in the pathogenesis insulin resistance (Samuel and Shulman 2012).

Excess LCFA-CoA in muscle cells in high-fat-fed animals are esterified to glycerol-3-phosphate and DAG is produced (Timmers et al. 2008). DAG has been shown to accumulate in muscles of high-fat-induced rodent models and of obese individuals (Moro et al. 2009). DAG can activate both protein kinase C (PKCz), and this is associated with inhibition of early steps in the insulin signal cascade (Savage et al. 2007, Gao et al. 2007, Ragheb et al. 2009). Mitochondrial acyl-CoA:glycerol-sn-3-phosphate acyltransferase (mtGPAT) catalyzes the formation of lysophosphatidic acid from fatty acyl CoA and glycerol 3-phosphate (Fig. 12.3). When mtGPAT-deficient (mtGPAT1_/_) mice are placed on a high-fat diet, they accumulate hepatic fatty acyl-CoA, but not hepatic diacylglycerol and triglyceride (Neschen et al. 2005). And these mice do not lead to insulin resistance. Similarly although hepatic mtGPAT overexpression does not decrease fatty acyl-Coa, it leads to hepatic insulin resistance due to the accumulation of lysophosphatidic acid, DAG, and TAG (Nagle et al. 2007). These studies suggest that fatty acyl CoAs do not cause insulin resistance.

Ceramides have been shown to play role in the pathogenesis of insulin resistance. Ceramides are

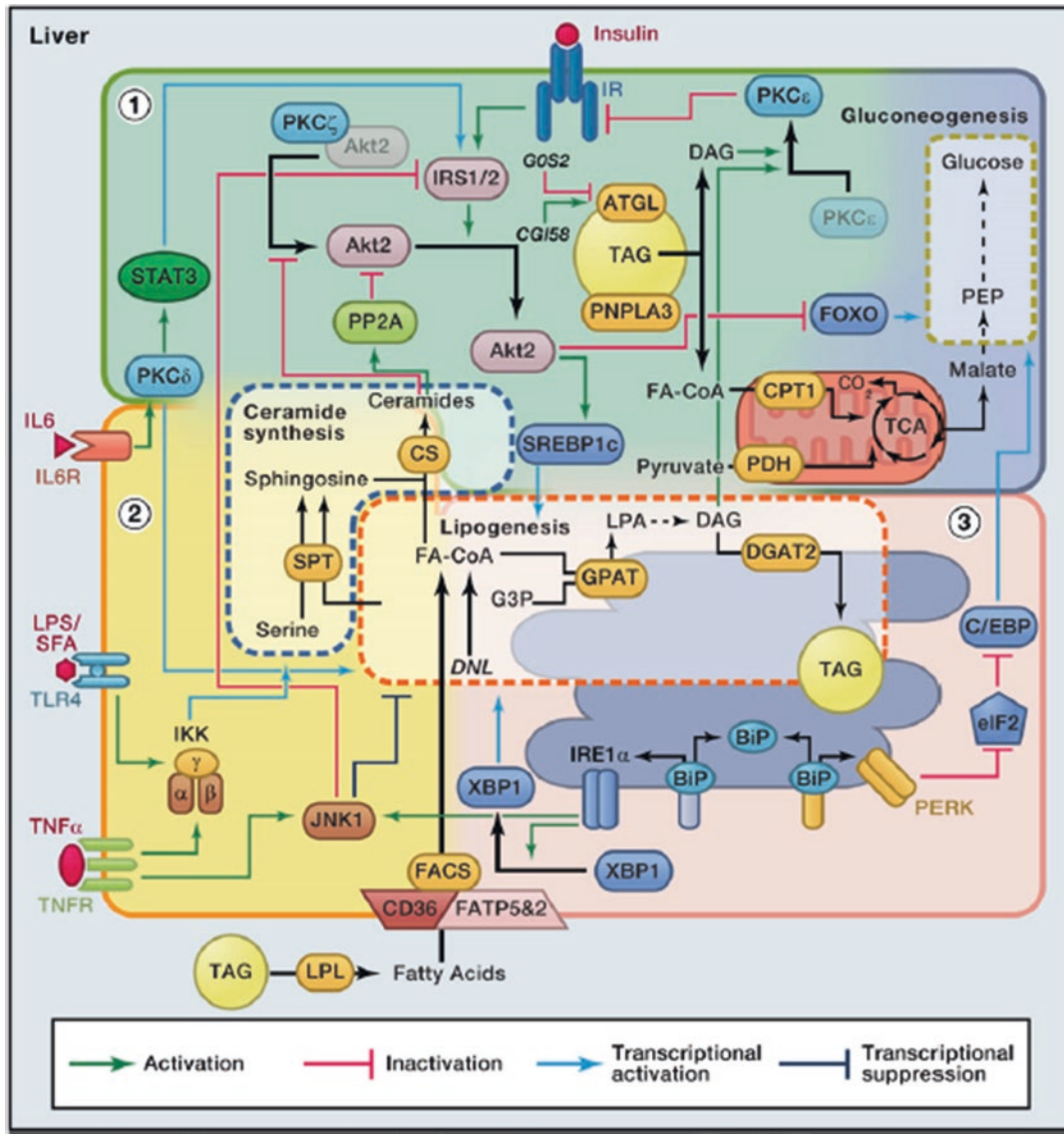

Fig. 12.3 Insulin activates the insulin receptor (IR) tyrosine kinase, which subsequently tyrosine phosphorylates IRS1 and 2. This leads to Akt2 activation. Akt2 can promote glycogen synthesis (not shown), suppress gluconeogenesis, and activate de novo lipogenesis (DNL). This central signaling pathway is connected to multiple other cellular pathways that are designated by numbers 1–3. Key lipid synthesis pathways are juxtaposed within this domain and are regulated by them. (1) The *green shaded areas* represent mechanisms for lipid-induced insulin resistance—notably, diacylglycerol-mediated activation of PKCε and subsequent impairment of insulin signaling, as well as ceramide mediated increases in PP2A and increased sequestration of Akt2 by PKCz. Impaired Akt2 activation limits the inactivation of FOXO1 and allows for increased expression of key gluconeogenesis enzymes. Impaired Akt2 activity also decreases insulin-mediated glycogen synthesis (not shown). (2) The *yellow areas* depict several intracellular inflammatory pathways—notably, the activation of IKK, which may impact ceramide synthesis, and the activation of JNK1, which may impair lipogenesis. (3) The *pink area* depicts activation of the UPR that can lead to increased lipogenesis via XBP1s and also increased gluconeogenesis via C/EBP. The ER membranes also contain key lipogenic enzymes and give rise to lipid droplets. Proteins that regulate the release from these droplets (e.g., ATGL and PNPLA3) may modulate the concentration of key lipid intermediates in discrete cell compartments (Samuel and Shulman 2012). *CS* ceramide synthase, *DNL* de novo lipogenesis, *FA-CoA* fatty acyl CoA, *G3P* glycerol 3-phosphate, *LPA* lysophosphatidic acid, *SPT* serine palmitoyl transferase, *TAG* triacylglycerol, *TCA* tricarboxylic acid cycle, *PEP* phosphoenolpyruvate. Samuel VT, Shulman G. 2012. Mechanisms for insulin resistance: common threads and missing links. *Cell,* 148, 852–71

primarily membrane lipids, a precursor in the formation of sphingomyelin (Samuel and Shulman 2012). Increases in hepatic and muscle ceramide content, along with diacylglycerols, have been associated with insulin resistance in obese Zucker (fa/fa rats, homozygous for a truncated, nonfunctional leptin receptor) (Turinsky et al. 1990). If fat-fed mice are treated with myriocin, an inhibitor of serine palmitoyl transferase 1, the increase in muscle ceramide content is attenuated in fat-fed mice without any change in long chain acyl-CoAs, diacylglycerols, or triglyceride. Treatment with myriocin improves glucose tolerance. The role of ceramides as a mediator of insulin resistance may be limited to saturated fats. Myriocin prevents acute skeletal muscle insulin resistance following infusion of palmitate, but not oleate (Holland et al. 2007). Saturated fatty acids lead to ceramide synthesis through inflammatory signals (Samuel and Shulman 2012). Palmitate infusion increases plasma cytokine concentrations, and these act through toll-like receptor 4 (TLR4). Mice lacking TLR4 are protected from ceramide accumulation and insulin resistance following lard, but not soy oil infusions (Holland et al. 2011). It has been suggested that saturated fatty acids themselves may also be the ligands for TLR4 (Shi et al. 2006). Thus, intracellular ceramides may also act as "second messengers" that play role in a cell's response to circulating cytokines or signals like saturated fatty acids.

Acylated FA's can esterify into DAG, metabolize into ceramides (only in the case of saturated FA), or enter ß-oxidation as acylcarnitine conjugates, when not stored as intramyocellular triglyceride (IMTG) (Consitt et al. 2009). If ß-oxidation as acylcarnitine conjugates is incomplete, acylcarnitines of diverse size will accumulate (Koves et al. 2008). These different forms of lipid accumulation are considered to be different causative agents of insulin resistance. Some of these FA metabolites have been related to interruptions in insulin signaling at different levels. This leads to reduction in insulin-dependent GLUT4 translocation that mediates stimulation of glucose uptake. It leads to insulin signalling through insulin receptor substrate-1 (IRS1), PIP-3 and bifurcation towards Akt activation (Zaid et al. 2008; Huang et al. 2005; Randhawa et al. 2008).

DAG, produced from phosphatidylinositol 4,5-bisphosphate by the action of phospholipase C is another class of lipid intermediates that can function as second messengers. It is incorporated into triacylglyerol or triglyceride hydrolysis pathways. Its accumulation in insulin resistance may be secondary to an imbalance between its synthesis and in its incorporation into tryglycerides. These lipid metabolites also have intracellular signaling properties. Acyl-CoA:diacylglycerol acyltransferase (DGAT) are the enzymes that acylate diacylglycerols into triglycerides. Mice that overexpress skeletal muscle DGAT1 (MCK-DGAT1) accumulate muscle triglyceride but do not exhibit muscle insulin resistance (Liu et al. 2007). Although muscle triglyceride content is increased in these mice, muscle DAG content is lower and this may explain the protection from lipid-induced insulin resistance. This is in line with the athlete's paradox in which endurance athletes have increased muscle triglyceride yet are insulin sensitive (Goodpaster et al. 2001; Krssak et al. 2000). DAGs can also be converted into phosphatidic acid, a major membrane lipid, by diacylglycerol kinases (DGKs) (Chibalin et al. 2008). DGKd expression is decreased in skeletal muscles of both hyperglycemic rodents and poorly controlled diabetic patients. DGKd haploinsufficient (DGKd+/_) mice have increased muscle DAG, but not triglyceride, and muscle insulin resistance. These studies all underline the fact that tissue fatty acyl CoA and triglyceride are not the pathogenic lipid species for insulin resistance (Samuel and Shulman 2012).

6 Intracellular Localization of Lipids Related to Insulin Resistance

It is estimated that where the lipid intermediates are accumulated intracellularly is important in the development of insulin resistance. Intracellular lipid droplets are called "adiposomes," (Liu et al. 2004) and are considered as sites of active lipid synthesis and lipolysis (Figs. 12.3 and 12.4).

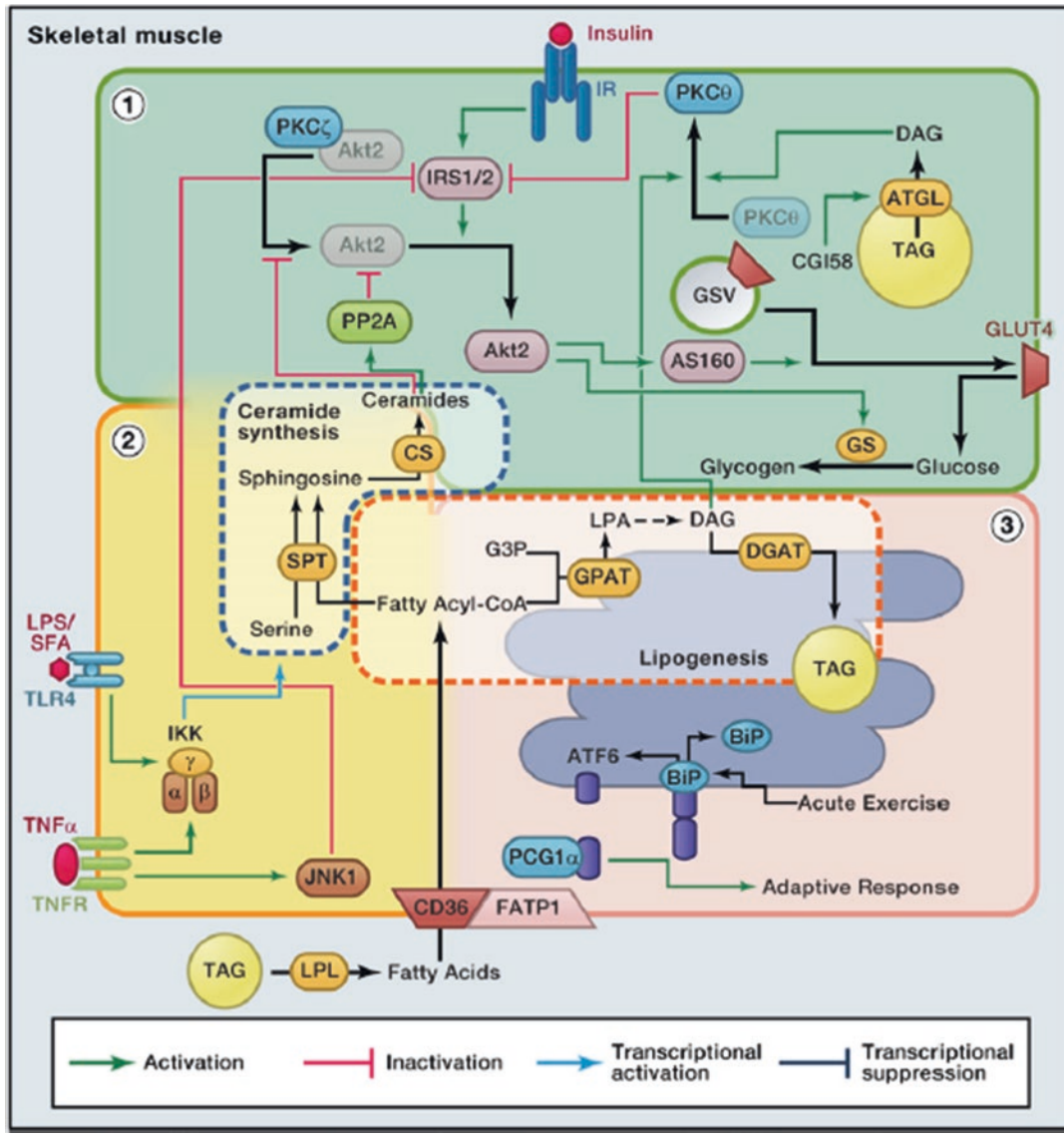

Fig. 12.4 Insulin activates the insulin receptor (IR) tyrosine kinase, which subsequently tyrosine phosphorylates IRS1. Through a series of intermediary steps, this leads to activation of Akt2. Akt2 activation, via AS160 and Rab-GTPase (not shown), promotes the translocation of GLUT4-containing storage vesicles (GSVs) to the plasma membrane, permitting the entry of glucose into the cell, and promotes glycogen synthesis via glycogen synthase (GS). This central signaling pathway is connected to multiple other cellular pathways that are designated by numbers 1–3. (1) The *green shaded areas* represent mechanisms for lipid induced insulin resistance, notably diacylglycerol (DAG)-mediated activation of PKCq and subsequent impairment of insulin signaling, as well as ceramide-mediated increases in PP2A and increased sequestration of Akt2 by PKCz. Impaired Akt2 activation limits translocation of GSVs to the plasma membrane, resulting in impaired glucose uptake. Impaired Akt2 activity also decreases insulin-mediated glycogen synthesis. (2) The *yellow areas* depict several intracellular inflammatory pathways—notably, the activation of IKK, which may impact ceramide synthesis, and the activation of JNK1, which may impair insulin signaling via serine phosphorylation of IRS1. (3) The *pink area* depicts activation of the unfolded protein response (UPR), which under some instances (such as acute extreme exercise) may lead to activation of ATF6 and a PGC1a-mediated adaptive response. The endoplasmic reticulum membranes also contain key lipogenic enzymes and give rise to lipid droplets. Proteins that regulate the release from these droplets (e.g., ATGL and PNPLA3) may modulate the concentration of key lipid intermediates in discrete cell compartments (Samuel and Shulman 2012). *CS* ceramide synthase, *G3P* glycerol 3-phosphate, *LPA* lysophosphatidic acid, *SPT* serine palmitoyl transferase, *TAG* triacylglycerol. Samuel VT, Shulman G. 2012. Mechanisms for insulin resistance: common threads and missing links. *Cell,* 148, 852–71

A single-nucleotide polymorphism (SNP), rs738409, in the gene encoding the lipid droplet protein patatin-like phospholipase domain containing protein 3 (PNLPA3) (Romeo et al. 2008) has been associated with non-alcoholic fatty liver disease (NAFLD). This protein, also called adiponutrin, possesses triglyceride lipase and transacylation activity (Jenkins et al. 2004). rs738409 polymorphism results in loss of lipolytic activity. Although carriers of this SNP have increased liver fat content, it is not associated with insulin resistance (Kantartzis et al. 2009; Kotronen et al. 2009) The exact role of PNPLA3 in regulating lipid metabolism remains unknown. Normally PNPLA3 functions as a lipase and increased hepatic PNPLA3 expression should promote lipolysis and decrease liver lipid accumulation. Overexpression of the wild-type PNPLA3 does not seem to alter liver lipid content (He et al. 2010; Qiao et al. 2011). PNPLA3 knockout mice do not develop hepatic steatosis, even when provoked with a variety of dietary challenges (Basantani et al. 2011; Chen et al. 2010). This enzyme also possesses transacylation activity and thus is considered to have a role in lipid synthesis (Jenkins et al. 2004). The fact that PNPLA3 expression in adipose tissue (Caimari et al. 2007; Kershaw et al. 2006) and liver (Huang et al. 2010) decreases with fasting and increases in response to insulin and glucose is consistent with a potential role in lipogenesis. Additional studies are needed to better understand the role of PNPLA3 in regulating lipid homeostasis and its relationship with the accumulation of key signaling lipid metabolites, e.g., diacylglycerol or ceramides.

Adipose triglyceride lipase (ATGL, also known as desnutrin and PNPLA2) is a potent lipase that catalyzes the hydrolysis of triglycerides into diacylglycerols. Mice lacking ATGL exhibit ectopic lipid accumulation in most tissues, manifesting dramatically with massive cardiac lipid accumulation leading to premature death (Haemmerle et al. 2006). Although there is increased adiposity and ectopic lipid accumulation, glucose tolerance is improved, again due to triglycerides not having role in the pathogenesis of insulin resistance. Human studies have demonstrated that ATGL protein expression in skeletal muscle is inversely related to whole-body insulin stimulated glucose disposal (Badin et al. 2011). Adipose-specific overexpression of ATGL also appears to protect mice from insulin resistance, probably due to an increase in lipid cycling and increased white adipose tissue uncoupling protein 1 (UCP1) expression, which increases energy expenditure (Ahmadian et al. 2009). In the liver, deletion of ATGL results in mice that are more prone to hepatic steatosis and decreased lipid oxidation but without alteration in glucose tolerance (Wu et al. 2011). ATGL seems to have a role both in regulating energy balance and in the generation of key lipid intermediates. Cells cannot readily access the energy stored in triglycerides without ATGL but at the same time cannot produce the intermediary lipid products causing insulin resistance.

Studies in mice that overexpressed DGAT2 have demonstrated that the intracellular localization of diacylglycerol is important (Jornayvaz et al. 2011). This enzyme is normally localized to the endoplasmic reticulum but, with lipid accumulation, it is highly expressed in the lipid droplet membranes (Stone et al. 2009). In the DGAT2 transgenic mice (specifically the "low-overexpressing" strain), DAG content in the cytosolic fraction containing the lipid droplets is increased nearly tenfold, while total DAG content is increased only modestly.

7 Role of Mitochandria in Insulin Lipotoxicity and Insulin Resistance

Mitochondria are the main site of lipid degradation. Cells protect themselves from lipotoxicity or death by either oxidizing FAs or sequestering them triacylglycerol (TAG) within lipid droplets (LDs). TAG is the major form of energy storage that with sterol esters. TAGs are synthesized by acyltransferases and phosphatases at the sarcoplasmic reticulum and mitochondrial membrane and then packaged into cytoplasmic LDs. Lipids are not stored as FAs but as TAGs produced by a series of esterification reactions that combine three FA molecules with glycerol 3-phosphate (Walther and Farese 2009, 2012). In oxidative tissues, TAG-derived FAs are utilized as an energy

source, but they also serve as signaling molecules as well as building blocks for membranes and complex lipids. Hepatocytes, heart, skeletal myocytes, adrenocortical cells, enterocytes and macrophages may all contain large amounts of LDs. Excessive LD accumulation is a hallmark of tip 2 DM, obesity, atherosclerosis, hepatic steatosis and other metabolic diseases (Aon et al. 2014). Development of tissue lipotoxicity and dysfunction is linked to alterations in LD biogenesis and regulation of TAG hydrolysis. The protein family of perilipins (Plin) is associated with LDs. Perilipins affect the spatial and metabolic interactions between LD and mitochondria. Plin 1 is the most abundant protein in adipocytes and plin 2 in the liver (Greenberg and Coleman 2011). Plin 1 protein enhances catecholamine-stimulated lipolysis and importantly a reduction in Plin 1 protein expression is associated with increased constitutive lipolysis which can promote systemic insulin resistance (Greenberg and Coleman 2011). Reduced expression of perilipins may promote both lipolysis and fat oxidation, resulting in reduced intracellular TAG and adipose mass. On the other hand excessive lypolysis and defective lipid storage may promote insulin resistance (Aon et al. 2014).

It is well established that mitochondrial function is required for normal glucose-stimulated insulin secretion from pancreatic beta cells (Lowell and Shulman 2005). Impaired ATP synthesis and mitochondrial dysfunction play a role in the pathogenesis of insulin resistance and type 2 diabetes (Lowell and Shulman 2005; Defronzo 2010). Peterson et al. have shown that decreased ATP synthesis is associated with increased intramyocellular fat. In isolated mitochondria from obese and type 2 diabetic individuals, ATP synthesis is reduced and correlates closely with decreased insulin-stimulated glucose disposal and increased fasting plasma NEFA (Petersen et al. 2003). Mitochondrial oxidative activity and mitochondrial adenosine triphosphate (ATP) synthesis decrease, these data support the hypothesis that insulin resistance in humans arises from defects in mitochondrial fatty acid oxidation, which in turn lead to increases in intracellular fatty acid metabolites (fatty acyl CoA and diacylglyerol) that distrupt insulin signaling (Lowell and Shulman 2005). Type 2 diabetic individuals and insulin-resistance offspring with normal glucose tolerance have reduced expression of multiple nuclear genes that encode enzymes involved in oxidative metabolism, including peroxisome proliferator-activated receptor (PPAR) coactivator (PGC-1alfa, 1beta), the master regulator of mitochondrial biogenesis (Patti et al. 2003; Puigserver and Spiegelman 2003). Studies support this idea, PGC-1-responsive genes are down-regulated in obese with impaired glucose tolerance and type 2 diabetic individuals. The reduction in mitochondrial oxidative-phosphorylation activity in insulin-resistance individuals is suggested to be due to a defect in mitochondrial function (Lowell and Shulman 2005). In one study, the activity of mitochondrial oxidative enzymes was found to be lower in type 2 diabetic patients who were also obese (Vondra et al. 1977). Obese individuals have also been shown to have a smaller mitochondria with reduced bioenergetic capacity compared with lean controls (Kelley et al. 2002).

8 Signaling Pathways Involved in Lipotoxicity and Insulin Resistance

FFA's act through many different signaling pathways. One of the pathways affected by saturated FA's is through the toll-like receptor 4 (TRL-4). TRL's are the receptors that recognize pathogen-associated molecular patterns and activate the innate immune response. LRT4 recognizes lipopolysaccahride (LPS), a component of cell wall in gram negative bacteria and activates inflammatory signaling pathways including nuclear factor kappa light chain enhancer of activated B cells pathway (NF-kappa B). Saturated FA residues in LPS are necessary for activation of LTR4 signaling.

FFA's activate TLR4 signaling in adipocytes, macrophages and skeletal cells and are considered to cause insulin resistance in mice (Shi et al. 2006). TRL4(-) mice are protected from the expression of proinflammatory cytokines in adipose tissue and insulin resistance induced by lipid infusion. C3H/HeL mice, with a loss of function mutation in the TLR4 gene are protected against the development of HFD-induced insulin

resistance (Tsukumo et al. 2007). Lipid infusion and/or a high fat diet activate inflammatory signaling within metabolic tissues, such as adipose tissue, partly through TLR4. This inflammatory activation contributes to insulin resistance.

The complex roles of immune cells in metabolic tissue have been shown. Adipose tissue contains different immune cells, which are constitutively present but accumulate further in obesity. These immune cells communicate with adipocytes within adipose tissue. Inflammatory cytokines secreted by macrophages activate lipolysis in adipocytes through supressing insulin signaling. FFA's that are released from the adipocytes further activate macrophages via TLR4. Macrophages and other immune cells contribute to the development of insulin resistance in the liver and skeletal muscle (Shi et al. 2006). Thus it is suggested that FFA's promote insulin resistance through activating inflammatory pathways via TLR4 expressed on immune cells. Other pathways also appear to have role in lipid-induced insulin resistance. Lipid infusion has been shown to increase the levels of lipid metabolites, like fatty-acyl-CaA and DAG (Yu et al. 2002). DAG in turn activates protein kinase C (PKC) and some PKC isoforms have been shown to interfere with insulin receptor signaling (Boden 2011).

9 Role of Novel Protein Kinase Cs

Protein kinase C family members, through their kinase activity sense lipid signals and affect cellular events in response. The PKC kinases are subdivided into three categories: conventional PKCs (cPKC: a, bI, bII, and g) that require both calcium and diacylglycerol for activation, novel PKCs (nPKC: d, ε, h, and q) that require only diacylglycerol and atypical PKCs (aPKC: z and l) that require neither calcium nor diacylglycerol. Increased plasma fatty acid concentrations due to an infusion of intralipid/heparin causes impaired insulin signaling and skeletal muscle insulin resistance, that is associated with activation of PKC-q (Griffin et al. 1999).

Increased activity of the novel-type PKCu and PKCe are involved in inhibition of muscle insulin action in response to elevated FA (Frangioudakis and Cooney 2008; Yu et al. 2002; Corcoran et al. 2007). Both PKCu and PKCe act upstream of the stress kinases IkBa kinase b (IKKb) and c-Jun NH2-terminal kinase (JNK) and thus they mediate phosphorylation of IRS1 on Ser307 (Gao et al. 2007). This phosphorylationof IRS1 at these serine residues reduces its tyrosine phosphorylation and downstream propagation of the insulin signal. PKCe may promote the degradation of insulin receptors (Ikeda et al. 2001). However there are several reports that do not verify these suggestions.

For instance impaired insulin signalling at the level of IRS1, that is thought to be the cause of insulin resistance, may not necessarily cause downstream insulin resistance. In an oxidative stress model of insulin resistance, although the defect in IRS1 was corrected, downstream insulin resistance persisted (Potashnik et al. 2003). These findings suggest that insulin resistance related to GLUT4 translocation may be involving mechanisms other than IRS1. Thus, the interelation between DAG, PKC and lipid induced muscle insulin resistance needs to be clarified by further research.

PKCq activation was found to be associated with decreased insulin-stimulated IRS-1 tyrosine phosphorylation along with increased IRS-1 serine phosphorylation (Li et al. 2004). Mice lacking PKCq are protected from skeletal muscle insulin resistance following acute lipid infusion (Kim et al. 2004b). In contrast, relatively chronic exposure to 14 weeks of high-fat feeding, PKCq knockout mouse are prone to diet-induced obesity and develop muscle, adipose, and hepatic insulin resistance (Gao et al. 2007).

PKCs are also implicated in the pathogenesis hepatic insulin resistance in non alcoholic fatty liver disease (NAFLD). High-fat feeding in rodents cause marked hepatic steatosis and hepatic insulin resistance without peripheral insulin resistance. Hepatic insulin resistance is thought to be associated with activation of PKCε. Decreasing PKCε expression enhances hepatic insulin response in fat-fed rats despite hepatic steatosis, demonstrating that PKCε is required for the development of hepatic insulin resistance in NAFLD (Samuel and Shulman 2012).

PKCd is another novel isoform that is also associated with hepatic insulin resistance. PKCd has been shown to be activated in the livers of rats subjected to a 6 h intralipid/heparin infusion, along with the development of hepatic insulin resistance (Lam et al. 2002). The effects of PKCd may be mediated through altered lipogenesis; expression of key lipogenic enzymes is decreased in PKCd knockout mice and increases following adenoviral overexpression. Although PKCd is important for the development of hepatic steatosis, whether it acts as part of an inflammatory signal or a mediator of lipid homeostasis, is unknown (Fig. 12.3). Early studies suggest that nPKCs impair insulin signaling through interferance with insulin receptor activation (Pillay et al. 1990; Takayama et al. 1988), while this was not confirmed with later studies (Kellerer et al. 1995). nPKC-mediated impairment of insulin receptor kinase activity is also evident in the liver, where, PKCε and the insulin receptor are closely associated with each other. These data may provide evidence to show that diacylglycerol-mediated activation of nPKCs directly impairs insulin signaling and insulin action, explaining insulin resistance in both muscle and liver with lipid excess.

10 Role of Akt2

Accumulation of ceramides has been associated with impaired Akt2 action. The proposed mechanisms are activation of protein phosphatase 2A (Teruel et al. 2001), which can dephosphorylate Akt2, effectively decreasing insulin signaling (Fig. 12.3). Ceramides may also impair insulin action via the atypical PKC isoform, PKCz (Powell et al. 2003), which prevents Akt2 activation (Fig. 12.4).

11 JNK Pathway in Insulin Resistance

One of the common pathways for the insulin resistance seen in inflammatory states is the activation of JNK1. Activation of JNK1 plays an important role in the mechanism whereby the unfolded protein response (UPR) may cause insulin resistance. In inflammatory signaling pathways (e.g., in response to TNFa), JNK1 activation follows a parallel pathway to NF-kB activation. JNK1 knockout mice are protected from diet-induced insulin resistance. JNK1 inactivation in macrophages and adipocytes have an increase in energy expenditure and are protected from diet induced obesity, hepatic steatosis, and glucose intolerance (Tuncman et al. 2006). JNK1 inactivation also increases energy expenditure, possibly via increased brown adipose tissue (BAT) expression of UCP1, decreased body weight gain and liver triglyceride content, decreased PKCε activation, and protection from insulin resistance. Tissue-specific manipulations of JNK1 provide additional insights. Liver-specific JNK1 deletion promotes hepatic steatosis, with increased expression of key lipogenic enzymes, impaired insulin signaling, and hepatic insulin resistance (Zhang et al. 2011b).

Adipose JNK1 knockout mice are protected from hepatic steatosis and specifically hepatic insulin resistance, but they exhibit similar weight gain and adiposity on a high-fat diet (Sabio et al. 2008). Fat-fed muscle-specific JNK1 knockout mice have similar weight gain and impairment of glucose tolerance as wild-type mice. However, they show a modest improvement in muscle-specific glucose uptake, with improvement in insulin signaling. Surprisingly, these mice also manifest increased adipose tissue inflammation and a modest increase in hepatic steatosis, though without differences in adipose or hepatic insulin sensitivity. Thus JNK 1 in adipocytes may be related with increased lipolysis, however in the liver it may supress lipogenesis. This may be a mechanism to coordinate shift of metabolic fuel substrates.

Thus JNK1 seem to have different effects in different tissues in the pathogenesis of insulin resistance. Alterations in JNK1 activity can alter insulin responsiveness, its role may be indirect and may instead be related to its ability to alter intracellular lipid metabolism (Samuel and Shulman 2012).

12 Lipoprotein Lipase

Lipoprotein lipase (LpL) is a key enzyme that hydrolyzes circulating triglyceride, allowing tissue uptake through specific fatty acid transport

proteins (FATPs) together with CD36. LpL expression in muscle leads to increased muscle lipid uptake and insulin resistance in muscle (Kim et al. 2001). In contrast, deletion of LpL (Wang et al. 2009) or of other proteins involved in fat transport, such as CD36 (Hajri et al. 2002) or FATP1 (Kim et al. 2004c), protects mice against muscle lipid accumulation and insulin resistance (Fig. 12.4). Hepatic LpL overexpression leads specifically to hepatic steatosis and hepatic insulin resistance (Kim et al. 2001).

The liver actively exports lipids. ApoC3 plays role, whereby inhibiting LpL activity and limiting peripheral fat uptake. This leads to postprandial hyperlipidemia (Samuel and Shulman 2012). When placed on a high-fat diet, ApoC3 tg mice develop hepatic steatosis with diacylglycerol accumulation, PKCε activation, and hepatic insulin resistance (Lee et al. 2011). A mismatch between hepatic lipid uptake and lipid export in the high fat-fed mice leads to the development of hepatic steatosis. While hepatic lipid uptake is increased, lipid export, via ApoB100 containing VLDL particles, is decreased in the hyperinsulinemic fat-fed mice due to suppression of ApoB100 expression. This is important for human disease. Individuals with body mass index of <25 kg/m^2, carrying polymorphisms of the ApoC3 gene-insulin response element region, have increased fasting plasma ApoC3 concentrations and fasting hypertriglyceridemia (Petersen et al. 2010). Thus, hepatic insulin resistance may result from ectopic lipid accumulation in liver either from increased delivery or decreased export (Samuel and Shulman 2012).

13 Lipids and the Unfolded Protein Response

A significant amount of work has focused on the role of UPR in the pathogenesis of hepatic insulin resistance and NAFLD (Fig. 12.3). Activation of the UPR, also termed endoplasmic reticulum stress plays important role in the etiology of insulin resistance, especially seen in NAFLD (Samuel and Shulman 2012). Lowered protein folding capacity of the ER causes accumulation of immature proteins in the ER lumen and this triggers a complex primarily adaptive signaling network called the unfolded protein response (UPR) in order to restore ER homeostasis. Unfolded proteins recruit increasing amounts of BiP, ER chaperone, which is thus detached from the luminal domains of three transmembrane ER stress sensor proteins: RNA-dependent protein kinase-like ER kinase (PERK), inositol-requiring enzyme 1 (IRE1) and activating transcription factor 6 (ATF6). When the UPR fails to restore the ER functions, apoptosis is stimulated mostly by induction of CCAAT/enhancer binding protein homologous protein (CHOP) and activation of JNK. JNK activation is also triggered by the oxidative stress in lipotoxicity (Gao et al. 2010). Besides its pro-apoptotic activity, JNK also interferes with insulin signaling by phosphorylating insulin receptor substrate-1 (IRS-1) at serine (Aguirre et al. 2000), which represents a key link between ER-stress and insulin resistance. Activation of the UPR was observed in the livers of leptin deficient ob/ob mice (Ozcan et al. 2004), suggesting that these pathways may be involved in the pathogenesis of insulin resistance in obese states in rodents as well as in humans (Gregor et al. 2009). Markers of the UPR are decreased after surgical induced weight loss (Gregor et al. 2009). Activation of the UPR mostly provides cells the ability to adapt to changing demands. This is especially evident in pancreatic beta cells in insulin-resistant states. The increased amount of insulin produced could overwhelm the capacity of the ER to process and secrete insulin. On the other hand, in other tissues, such as liver and adipose tissue, activation of the UPR is maladaptive; IRE1-mediated activation of JNK1 leads to serine phosphorylation of IRS-1, which impairs insulin signaling (Ozcan et al. 2004; Tuncman et al. 2006). The UPR regulates lipogenesis, allowing for expansion of the ER membrane and increasing the capacity of the ER to handle proteins (Samuel and Shulman 2012). The ability of the UPR to cause hepatic insulin resistance may ultimately depend on whether UPR activation alters the balance of lipogenesis and lipid export. The programmed cell death caused by fatty acids, that is at least partly due to ER stress, is referred to as lipoapoptosis. The role of PERK/ATF4/CHOP signaling pathway has been demonstrated

in saturated fatty acid-induced ER stress and lipoapoptosis in L02 and HepG2 human liver cell lines (Cao et al. 2012). Increased levels of palmitate has been shown to disrupt ER homeostasis by decreasing the expression of Bip in HepG2 cells.

In adipose tissue, UPR activation appears to regulate energy balance. Mice with a heterozygous deletion of Grp78 had an activation of "adaptive" UPR factors and are protected against diet-induced obesity, hepatic steatosis, and insulin resistance (Ye et al. 2010). Activation of UPR following loss of the Grp78 may alter adipogenesis and energy balance to improve insulin sensitivity. The UPR also has role in skeletal muscle (Fig. 12.4). Chronic high-fat feeding activates the UPR in both early (e.g., 6 weeks high-fat feeding) and late stages (20 weeks) in mice. However, 6 weeks of high-fat feeding in healthy human subjects did not activate the UPR in skeletal muscle, even though there were the expected changes in glucose tolerance and intramyocellular lipid accumulation. Regarding the effect of UPR in insulin resistance, different models, with different challenges employed (e.g., varying diets, chemical inducers) and different assessments of glucose and insulin action are conducted. It is difficult to conclude that the UPR directly interferes with insulin signaling and leads to insulin resistance. However, the data suggest that the UPR functions as a part of cellular response to balance metabolic needs. Some aspects of the UPR clearly regulate lipogenesis (e.g., the IRE1a-XBP1s arm), lipid droplet formation, and lipid storage (e.g., through ATF6) and also regulate glucose metabolism (e.g., signaling through eiF2a and CREBH (Lee et al. 2010). Thus, activation of the UPR may primarily alter cellular lipid balance and, via accumulation of lipid intermediates, alter insulin signaling (Samuel and Shulman 2012).

14 Lipotoxicity and Hepatic Insulin Resistance

Lipotoxic derangement of ER functions is likely to be due to oxidative stress, disturbed calcium homeostasis and altered membrane saturation. Inhibition of fatty acid oxidation has been shown to protect hepatocytes from ER stress, as a result of elevated ratio of oxidized to reduced glutathione and enhanced oxidative folding in the ER (Tyra et al. 2012). ER lipid perturbation can trigger the UPR directly and independently of luminal accumulation of unfolded proteins. Two of the ER stress receptors, IRE1α and PERK have been revealed to respond to increased lipid saturation (Volmer et al. 2013). This novel mechanism is in accordance with the greater toxicity of saturated vs. unsaturated fatty acids as well as with the protective effect of unsaturated fatty acids against saturated fatty acid induced toxicity (Mantzaris et al. 2011; Nivala et al. 2013; Zhang et al. 2011c).

On the other hand, several genes related to lipoprotein are controlled by IRE1α, and hence induction of the UPR upon membrane lipid perturbation might contribute to the prevention of hepatic steatosis (Zhang et al. 2011a). Mice with hepatocyte-specific IRE1α deletion without ER stress display modest hepatosteatosis, and this is aggravated after induction of ER stress (Zhang et al. 2011a). Disturbed lipid metabolism can lead to ER stress and trigger the UPR, and the ER stress-dependent alteration in lipid homeostasis might underlie the hepatic steatosis (Zámbó et al. 2013).

Increased reactive oxygen species (ROS) generation has been shown to be involved in hepatic lipotoxicity. Palmitate induces oxidative stress and this contributes to insulin resistance in H4IIEC3 rat hepatocytes (Nakamura et al. 2009; Seifert et al. 2010). Fatty acid accumulation causes ROS generation in the liver presumably through enhanced β-oxidation and electron overflow in the mitochondrial electron transfer chain (Seifert et al. 2010). A decrease in mitochondrial quinone pool and a related inhibition of mitochondrial oxidative metabolism were also suggested to be mechanisms underlying the increased mitochondrial ROS production in high fat diet (Vial et al. 2011). In HepG2 and McNtcp.24 liver cells exposed to saturated FFAs, mitochondrial depolarization, cytochrome c release, and increased ROS production were detected. The role of lysosomal disruption was also suggested (Li et al. 2008). Increased expression (Weltman et al. 1998) and activity (Videla et al. 2004;

Orellana et al. 2006) of cytochrome P450 2E1 (CYP2E1) monooxygenase likely contributes to the oxidative stress in lipotoxicity. CYP2E1 is an integral membrane protein of the ER and an important source of oxidative intermediates including free radicals. CYP2E1 activity has been shown to be positively correlated with body mass index and with the degree of steatosis (Chtioui et al. 2007). Increased ROS production causes damage by injuring DNA, proteins and lipids. It activates certain stress-sensitive signaling pathways, such as nuclear factor κB, p38MAPK, and JNK (Ghosh et al. 2009), which in the long run favor cell death. Increased oxidative stress also affects the redox homeostasis in the ER lumen (Banhegyi et al. 2007, 2012).

15 Lipotoxicity and Insulin Resistance in Muscle

Three mechanism are considered to play role in the way lipid metabolites cause insulin resistance in muscle. These are altered intramuscular lipid metabolism, circulating cytokines and macrophage infiltration of muscle tissue (Kewalramani et al. 2010). FA's that are released into the circulation may accumulate in the skeletal muscle (Kraegen et al. 1991). Within muscle FA's become activated to acyl-CoA derivatives. The majority of them are esterified into tryglycerides. Although IMTG accumulation has earlier been proposed to be related to insulin resistance in muscle, the other fat metabolites such as DAG's, ceramides, LCFA-CoA's and acylcarnitine products of incomplete FA oxidation, are more directly responsible for insulin resistance in skeletal muscle (Muoio 2010).

Numerous studies have indicated that ceramides play role in the development of impaired insulin-stimulated muscle glucose uptake through acting downstream of IRS1 (Holland et al. 2007; Watson et al. 2009). Ceramides can impair insulin signaling in muscle by reducing Akt activation (Thrush et al. 2009; Summers 2006). However, the reductions observed in Akt activity may not be the cause of insulin resistance in muscle, since GLUT4 translocation would need as little as 20% of Akt activation (Bilan et al. 2009). This suggests that targets other than Akt may contribute to insulin resistance to ceramides in muscle. Muscle ceramide levels are elevated only when muscle is exposed to saturated FA (palmitate) and not unsaturated FA (linoleate). Inhibition of ceramide biosynthesis prevents palmitate-induced insulin-resistant glucose uptake, but not the insulin resistance caused by linoleate (Holland et al. 2007). These results indicate that palmitate leads to insulin resistance primarily via ceramides, provided its metabolism is not diverted to DAG or IMTG. Whether ceramides directly influence insulin sensitivity in human skeletal muscle in vivo remains unclear (Boden 2008; Skovbro et al. 2008; Straczkowski et al. 2007).

The increased amount of acyl-CoAs in muscle of high-fat fed animals may induce upregulation of ß-oxidative metabolism. However, downstream pathways such as the TCA cycle or the electron transport chain activity may not compensate for this increased metabolism. This leads to the accumulation of LCFA-CoAs and acylcarnitine conjugates that are not completely oxidized (Muoio 2010). Moreover levels of LCFA-CoAs are elevated in conjunction with muscle insulin resistance (Bruce et al. 2009). LCFA-CoAs may impair muscle insulin action by acting as precursors to other lipid intermediates as ceramides and DAG. A direct target of LCFA-CoAs in the insulin-signalling pathway has not been identified yet. The improved muscle insulin action in rat muscle with overexpression of carnitine palmitoyltransferase-1 provides some proof that FA oxidation is a suitable target to improve muscle insulin action (Bruce et al. 2009). Indeed, carnitine supplementation can improve insulin action in high-fat-fed animals, suggesting that carnitine availability may be the limiting factor for appropriate fat oxidation (Muoio 2010). Acylcarnitines themselves may be toxic or the incomplete FA oxidation generates oxidative radicals that interfere with the insulin-signalling cascade (Samocha-Bonet et al. 2010). Direct exposure of muscle cells to low levels of peroxide causes insulin resistance through insulin-induced Akt phosphorylation and Rac activation (Jebailey et al. 2007), and in the long

term reduces tyrosine phosphorylation of IRS1 (Bashan et al. 2009).

In summary, FA's cause muscle insulin resistance through changes in the levels of DAG, ceramide, LCFA-CoA, acylcarnitines and possibly oxidative radicals. Each lipid metabolite interfere with distinct steps in the insulin signaling cascade, reducing GLUT4 translocation to the muscle membrane.

16 Role of Macrophages in Skeletal Muscle Insulin Resistance

Macrophages are an important component of skeletal muscle. Macrophages are mostly in their M2 (anti-inflammatory) phenotypic polarization (Olefsky and Glass 2010) in muscle, where they contribute to regeneration and revascularization. If they become M1 polarized (inflammatory), especially in close vicinity of muscle fibres, these macrophages release cytokines onto the muscle cells. It has been postulated that this may ocur in response to circulating FA's (Olefsky and Glass 2010; Bilan et al. 2009). These macrophages may also be directed to muscle through adipocytes that are found among muscle fibers in obesity (Vettor et al. 2009).

Activated macrophages have been shown to be increased within muscle tissue through different techniques (Weisberg et al. 2003; Nguyen et al. 2007). Increased infiltration of macrophages in skeletal muscle of obese insulin resistant individuals has been demonstrated by Varma et al. (Varma et al. 2009). On the other hand, two studies found very low expression of macrophage-specific markers (CD68 and CD14) in severely obese patients with muscle biopsies (Bruun et al. 2006; Di Gregorio et al. 2005). No change in the mRNA levels of these markers in muscle was observed with lifestyle intervention which seemed to improve insulin action (Bruun et al. 2006). In vitro studies have demonstrated that FA can activate macrophages to secrete cytokines that cause insulin resistance in muscle. In an in vitro system of co-culture of primary human skeletal muscle myotubes with THP-1 macrophages, palmitate increased the expression of a variety of cytokines and chemokines (TNFa, IL-6, IL-10, IL-1b and MCP-1) in the myotubes, caused IkBa protein degradation, elevated JNK phosphorylation and diminished insulin-dependent phosphorylation of Akt.

Adipose tissue macophages (ATM) may actually adjust lipid release and, in doing so, prevent excess lipid delivery that would further enhance ectopic lipid accumulation and exacerbate insulin resistance. The interaction between macrophages and adipose tissue may also optimize energy usage in the organism, providing that lipid release is in balance with lipid utilization (Samuel and Shulman 2012). ATMs secrete cytokines to provide this balance through a complex series of signaling pathways. TNFα binding can lead to activation of the mitogen-activated protein kinase pathways (e.g., JNK1) as well as the inhibitor of nuclear factor k-B kinase pathway (IKK) pathway. Adipose-specific expression of p65 (aP2-p65) activates inflammation in the adipose tissue, causing ATM recruitment and cytokine production, etc., but at the same time protects mice from weight gain when placed on a high-fat diet, probably due to an increase in energy expenditure (Tang et al. 2010). Thus, ap2-p65 transgenic mice are protected from diet-induced insulin resistance, with improved hepatic and peripheral insulin sensitivity (Samuel and Shulman 2012).

16.1 Inflammatory Cytokines Taking Role in Muscle Insulin Resistance

Numerous studies have demonstrated that cytokines increase adipose lipolysis (Kawakami et al. 1987; Stone et al. 1969), but the underlying mechanism is unclear. Earlier studies examined the possibility that cytokines may increase expression of key lipases. However, TNFa actually decreases expression of ATLG, which is thought to be secondary to decreased PPARϒ expression (Kim et al. 2006). IL-6 infusion in healthy humans increases plasma fatty acid and glycerol concentrations (indirect measures of lipolysis). Recent studies have suggested that

cytokines may affect the stability of lipid droplets in adipocytes by influencing the proteins that stabilize the lipid droplet. TNFα decreases perilipin expression, presumably enhancing the ability of lipases to access triglyceride within the lipid droplets (Laurencikiene et al. 2007). Adipose tissue inflammation may increase lipolysis. This, in turn, may also contribute to insulin resistance; excess lipolysis could drive fatty acid re-esterification and ectopic lipid accumulation, leading to the associated impairments in insulin signaling. Calorie restriction in fat-fed mice acutely increases ATMs and may represent a response to the initial increase in ATGL-mediated lipolysis (Kosteli et al. 2010). ATMs may play a role in regulating the rate of adipose lipolysis. Thus, ATMs may actually regulate lipid release and prevent excess lipid delivery that would further enhance ectopic lipid accumulation and exacerbate insulin resistance. The interaction between macrophages and adipose tissue may also organize energy usage in the organism, permitting lipid release that can be coordinated with lipid utilization.

ATMs secrete cytokines to orchestrate these metabolic changes in target tissues through a complex series of signaling pathways. For example, TNFα binding to the TNF receptor can lead to activation of the mitogen-activated protein kinase pathways (e.g., JNK1,) as well as the inhibitor of nuclear factor k-B kinase pathway (IKK).

FA's have been suggested to trigger the immune response in macrophages through TLR's, but they may also have direct effects. FAs lead to activation of inflammatory signals in adipocytes, in macrophages (Shi et al. 2006) and in muscle cells (Bilan et al. 2009), which leads to the release of proinflammatory cytokines. Cytokines can affect insulin action. Tumour necrosis factor-α (TNFa), interleukin (IL)-6 and IL-10 affect insulin responses directly in skeletal muscle. The balance between proinflammatory and anti-inflammatory cytokines are fundamental in the development of muscle insulin resistance.

Cytokines also increase adipose lipolysis (Kawakami et al. 1987; Stone et al. 1969), but the underlying mechanism is unclear. They may affect the stability of lipid droplets in adipocytes by influencing the proteins that stabilize the lipid droplet (Samuel and Shulman 2012).

16.1.1 Tumour Necrosis Factor-α

Obese rodent models of insulin resistance demonstrated an increase in TNFα in their adipose tissue (Hotamisligil et al. 1993). TNFα expression was also increased in the adipose tissue obtained from obese subjects; this was related to insulin resistance, and decreased with weight reduction (Hotamisligil et al. 1995; Kern et al. 1995). Adipose tissue TNFα secretion is thought to originate from activated macrophages (Weisberg et al. 2003; Xu et al. 2003). Palmitate augments TNFα gene expression in muscle cells, through stimulation of PKCu and activation of NF-kB (Jove et al. 2006; Hommelberg et al. 2009). Exposure of primary human muscle cell cultures to TNFα causes insulin resistance through decreasing glucose uptake (Austin et al. 2008; Bouzakri and Zierath 2007). The mechanisms beyond this involves activation of extracellular signal-regulated kinase-1/2 (ERK1/2), JNK, or the NFkB pathway.

16.1.2 Interleukin-6

In mouse C2C12 myoblasts, palmitate activates NF-kB and augments IL-6 production in parallel to reducing insulin-dependent glucose uptake (Jove et al. 2006). It has been argued that, IL-6 acutely may potentiate insuin actions on muscle glucose uptake, but with prolonged exposure may cause insulin resistance (Nieto-Vazquez et al. 2008). In rats, sustained IL-6 administration increased muscle insulin signalling at the level of IRS1 and Akt (Holmes et al. 2008). On the other hand, overexpression of IL-6 in mouse skeletal muscle impaired insulin-stimulated muscle glucose uptake (Franckhauser et al. 2008). Several pathways (AMPK, activation of JNK1/2, suppressor of cytokine signalling-3 expression and activation of the IRS1 tyrosine phosphatase, protein tyrosine phosphatase-1B) have been suggested regarding the effect of IL-6, but in vivo effects have not been delineated. IL-6 may need to interact with other pro-inflammatory and anti-inflammatory cytokines to modulate muscle insulin action (Samuel and Shulman 2012).

16.1.3 Interleukin-10

IL-10 is a classical anti-inflammatory cytokine expressed in muscle. It that can modulate lipid-induced insulin resistance. As well as directly inhibiting effects of excess lipid, it also protects against IL-6 induced muscle insulin resistance (Kim et al. 2004a). IL-10 partially prevents the harmful effects of palmitate-treated macrophages on insulin-induced GLUT4 translocation in L6 muscle cells (Samokhvalov et al. 2009). Although in vivo studies of IL-10 administration (Kim et al. 2004a) support the hypothesis that IL-10 reduces insulin resistance in muscle cells, the major cellular targets and the mechanisms involved remain uncertain.

In summary, TNFa, IL-6 and IL-10 directly act on muscle cells to alter insulin signalling and the stimulation of glucose uptake. TNFα generally has negative actions and IL-10 has positive actions and the response to IL-6 varies depending on the amount of exposure. The studies show that cultured muscle respond to cytokines, which in vivo may originate from adipose tissue, innate immune cells or even from autonomous production by the muscle cells themselves.

17 Pancreatic Beta Cell Dysfunction

Lipotoxicity further contributes to pancreatic beta cell dysfunction. Suggested mechanism like activation of reactive oxygen species (ROS) and endoplasmic reticulum (ER) stress have been identified as the mechanisms leading to beta cell lipotoxicity (Cnop 2008; Fonseca et al. 2011). It has been demonstrated that palmitate is involved in the development of pancreatic beta cell dysfunction (Gillingham et al. 2011). Palmitate does this through activating TLR4 on pancreatic beta cells and through the activation of chemokines. As a result, M1-type macrophages accumulate within the islets. Thus inflammation contributes to lipotoxicity in vivo. Also M1 macrophages have been shown to accumulate within islets in obese mice (Cucak et al. 2014; Jourdan et al. 2013). In mice fed on a high-fat diet (HFD), TRL4 receptor has been involved in the development of islet cell inflammation and beta cell dysfunction.

In obese type 2 diabetic individuals, beta cells don't secrete enough insulin to compensate for the increased demand (Rhodes 2005; Deng et al. 2004) and beta cell mass decrease (Butler et al. 2003). Increased rates of apoptosis play an important role (Pick et al. 1998). Numerous studies have documented that, in individuals with type 2 diabetes, beta cells don't sense glucose (Gerich 2003). Glucose sensing requires oxidative mitochondrial metabolism, leading to the generation of ATP (Maechler and Wollheim 2001). The increase in the ratio of ATP to adenosine diphosphate (ADP) in the beta cell, which then initiates the following chain of events: inhibition of the cell's ATP/ADP-regulated potassium channel, plasma membrane depolarization, opening of a voltage-gated calcium channel, calcium influx and secretion of insulin (Lowell and Shulman 2005). It is possible that decreased mitochondrial function in beta cells, might lead to beta cell dysfunction and type 2 diabetes (Lowell and Shulman 2005). Beta cell dysfunction in type 2 diabetes is thought to be secondary to glucotoxicity and/or lipotoxicity (Unger 1995; Poitout and Robertson 2002; Prentki et al. 2002). A number of hypotheses have been proposed to explain how these conditions induce beta cell dysfunction. One of these hypotheses, focuses on changes in the expression and function of a mitochondrial inner membrane protein called uncoupling protein-2 (UCP2) (Chan et al. 2001; Joseph et al. 2002; Krauss et al. 2003). UCP2 exerts substantial negative control over glucose-stimulated insulin secretion (Lowell and Shulman 2005). UCP2 is expressed in human beta cells and its expression is increased by hyperglycemia (Brown et al. 2002). Stimulation of UCP2 activity by superoxide is relevant to the development of beta cell dysfunction, because superoxide production is increased in beta cells of rodents with type 2 diabetes and in cultured beta cells exposed to hyperglycemia and elevated levels of lipids (Krauss et al. 2003, Bindokas et al. 2003). The superoxide-UCP2 proton leak pathway is an important contributor to beta cell dysfunction and may play an important role in the pathogenesis of type 2 diabetes (Lowell and Shulman 2005).

18 Conclusion

Excess nutrient intake seen in obesity, results in depositon of fat in non-adipose tissue. Functional impairments associated with increased circulating levels of lipids and the consequent metabolic alterations in fatty acid utilization and intracellular signaling have been termed lipotoxicity. Lipotoxicity has been implicated in insulin resistance and pancreatic beta cell dysfunction. Various lipid moities, such as diacylglycerols and ceramides, have been implicated in the development of insulin resistance. Several different pathways are involved in the effects of different lipid moieties leading to insulin resistance in nonadipose tissue organs, such as liver and muscle. Mitochondria are the main site of lipid degradation and mitochondrial dysfunction plays a role in the pathogenesis of insulin resistance. Effects through the toll like receptor 4, through novel protein kinase c pathways and the JNK-1 pathway have all been involved as the mechanisms whereby lipid moieties lead to insulin resistance. Activation of the unfolded protein response, also termed endoplasmic reticulum stress, through mainly increased oxidative stress, plays important role in the etiology of insulin resistance, especially seen in NAFLD. Fatty acids cause muscle insulin resistance through changes in the levels of DAG, ceramide, LCFA-CoA, acylcarnitines and possibly oxidative radicals. The balance between proinflammatory and anti-inflammatory cytokines are also fundamental in the development of muscle insulin resistance. TNFα generally has negative actions and IL-10 has positive actions and the response to IL-6 varies depending on the amount of exposure. Besides leading to insulin resistance, lipotoxicity also has role in pancreatic β cell dysfunction. Activation of reactive oxygen species (ROS) and endoplasmic reticulum (ER) stress have been identified as the mechanisms leading to beta cell lipotoxicity.

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13 Adipose Tissue Hypoxia in Obesity and Its Impact on Preadipocytes and Macrophages: Hypoxia Hypothesis

Atilla Engin

Abstract

Obese subjects exhibit lower adipose tissue oxygen consumption in accordance with the lower adipose tissue blood flow. Thus, compared with lean subjects, obese subjects have 44% lower capillary density and 58% lower vascular endothelial growth factor (VEGF). The VEGF expression together with hypoxia-inducible transcription factor-1 (HIF-1) activity also requires phosphatidylinositol 3-kinase (PI3K)- and target of rapamycin (TOR)-mediated signaling. HIF-1alpha is an important signaling molecule for hypoxia to induce the inflammatory responses. Hypoxia affects a number of biological functions, such as angiogenesis, cell proliferation, apoptosis, inflammation and insulin resistance. Additionally, reactive oxygen radical (ROS) generation at mitochondria is responsible for propagation of the hypoxic signal. Actually mitochondrial ROS (mtROS) production, but not oxygen consumption is required for hypoxic HIF-1alpha protein stabilization. Adipocyte mitochondrial oxidative capacity is reduced in obese compared with non-obese adults. In this respect, mitochondrial dysfunction of adipocyte is associated with the overall adiposity. Furthermore, hypoxia also inhibits macrophage migration from the hypoxic adipose tissue. Alterations in oxygen availability of adipose tissue directly affect the macrophage polarization and are responsible from dysregulated adipocytokines production in obesity. Hypoxia also inhibits adipocyte differentiation from preadipocytes. In addition to stressed adipocytes, hypoxia contributes to immune cell immigration and activation which further aggravates adipose tissue fibrosis. Fibrosis is initiated in response to adipocyte hypertrophy in obesity.

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Obesity • Hypoxia-inducible transcription factor-1 (HIF-1) alpha • Vascular endothelial growth factor (VEGF) • Angiogenesis • Reactive oxygen species (ROS) • Mitochondrial ROS (mtROS) • CAAT/enhancer binding protein (C/EBP) • CAAT/enhancer binding protein (C/EBP)-homologous protein (CHOP) • Adipocyte differentiation • Adipose tissue blood flow • Phosphatidylinositol 3-kinase (PI3K) • Inducible nitric oxide synthase (iNOS)

1 Introduction

Obesity is associated with a low-grade inflammation of white adipose tissue resulting from chronic activation of the innate immune system (Bastard et al. 2006). Although there is an enhanced secretion of some interleukins and inflammatory cytokines in adipose tissue of the obese individuals, adipose tissue macrophages are responsible for almost all adipose tissue tumor necrosis factor-alpha (TNF-alpha) expression and significant amounts of inducible nitric oxide synthase (iNOS) and interleukin (IL)-6 expression (Fain 2006; Weisberg et al. 2003). Among normal-weight healthy young adults, IL-6 is positively associated with quantitative insulin resistance check index and capillary density but the inverse is true among obese individuals (Cheng and Daskalakis 2015). Actually, angiogenesis and adipogenesis is expected to be spatially and temporally coupled during the expanding of adipose tissue (Crandall et al. 1997). However, in morbid obesity, angiogenesis is insufficient to achieve an appropriate level of vascularization for the expanded adipose tissue (Gealekman et al. 2011). Thus, adipose tissue blood flow is downregulated in obesity and its responsiveness to meal intake is reduced. However, there is little evidence indicating that this leads to adipose tissue hypoxia in human obesity (Frayn and Karpe 2014). In fact, perivascular adipose tissue in obesity becomes highly inflamed and induces vascular dysfunction by augmented secretion of vasoconstriction factors and pro-inflammatory adipokines. Furthermore, several adipocyte-derived adipokines also impair vascular function indirectly (Gu and Xu 2013). Inflammation in the visceral adipose tissue resulting from diet-induced obesity impairs endothelial function and nitric oxide (NO) bioavailability in the associated resistance arteries (Donato et al. 2012). Hereby, in this chapter, cellular hypoxia as an underlying cause of obesity-related-adipocyte dysfunction is reviewed.

2 Adipose Tissue Oxygenation in Obesity

Adipose tissue blood flow and muscle blood flow rates are approximately 30–40% lower in obese versus non-obese subjects (Bolinder et al. 2000). Compared with lean subjects, overweight/obese subjects have 44% lower capillary density and 58% lower vascular endothelial growth factor (VEGF). This might be due to lower peroxisome proliferator-activated receptor gamma1 (PPAR-gamma 1) and higher collagen VI mRNA expression, which correlates with the lower adipose tissue oxygen partial pressure. In this case, negative correlation between the adipose tissue oxygen partial pressure, CD68 mRNA and macrophage inflammatory protein 1alpha (MIP-1alpha) secretion indicates that lower adipose tissue oxygen partial pressure may be the cause of adipose tissue inflammation in obesity (Pasarica et al. 2009b).

Despite lower adipose tissue blood flow and reduced oxygen consumption, Goossens et al. found a surprisingly higher adipose tissue oxygen partial pressure in obese subjects when compared with lean individuals. This is also accompanied by insulin resistance, impaired adipose tissue

capillarization and higher adipose tissue gene expression of inflammatory cell markers (Goossens et al. 2011). Pasarica et al. controversially proposed that reduced adipose tissue oxygen partial pressure in obese subjects is not sufficient to activate the hypoxia target genes of adipose tissue. In addition to increase in the CD68 and galectin-3 (MAC2)/CD163 macrophage infiltration, lower adipose tissue oxygen partial pressure in obese humans is strongly correlated with the percentage of body fat (Pasarica et al. 2009b). Actually, obese subjects exhibit lower adipose tissue oxygen consumption in accordance with the lower adipose tissue blood flow. Furthermore, contrary to lean individuals, postprandial increase in adipose tissue blood flow and adipose tissue oxygen partial pressure is blunted in obese individuals (Goossens et al. 2011). In any way, it appears that oxygen delivery to adipose tissue is impaired in obesity. Consequently, adipocytes do not receive enough oxygen (Goossens and Blaak 2015). Thus, it has been put forward that diameter of hypertrophic adipocytes in obesity exceeds the normal diffusion distance of oxygen across tissues (100–200 μm). In this manner, it is expected that the large adipocytes will form a barrier to block oxygen diffusion (Brahimi-Horn and Pouysségur 2007). In fact, in human adipose tissue, there seems to be only a very small proportion of adipocytes with a diameter more than 100 μm in obese subjects (Goossens et al. 2012). This suggested that hypoxia is a result of reduction in adipose tissue blood flow. Therefore, the contribution of increased adipocyte size to the hypoxia response is controversial (Goossens and Blaak 2015). Albeit adipose tissue hypoxia has been proposed as a key underlying mechanism triggering tissue dysfunction, but data from human to support this hypothesis is very limited (Hodson 2014). Adipose tissue hypoxia may occur when body fat content exceeds more than 20% of body weight (Yin et al. 2009). Although adipose tissue blood flow is reduced by 40% in obesity, oxygen tension reduces by 75% in adipose tissue. The changes in blood flow and oxygen tension are not proportional in obese adipose tissue. Nevertheless, the barrier effect of large adipocytes presumably may contribute to the difference between the two parameters (Ye et al. 2007).

There are spatial and temporal interrelationships between blood vessel formation and adipogenesis. Theoretically due to adverse effect of increased diffusion distance for oxygen supply in obesity, angiogenesis may have a critical role in providing to expand adipose tissue with adequate oxygen and nutrients (Nishimura et al. 2007). Actually, human adipocytes are too sensitive to small changes in oxygen tension. The alterations in substrate transport are accompanied by parallel changes in glucose transporter 1 (GLUT1) and monocarboxylate transporters (MCT) mRNA expression (Wood et al. 2011). Increased glucose transport into adipocytes is also observed with low oxygen tension, as a result of the up-regulation of GLUT-1 expression (Wood et al. 2009). However, in hypoxic conditions, a switch is formed from oxidative metabolism to anaerobic glycolysis. Thereby, when the glucose utilization is increased in hypoxic adipocytes, corresponding increase in lactate production occurs (Trayhurn 2013). Exposure of the human pre-adipocyte cell line to 1% oxygen for 24 h results in a 2.3-fold increase in lactate in the medium compared with cells incubated under normal oxygen concentration. Hypoxia increases lactate release from adipocytes and modulates MCT expression in a type-specific manner, with 8.5-fold increase in MCT1 and 14.3-fold increase in MCT4 messenger RNA levels in human adipocytes being hypoxia-inducible transcription factor-1 (HIF-1)-dependent (Pérez de Heredia et al. 2010) (Fig. 13.1).

3 Hypoxia-Inducible Transcription Factor-1

HIF-1 is a heterodimer composed of alpha and beta subunits (De Ponti et al. 2007). The beta subunit is identical to the aryl hydrocarbon receptor nuclear translocator (ARNT) that also serves as a heterodimeric partner with the aryl hydrocarbon receptor (AHR) (Hoffman et al. 1991). The activated AHR and the ARNT represent a novel class of basic helix-loop-helix-containing tran-

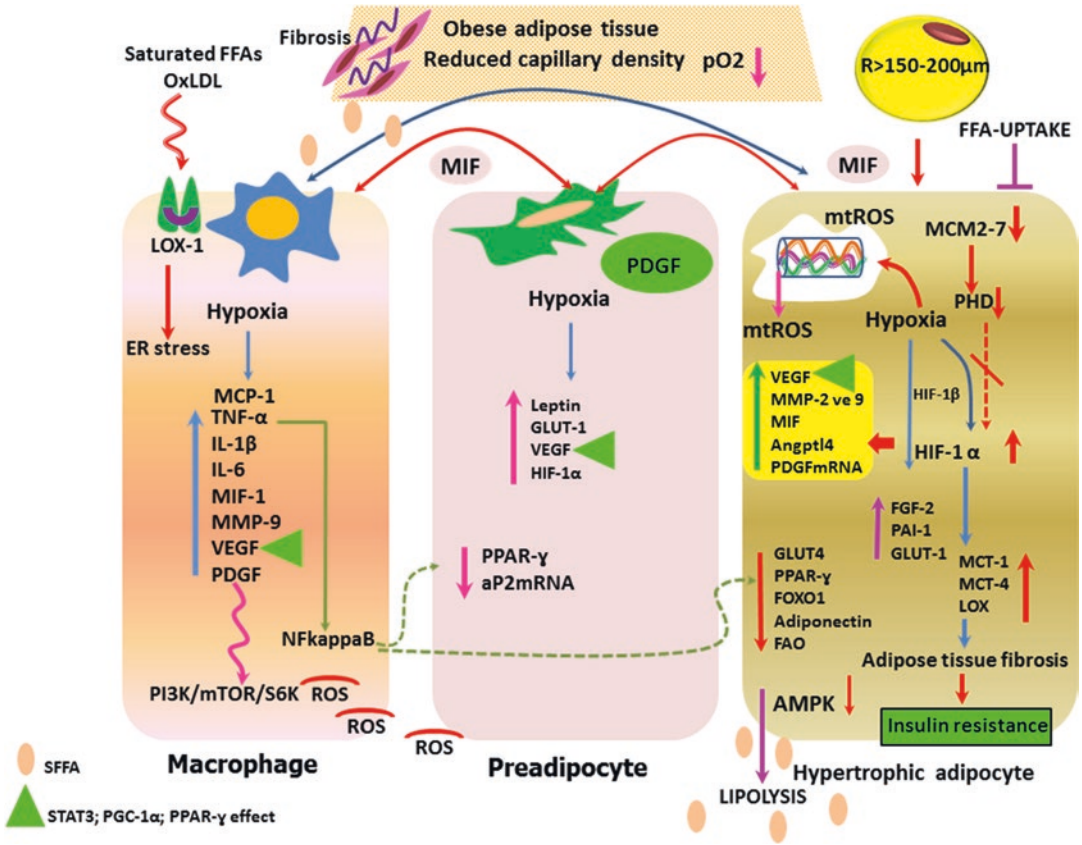

Fig. 13.1 Hypoxia affects a number of biological functions in obesity and also stimulates the inflammatory response of macrophages and inhibits adipocyte differentiation from preadipocytes. Several factors are recognized to influence the hypoxic pathways (*TNF-α* Tumor necrosis factor-alpha, *IL-1β* Interleukin-1beta, *IL-6* Interleukin-6, *MIF* Macrophage migration inhibitory factor, *MMP* Matrix metalloproteinase, *VEGF* Vascular endothelial growth factor, *PDGF* Platelet-derived growth factor, *PI3K* Phosphatidylinositol 3-kinase, *mTOR* Mammalian target of rapamycin, *S6K* S6 kinase, *NF-kappaB* Nuclear factor-kappa B, *GLUT* Insulin-responsive glucose transporter, *HIF-1* Hypoxia-inducible factor-1, *PPAR-γ* Peroxisome proliferator-activated receptor-gamma, *P2 (aP2)* Proximal promoter region of the adipocyte P2 (aP2) gene mRNA, *Angptl4* Angiopoietin-like protein 4, *FGF* Fibroblast growth factor, *PAI-1* Plasminogen activator inhibitor-1, *MCT* Monocarboxylate transporter (lactate transport), *LOX* Lectin-like ox-LDL receptor, *LDL* Low-density lipoprotein, *MCM* Minichromosome maintenance proteins, *PHD* Prolyl hydroxylase domain, *FFA* Free fatty acid, *SFFA* Saturated free fatty acid, *ROS* Reactive oxygen species, *mtROS* Mitochondrial reactive oxygen species, *OxLDL* Oxidized low density lipoprotein, *MCP-1* Macrophage chemoattractant protein-1, *ER* Endoplasmic reticulum, *FOXO1* Forkhead box transcription factor O1, *FAO* fatty acid oxidation, *AMPK* AMP-activated protein kinase, *STAT-3* Signal transducer and activator transcription 3, *PGC-1α* PPAR-gamma coactivator-1alpha)

scription factors. Basic helix-loop-helix domain is necessary for DNA binding as heterodimers (Reisz-Porszasz et al. 1994). Critical function of alpha subunit is mediation of the response to hypoxia. HIF-1alpha is an important signaling molecule for hypoxia to induce the inflammatory responses. Under normal oxygen conditions, half-life of HIF-1alpha protein is less than 5 min, whereas under hypoxic conditions, HIF-1alpha protein increases dramatically through inhibition of ubiquitination-proteasome mediated degradation of HIF-1alpha (Ye 2011). Multiple mini-chromosome maintenance (MCM) proteins constitute the core of the replicative DNA helicase, and bind directly to the HIF-1alpha subunit and synergistically inhibit HIF-1 transcriptional

activity via distinct oxygen-dependent mechanisms. MCM3 inhibits transactivation prolyl hydroxylase domain (PHD) function, whereas MCM7 enhances HIF-1alpha ubiquitination and proteasomal degradation. HIF-1 activity decreases when quiescent cells re-enter the cell cycle, and this effect is MCM dependent. Exposure to hypoxia leads to MCM2-7 downregulation (Hubbi et al. 2011; Ibarra et al. 2008). Indeed, HIF-1alpha interacts directly with MCM7 and MCM3. Overexpression of MCM7 and MCM3 leads to a decrease in HIF activity. Contrariwise, exposure to hypoxia leads to coordinated downregulation of the MCM proteins. Actually, MCM2–7 downregulation is a general feature of the hypoxic response mediated by the HIF transcription factors. During the normal oxygen supply, the MCM proteins inhibit HIF-1 activity in an oxygen- and hydroxylation-dependent manner: prolyl hydroxylation by PHD is necessary for the effect of MCM7 on HIF-1alpha stability and asparaginyl hydroxylation is necessary for the effect of MCM3 on HIF-1alpha transactivation (Hubbi et al. 2011). HIF-1 activity is primarily determined by hypoxia-induced stabilization of HIF-1alpha, which is otherwise rapidly degraded in oxygenated cells (Huang et al. 1996). Oxygen-dependent degradation domain within the central region of HIF-1alpha controls its degradation by the ubiquitin-proteasome pathway (Huang et al. 1998). After prolyl hydroxylation, HIF-1alpha is ubiquitinated and degraded by the 26S proteasome. Under hypoxic conditions HIF-1-prolyl hydroxylases are inactive and HIF-1alpha escapes ubiquitination and proteasomal degradation, and can be transported to the nucleus (Zagórska and Dulak 2004). The chaperone heat-shock protein 90 (HSP90) binds to HIF-1alpha under normoxic conditions, whereas ARNT binds to HIF-1alpha under hypoxic conditions. In this case ARNT displaces HSP90 from HIF-1alpha following nuclear translocation (Katschinski et al. 2004). After translocation of HIF-1alpha to the nucleus, it dimerizes with HIF-1beta and binds to ARNT to form the active HIF-1 protein, which binds to the promoter DNA of target genes and induces multiple hypoxia responsive genes transcription (Ye 2011). Thus, during hypoxic conditions, HIF-1 triggers the overexpression of genes coding for glycolytic enzymes and angiogenic factors. In addition, HIF-1 is a substrate for various kinase pathways including phosphatidylinositol-3 kinase (PI3K) and the mitogen-activated protein kinase (MAPK), extracellular signal-regulated kinase (ERK) and p38 (Minet et al. 2001). The expression of an active form of protein kinase B (PKB, Akt) and treatment of cells with specific inhibitors of PI3K, MAPK, and mammalian target of rapamycin (mTOR) show that mainly PI3K and to a lesser extent mTOR are required for insulin-induced HIF-1alpha expression. The VEGF expression together with HIF-1 activity also requires PI3K- and TOR-mediated signaling (Treins et al. 2002). Thereby, hypoxia affects a number of biological functions, such as angiogenesis, cell proliferation, apoptosis, inflammation and insulin resistance (He et al. 2011; Trayhurn et al. 2008) (Fig. 13.1).

Although adipose tissue HIF-1alpha activity is influenced on a large scale by hypoxia, the HIFs are not the only transcription factors that signal the hypoxic response. Several other factors are recognized to influence the hypoxic pathways, including nuclear factor-kappaB (NF-kappaB), cyclic adenosine monophosphate (cAMP) response element-binding protein (CREB), CAAT/enhancer binding protein (C/EBP)-homologous protein also identified as growth arrest and DNA damage 153 (CHOP/GADD153), 11beta-Hydroxysteroid dehydrogenase type 1 (11beta-HSD1), reactive oxygen species (ROS) generation at mitochondria and decrease in sterol regulatory element-binding protein-1c (SREBP-1c) mRNA expression (Blanchet et al. 2015; Carrière et al. 2004).

As mentioned above, first of all, NF-kappaB activation and transcriptional activation of TNF-alpha gene promoter occurs in response to hypoxia. It is generally believed that NF-kappaB is directly activated by hypoxia. However, it also is activated in adipocytes by an indirect effect of hypoxia, through the secretion of TNF-alpha (Ye et al. 2007). cAMP promotes cellular gene expression via the PKA-mediated phosphorylation of the CREB family of activators. Subsequently

CREB promotes the islet function by upregulating the insulin receptor substrate 2 (IRS2) in beta cells (Jhala et al. 2003), feeding conditions, the incretin hormone glucagon-like peptide 1 (GLP-1) promotes pancreatic islet viability through the upregulation of HIF-1alpha. GLP-1 stimulates HIF-1alpha accumulation via the cAMP-mediated induction of the mTOR pathway in beta cells. cAMP appears to trigger mTOR activation in part via the CREB-mediated induction of IRS2-AKT signaling (Van de Velde et al. 2011). In a prolonged insulin resistance state, HIF1 inhibits CREB activity by stimulating the expression of protein kinase inhibitor B (PKIB) and blocking the activation of protein kinase A (PKA) (Blanchet et al. 2015). While adiponectin mRNA expression decreases, mRNA of CHOP, which is an endoplasmic reticulum stress-mediated protein, is significantly increased in adipose tissue of obese animals. Expression of CHOP attenuates the adiponectin promoter activity. This interference of CHOP reverses hypoxia-induced suppression of adiponectin mRNA expression in adipocytes. Thus, hypoxia also increases instability of adiponectin mRNA (Hosogai et al. 2007). Actually, C/EBPalpha accesses the adiponectin promoter through two forkhead box transcription factor O1 (FOXO1) binding sites and acts as a co-activator. On the other, hand sirtuin (SIRT)1 increases adiponectin transcription in adipocytes by activating FOXO1 and enhancing FOXO1 and C/EBPalpha interaction. Both FOXO1 and SIRT1 protein levels are significantly lower in high fat diet-induced obese mice compared with normal subjects (Qiao and Shao 2006). Therefore, reduction of FOXO1 levels by hypoxia may contribute to the inhibition of adiponectin synthesis. In addition to C/EBPalpha, inhibition of C/EBPbeta is also proposed to be responsible for the hypoxia-mediated adiponectin reduction (Hosogai et al. 2007). In fact, CHOP-10/GADD153 (C/EBP homologous protein) is one of the three members of the C/EBP family and a dominant negative regulator of C/EBP (Ron and Habener 1992). Expression of C/EBPalpha and PPAR-gamma triggers adipocyte differentiation (Fajas et al. 1998). Eventually, hypoxia-dependent inhibition of adipocyte differentiation is associated with an increase of HIF-1alpha and CHOP-10/GADD153 protein content (Blanchet et al. 2015). Similarly, 11beta-HSD1 expression is induced by hypoxia through NF-kappaB activation. NF-kappaB activity likely becomes dominant in the system to increase 11beta-HSD1 expression which converts the inactive glucocorticoids to active glucocorticoids. Actually 11beta-HSD1 reduction is associated with hypoxia during adipose tissue expansion in diet-induced obesity. This means that HIF-1alpha is a negative regulator of 11beta-HSD1 expression. In response to hypoxia, HIF-1alpha protein level is elevated in the cytoplasm, as a result of HIF-1alpha protein stabilization. Later HIF-1alpha translocates to the nucleus to induce transcription of angiogenic factors (Lee et al. 2013).

4 Reactive Oxygen Radical Generation in Hypoxia

Beside their key role in ATP production, mitochondria constitute the primary source of ROS generation in many cells (Turrens 1997). ROS generation at mitochondria is responsible for propagation of the hypoxic signal (Chandel et al. 1998). In this context two alternatives can be put forward as hypotheses, first; hypoxia-induced HIF-1alpha expression involves a cascade of signaling events including ROS generation, activation of PI3K and ERK signaling, and subsequent activation of small Rho family hydrolase, guanosine triphosphatase (GTPase) (Rac1) (Du et al. 2011). Rac1 may be able to directly control the transcriptional stabilization of HIF-1alpha in hypoxia (Xue et al. 2006). Additionally, trans-membrane nicotinamide adenine dinucleotide phosphate, reduced (NADPH) oxidases or NADH-oxidoreductase complex is activated to release superoxide by hypoxic conditions (Marshall et al. 1996). Second; hypoxia partially inhibits mitochondrial electron transport, producing redox changes in the electron carriers that increase ROS generation (Chandel et al. 2000). Actually, mtROS acts as a second messenger in the oxygen-sensing mechanism to trigger eryth-

ropoietin and vascular endothelium growth factor gene transcription via HIF-1 (Chandel et al. 2000). Further, mtROS controls CHOP-10/GADD153 expression and adipocyte differentiation (Blanchet et al. 2015). Actually, mROS production, but not oxygen consumption is required for hypoxic HIF-1alpha protein stabilization. Conversely antioxidants maintain the hydroxylation of HIF-1alpha protein and prevent stabilization of HIF-1alpha protein during hypoxia (Bell et al. 2007). Thus, the expression of glutathione peroxidase or catalase prevents the hypoxic stabilization of HIF-1 alpha. Hence oxygen sensing is dependent on mitochondria-generated ROS but independent of oxidative phosphorylation (Brunelle et al. 2005). Eventually, mtROS are essential for oxygen sensing and subsequent HIF alpha stabilization in hypoxia. In the absence of hypoxic signal, HIF alpha subunits continue to be hydroxylated and degraded via the proteasome (Simon 2006). Mitochondrial inhibition is the cause of inability to produce mtROS from Complex III during hypoxia that results in the oxygen sensing defect. However, exogenous ROS can lead to the stabilization of HIF-alpha even when oxygen levels are high (Mansfield et al. 2005). In addition to reduced number of mitochondria, mitochondrial respiration and biogenesis are inhibited by hypoxia in obese individuals. Actually, initial adipose failure following long-term excess energy intake may be the result of reduced mitochondrial capacity associated with altered mtROS signaling and adipose tissue hypoxia (Keijer and van Schothorst 2008). Under normal physiological conditions, PPAR-gamma is mainly expressed in adipose tissue and regulates diverse functions such as the development of fat cells and their capacity to store lipids (Medina-Gomez et al. 2007). The reduction in PPAR-gamma expression correlates with the increase in free fatty acids. Thus the high-fat meal results in a significant decrease in plasma superoxide dismutase (SOD) activity, glutathione reductase (GSH-R) activity, and mRNA expression of PPAR-gamma in morbidly obese individuals (Garcia-Fuentes et al. 2010). Moreover, PPAR-gamma promotes elimination of ROS by reducing NAD(P)H oxidase-derived superoxide production and enhancing catalase activity but it does not affect the SOD activity (Bagi et al. 2004).

5 Fatty Acids and Hypoxia

Visceral adipose tissue from morbidly obese individuals has a higher level of HIF-1alpha. In these patients visceral adipose tissues also show a decrease in SREBP-1c mRNA expression (García-Fuentes et al. 2015). SREBPs are transcription factors in adipogenesis and fatty acid biosynthesis. In particular, SREBP-1c controls fatty acid synthesis in the adipose tissues (Shimano 2009). Besides the SREBP-1c, SREBP-2 mRNA levels are also significantly lower in obese than in never-obese and post-obese subjects. However, only SREBP-1c gene expression is associated with fatty acid synthase and acetyl-CoA carboxylase alpha gene expression in the intraperitoneal adipose tissue of obese humans (Oberkofler et al. 2002). The liver kinase B1-AMP-activated protein kinase cascade is switched on by metabolic stresses that either inhibits ATP production in hypoxia or that accelerates ATP consumption (Hardie et al. 2006). Activation of AMP-activated protein kinase (AMPK) by hypoxia occurs in many tissues under pathological conditions. A rise in the cellular AMP/adenosine triphosphate (ATP) ratio activates AMPK and thereby evokes Ca^{2+} signals in O_2-sensing cells (Evans et al. 2005). The insulin sensitive and resistant patients differ with respect to AMPK activity and oxidative stress in all of their fat depots. Thus, adipose tissues of morbidly obese insulin resistant individuals uniformly show decreased AMPK activity and increased oxidative stress compared with insulin sensitive patients (Xu et al. 2012). Actually, AMPK activation suppresses SREBP-1 mRNA and nuclear SREBP-1 protein (Yang et al. 2009). On the other hand, insulin-resistance is the main factor responsible for the decreased SREBP1c mRNA expression of white adipose tissue in morbidly obese individuals. Thereby, insulin-induced gene 1 (Insig1) mRNA expression is decreased in the subcuta-

neous depot of morbidly obese individuals compared with obese patients and is further reduced in the presence of insulin-resistance. Adaptive response in human white adipose tissue involves changes in the set point of the Insig1/SREBP1/ stearoyl-CoA desaturase 1 (SCD1) axis. In this context, optimizing adaptive lipogenic gene expression ensures availability of essential unsaturated lipids in obesity or insulin resistance(Carobbio et al. 2013). Visceral adipose tissue and subcutaneous adipose tissue SCD1 mRNA expression levels in the morbidly obese patients are significantly lower than in the controls (García-Serrano et al. 2011).

Most of non-esterified fatty acids (NEFAs) delivered from adipose tissue originate from intracellular hydrolysis of chylomicron triglycerides via adipose triglyceride lipase (ATGL)/hormone-sensitive lipase (HSL)-mediated lipolysis (McQuaid et al. 2011). In response to hypoxia, while free fatty acid (FFA) uptake is reduced in adipocytes, lipolysis is increased. Decreased fatty acid uptake may be related to the inhibition of fatty acid transporters (FATP1 and CD36) and transcription factors, PPAR-gamma and C/EBPalpha, by hypoxia. In particular, the inhibition of PPAR-gamma expression seems to be an important mechanism in hypoxia-induced lipolysis. Eventual response to hypoxia in adipocytes is inhibition of the insulin-signaling pathway and induction of cell death (Yin et al. 2009; Yun et al. 2002). Additionally, expression of miR-27 results in blockade of expression of PPAR-gamma and C/EBPalpha, the two master regulators of adipogenesis. Importantly, expression of miR-27 is increased in fat tissue of obese mice and is regulated by hypoxia which is an important extracellular stress associated with obesity (Lin et al. 2009). Prolyl hydroxylase enzymes (PHDs) sense cellular oxygen upstream of HIF signaling. Adipose PHD2 deficiency increases adiposity in accordance with the increased adipose tissue vascularization, meanwhile maintains suppressed human adipocyte lipolysis and reduced ectopic lipid accumulation (Michailidou et al. 2015). HIF-1alpha as a central regulator of adipocyte lipid catabolism and energy expenditure, mediates these effects by interfering with the function of the SIRT2, thereby creating a metabolic state permissive for the development of obesity in the face of nutrient overload. SIRT2 is predominantly localized to the nucleus in adipocytes. HIF-1alpha protein accumulation is restricted to adipose depots of pathologically obese and diabetic humans that this accumulation results in an inhibition of fatty acid oxidation (FAO) and energetic uncoupling via transcriptional repression of SIRT2 (Krishnan et al. 2012). PPAR-gamma coactivator 1alpha (PGC1alpha) has been prominently associated with the regulation of the FAO gene program and energy expenditure (Handschin and Spiegelman 2006). PGC-1alpha-responsive promoters are negatively affected by HIF-1alpha activation in a SIRT2-dependent manner. PGC-1alpha is a SIRT2 substrate. Therefore, HIF-1alpha regulates adipocyte mitochondrial biogenesis and FAO by affecting SIRT2-mediated PGC-1alpha substrate acetylation state (Krishnan et al. 2012).

For both omental and subcutaneous adipocytes, oxygen consumption rates are significantly reduced in cells from obese compared with non-obese volunteers, even when matched for cell size by comparing large adipocytes from non-obese and small adipocytes from obese. This difference between obese and non-obese volunteers is not depended on quantity of mitochondria in adipocyte. Also, mitochondrial functions of the small and large cells are not different between adipocytes from the different adipose depots of the same person. However, adipocyte mitochondrial oxidative capacity is reduced in obese compared with non-obese adults. For this reason, mitochondrial dysfunction of adipocyte in obesity may be associated with the overall adiposity rather than adipocyte hypertrophy (Yin et al. 2014). Actually, mitochondrial respiratory capacities in adipocytes are inversely associated with body mass index (BMI) values but are independent of cell size. Thus, obese individuals have significantly fewer complex-I and IV components in adipose tissues compared with the non-obese subjects. These differences at the level of respiratory chain complexes might be responsible for the deterioration of respiratory capacity in obese individuals (Fischer et al. 2015).

Moreover, hypoxia significantly increases saturated fatty acid-induced mRNA expression and protein secretion of IL-6 and IL-1beta. However, it is proposed that saturated fatty acid-induced endoplasmic reticulum stress and NF-kappaB pathway activation are not enhanced by hypoxia alone. Hypoxic and saturated fatty acid-treated macrophages promote IL-6 and macrophage chemoattractant protein-1 (MCP-1) expression from primary human adipocytes. Hence, coexistence of hypoxia along with saturated fatty acid exacerbates macrophage-mediated inflammation (Snodgrass et al. 2016). As explained above, development of adipose tissue hypoxia is a multifactorial process. Elevation of saturated fatty acid levels in adipose tissue directly leads to increased oxygen consumption by activation of adenine nucleotide translocase 2 (ANT2), which is an inner mitochondrial membrane protein. ANT2-mediated uncoupled respiration is a key contributor to relative adipocyte hypoxia in obesity triggering the HIF-1alpha response. HIF-1alpha mRNA and protein levels are highly induced early in the course of high fat diet-induced obesity. In this context, saturated fatty acid stimulation of ANT2 causes increased adipocyte oxygen consumption due to uncoupled mitochondrial respiration. Subsequent relative cellular hypoxia is associated with the HIF-1alpha induction and the obesity-related chronic adipose tissue inflammation (Lee et al. 2014).

6 Hypoxia and Adipose Tissue Macrophages

Human preadipocytes and mature adipocytes from different depots spontaneously release substantial amounts of macrophage migration inhibition factor (MIF). Expression levels are positively associated with donor body mass index (Skurk et al. 2005). Expression of MIF in response to hypoxia promotes macrophage infiltration in hypoxic areas of adipose tissue (Ye et al. 2007). Furthermore, hypoxic areas colocalize predominantly with F4/80+ macrophages (Rausch et al. 2008). Frequency of adipocyte death coincides with increases in weight of fat stores accumulation of adipose tissue macrophages expressing F4/80 and CD11c, mRNA for TNF-alpha, MCP-1, IL-10 and insulin resistance. Adipose tissue macrophages are located around dead adipocytes and form crown-like structures surrounding dead adipocytes (Strissel et al. 2007). Thereby, hypoxia inhibits macrophage migration from the hypoxic region to the other side (Turner et al. 1999). Interferon-gamma (IFN-gamma) induction is mediated through HIF-1alpha binding to its promoter on the functional hypoxia response element. In this case, hypoxic macrophages generate synapses with CD8+ T cells that are more efficient for activation of T cell receptor (TCR)/CD3epsilon conformation, CD3zeta and linker for activation of T cell phosphorylation. Thereby in hypoxic conditions, T cell cytokine production is higher compared with normoxic macrophages. Otherwise hypoxia-induced immune responses are markedly reduced in HIF-1alpha- and in IFN-gamma-silenced macrophages (Acosta-Iborra et al. 2009). The TCR/CD3 complex is composed of six subunits which are expressed on the cell surface. Two TCR alpha/beta heterodimers are present in a TCR/CD3 complex. Further CD3 zeta mediates the interaction between both TCR alpha/beta heterodimers contained in the double TCR complex (San José et al. 1998). The efficient IFN-gamma- and IL-12-producing immune synapse correlates with the high induction of the TCR/CD3 epsilon active conformation by hypoxia (Acosta-Iborra et al. 2009). Alterations in oxygen availability of adipose tissue directly affect the macrophage polarization (Escribese et al. 2012). The expression level of hypoxia-related genes, as well as inflammation-related genes, is also higher in M1 adipose tissue macrophages than in M2 adipose tissue macrophages. Thus, the expression of IL-6, IL-1beta and inducible nitric oxide synthase (iNOS) in macrophages is increased in the condition of hypoxia (Fujisaka et al. 2013). Reactive nitrogen species (RNS), ROS, cytokines, and growth factors expressed by hypoxia-stimulated macrophages participate in stability regulation of HIF-1alpha and HIF-1 transactivation even during the normal oxygen saturation. Especially endogenous nitric oxide (NO) formation induces HIF-1alpha accumula-

tion, HIF-1-DNA binding, and activation of its downstream target gene expression (Brüne and Zhou 2003). Hypoxia enhances saturated fatty acid-induced pro-inflammatory cytokine production in primary human macrophages through ROS-dependent modulation of Jun N-terminal kinase (JNK) activity. Consequently, adipocytokine production is modulated in primary human adipocytes. The coexistence of low oxygen along with excess FFA release in obese adipose tissue promotes an inflammatory shift of the adipose tissue macrophage phenotype (Snodgrass et al. 2016). Saturated fatty acid upregulates oxidized low density lipoprotein (LDL) receptor-1 (LOX-1) and enhances oxidized LDL (oxLDL) uptake in macrophages. This results in activation of endoplasmic reticulum (ER) stress markers and phosphorylation of protein kinase RNA-activated-like ER kinase (PERK), translation initiation factor eIF2alpha, and JNK. Both saturated fatty acid over-loading and hypoxia have been shown to induce ER stress-related unfolded protein response (UPR) (Ishiyama et al. 2011; Koumenis et al. 2002). In this case, increased demands on the protein folding capacity of the ER triggers the UPR (Brewer 2014). Hypoxia lead to phosphorylation of translation initiation factor eIF2alpha. The ER-resident eIF2alpha kinase PERK is hyperphosphorylated upon hypoxic stress. Activation of PERK and phosphorylation of eIF2alpha indicates the adaptation of cells to hypoxic stress. This may be linked to the UPR (Koumenis et al. 2002). ER transmembrane receptors; an ER-resident integral membrane protein, inositol requiring enzyme 1 (IRE1), ER stress sensor PERK and activating transcription factor 6 (ATF6) sense the accumulation of unfolded proteins. Their activation due to abnormal conditions in the ER lumen transmits the information across the membrane into the cytosol, consequently triggers specific adaptive responses to resolve this stress (Hetz 2012; Jäger et al. 2012; Walter and Ron 2011). CREB has an important role in the proper response to prolonged hypoxia. Activation of CREB and the UPR pathway is in a coordinated manner in response to prolonged hypoxia-induced ER stress. Depletion of CREB decreases the two critical UPR signaling molecules expression, IRE1alpha and PERK, (Kikuchi et al. 2016). In fact, the UPR is a complex signal transduction pathway that conveys information about protein folding status in the ER lumen to increase protein folding capacity and decrease unfolded protein load (Hetz et al. 2011). Although the UPR is cytoprotective, which allows adipocytes to adapt to developmental and environmental conditions, the UPR can become cytotoxic during the severe and prolonged ER stress (Lin et al. 2007). White adipose tissue of obese animals is hypoxic and that hypoxia dysregulates the production of adipocytokines in adipocytes. This effect is mediated by ER stress and posttranscriptional regulation of gene expression by hypoxia. Adiponectin and PPAR-gamma mRNA expression levels are reduced while plasminogen activator inhibitor-1 (PAI-1) level is increased in hypoxic adipocytes (Hosogai et al. 2007). Hypoxia-related ER stress in obesity in turn leads to suppression of insulin receptor signaling through hyperactivation of JNK and subsequent serine phosphorylation of IRS-1 (Ozcan et al. 2004) (Fig. 13.1).

Glucose-dependent insulinotropic polypeptide (GIP) is a gut hormone secreted in response to dietary fat and glucose. GIP receptor (GIPR) and HIF-1alpha expressions are positively correlated in the adipose tissue. HIF-1alpha gene silencing diminishes both macrophage- and hypoxia-induced GIPR expression and GIP-induced IL-6 expression in adipocytes (Chen et al. 2015). GIP promotes the interaction of G protein-coupled receptor kinase 2 (GRK2) with GIPR and decreases the association of GRK2 to IRS-1 in human fat cells. This effect of GIP is lost under hypoxia. Thereby GIP/GIPR signaling is disrupted in insulin-resistant obese humans (Ceperuelo-Mallafré et al. 2014).

7 Hypoxia and Plasminogen Activator Inhibitor-1

Obesity and in particular, an abdominal type of body fat distribution are associated with elevated PAI-1 antigen and activity levels. The greater the fat cell size and the adipose tissue mass is, the

greater the contribution of adipose production to circulating PAI-1 is. Impaired fibrinolysis in obesity is probably also due to an increased expression of PAI-1 in adipose tissue thereby increase the risk for cardiovascular disease (Skurk and Hauner 2004). Hypoxia in adipose tissue of obesity can promote elaboration of sphingosine-1-phosphate (S1P) that binds to S1P2 receptors in an autocrine or a paracrine manner. S1P potentially contributes toward increased expression of PAI-1 and consequent constraints on fibrinolysis (Ito et al. 2013). mRNA levels of PPAR-gamma and adiponectin are decreased, whereas mRNA level of PAI-1 is increased in hypoxic adipocytes. The mRNA expression levels of ER stress marker genes, CHOP and glucose-regulated protein, 78 kD (GRP78) are significantly higher in adipocytes under hypoxic conditions than those under normoxia. In this case, expression of CHOP dose-dependently inhibits adiponectin promoter activity. Collectively all these suggest that tissue hypoxia in white adipose tissue leads to ER stress and suppression of adiponectin transcription in obesity (Hosogai et al. 2007). Hypoxia markedly suppresses adiponectin mRNA expression and its protein secretion, and increases PAI-1 production in mature adipocytes. Hypoxia causes a modest elevation of ROS in adipocytes. However, ablation of intracellular ROS by antioxidants fails to alleviate hypoxia-induced aberrant production of adiponectin and PAI-1. The antioxidants could reverse H_2O_2-induced dysregulation of adiponectin and PAI-1 production. H_2O_2 treatment decreases the expression levels of PPAR-gamma and C/EBPalpha, but has no effect on HIF-1alpha, whereas hypoxia stabilizes HIF-1alpha and decreases expression of C/EBPalpha, but not PPAR-gamma (Chen et al. 2006).

8 Vascular Endothelial Growth Factor and Platelet-Derived Growth Factor Transcription in Hypoxia

Impaired angiogenesis in fat has been implicated in the development of adipose tissue hypoxia. Impaired adipose tissue angiogenesis is associated with overexpression of antiangiogenic factors. Endogenous anti-angiogenic isoform of VEGF-A, VEGF-A165b is over-expressed in human visceral fat and associated with impaired angiogenesis in adipose tissue. The extent of VEGF-A165b expression correlates negatively with capillary growth (Ngo et al. 2014). Overexpression of VEGF results in increased blood vessel number and size in both white adipose tissue and brown adipose tissue and protection against high-fat diet-induced hypoxia and obesity (Elias et al. 2012). VEGF signaling is necessary for adequate adipose tissue function (Sung et al. 2013). However, VEGF synthesis in human visceral adipose tissue is inefficient as it is not followed by angiogenesis that counterbalances tissue hypoxia (Fusaru et al. 2012). Actually, adipocytes exhibit extensive functional changes in response to hypoxia, which alters the expression of up to 1300 genes. These include genes encoding key adipokines such as leptin, IL-6, VEGF, and matrix metalloproteinase-2 (MMP-2), which are upregulated (Trayhurn 2014). Angiopoietin-like protein 4/fasting-induced adipose factor (ANGPTL4) is a major hypoxia-sensitive gene. The expression and secretion of ANGPTL4 by human adipocytes is upregulated by both hypoxia and fatty acids (González-Muniesa et al. 2011). Interestingly, hypoxia markedly enhances the expression of leptin, VEGF and MMPs (-2 and -9) and stimulates the accumulation of HIF-1alpha protein in the hypoxic adipocyte nuclei (Lolmède et al. 2003). VEGF release from preadipocytes is also increased by hypoxia. Hypoxia also induces human preadipocytes to synthesize and secrete leptin. Actually, preadipocytes and adipocytes differ in their responsiveness to hypoxia (Wang et al. 2008). Although HIF-1alpha is a major transcriptional activator of VEGF gene in adipocytes, HIF-1alpha activity is not sufficient to induce VEGF expression in obesity (He et al. 2011). Thus VEGF transcription is controlled by several other transcription factors in addition to HIF-1alpha, such as signal transducer and activator transcription 3 (STAT3) protein (Niu et al. 2002), PGC-1alpha (Arany et al. 2008), and PPAR-gamma (Wang

et al. 2008). The capillary density is reduced in adipose tissue in obese subjects and found to contribute to adipose tissue hypoxia (Pang et al. 2008). Compared with lean subjects, overweight/obese subjects have lower capillary density and lower VEGF expression. Strong positive correlations are identified between PPAR-gamma1, VEGF and adipose tissue oxygen partial pressure. Adipose tissue hypoxia in obese subjects most likely depends on the decreased capillary density and reduced expression of the angiogenic factors like VEGF and PPAR-gamma1 (Pasarica et al. 2009b). In linking leptin receptor activation to alphavbeta5 integrin phosphorylation, cytoplasmic protein kinase Src has an important role. However, constantly elevated leptin levels in obesity are accompanied by the elevated levels of protein tyrosine phosphatase 1 (PTP1)B. Phosphorylation of the alphavbeta5 integrin chain by leptin requires the Janus kinase2 (JAK2)-mediated activation of Src kinase. Overexpression of B in circulating angiogenic cells (CAC) from obese is a negative regulator of leptin signaling and an attenuated angiogenic response of CAC to leptin (Heida et al. 2010). Overexpression of integrin alphavbeta5 enhances the capacity of CACs to adhere to endothelial cell to promote new blood vessel formation. Increase in the phosphorylation of integrin alphavbeta5 and Src kinase results in the cytoplasmic transcription factor STAT3 activation, nuclear translocation and upregulation of STAT3-dependent angiogenic gene expression. Additionally alphavbeta5 increases the secretion of the angiogenic chemokines CXCL8 and CCL2 (Leifheit-Nestler et al. 2010).

Furthermore, in response to the reduced vascular density, macrophages express platelet-derived growth factor (PDGF) in adipose tissue to facilitate capillary formation in obesity. PDGF expression is induced by hypoxia, and tube formation of endothelial cells is induced by PDGF (Pang et al. 2008). A balance between these two angiogenic factors is required for the formation and function of new capillaries. Angiogenesis is coordinated by VEGF and PDGF through their related receptors on endothelial cells and vascular smooth muscle cells, respectively (Greenberg et al. 2008).

In obesity, at least three factors may be proposed to stimulate the VEGF transcription in adipocytes. Firstly, adipogenesis leads to VEGF expression through HIF-1alpha. Secondly, obesity-related hyperinsulinemia induces HIF-1alpha expression in mRNA and protein in adipocytes, which is translated into the VEGF transcription. Finally, HIF-1alpha activity is increased in adipocytes by hypoxia which causes VEGF expression. In obesity, although HIF-1alpha is an activator of VEGF gene, it is not sufficient to induce VEGF expression in adipocytes of obese subject. In this context, the PI3K-Akt pathway is required for HIF-1alpha activation in addition to these factors (He et al. 2011).

Adipose tissue contains preadipocytes, adipocytes, macrophages, and endothelial cells. PDGF is expressed in all of these types of cells; however, the expression levels are different. Preadipocytes express more PDGF than mature adipocytes. In obesity, most of the preadipocytes are differentiated into mature adipocytes. Thereby, preadipocyte number is reduced and local PDGF level decreases. To meet the demand for PDGF, adipose tissue macrophages increase the PDGF production. Actually, hypoxia in adipose tissue is likely to induce PDGF expression in macrophages. Substantially the activity of PDGF in angiogenesis is dependent on VEGF activity. Angiogenesis requires endothelial cell proliferation and tube formation. Endothelial cell proliferation is dependent on pro-angiogenic factor VEGF, which stimulates cell proliferation through VEGF receptor 2 in the endothelial cells. Additionally, hyperinsulinemia may serve to stimulate angiogenesis in adipose tissue in the obesity. The signaling pathway PI3K/Akt/mTOR/S6K is used by PDGF in the induction of tube formation (Pang et al. 2008). Thus, PI3K pathway is exclusively activated by PDGF (Kratchmarova et al. 2005). Similarly, saturated fatty acid-loaded hypertrophied adipocytes release VEGF, which is enhanced through PI3K pathways activated by oxidative stress (Takahashi et al. 2013) (Fig. 13.1).

9 Adipose Tissue Hypoxia and Adipogenesis

White adipose tissue expansion is based on both cellular hypertrophy and hyperplasia in obesity. However, white adipose tissue becomes hypoxic during obesity that leads to adipose tissue dysfunction (Sun et al. 2011). An oxygen-sensitive signaling mechanism regulates adipogenesis (Yun et al. 2002). However, in hypoxic circumstances, biphasically activated AMPK and concomitantly blocked clonal expansion of preadipocytes is an indispensable step for early phase of adipocyte differentiation (Kim et al. 2005). Mesenchymal stem cells (MSCs) can differentiate into adipocytes following the induction of transcription factors C/EBPbeta that induce the expression of PPAR-gamma, the emerging master regulator of adipogenesis (Lefterova and Lazar 2009). Activator protein (AP)-1 family member Fra-2 transcriptionally regulates PPAR-gamma2 expression and adipocyte survival by the modulation of HIF expression. PPAR-gamma2 is not only a master regulator of adipogenesis, but can also display a role in apoptotic signal transduction in adipocytes (Herold et al. 2013). Fra-2 inhibits the expression of PPAR-gamma2, a key regulator of adipocyte differentiation. By repressing PPAR-gamma2, Fra-2 also represses HIFs that control hypoxia and adipocyte survival. Increased PPAR-gamma expression in the absence of Fra-2 not only enhances adipocyte differentiation but also apoptosis (Luther et al. 2014). Adipogenesis requires the sequential activation of numerous transcription factors, including the C/EBP gene family and PPAR-gamma (Ali et al. 2013). Indeed, cAMP-dependent signaling along with C/EBPbeta leads to the stimulation of PPAR-gamma activity by mechanisms that involve production of PPAR-gamma ligands during adipogenesis. A crosstalk between PPAR-gamma and beta-catenin signaling has been shown. Activation of PPAR-gamma induces the degradation of beta-catenin during preadipocyte differentiation by mechanisms that require glycogen synthase kinase 3 beta (GSK3beta) and the proteasome (Farmer 2005). PPAR-gamma deficiency attenuates induction of hypoxia-responsive genes while increasing expression of inflammatory genes in mature hypoxic adipocytes (Pino et al. 2012). Furthermore, the hypoxia-induced suppression of adipogenesis is accompanied by reduced acetylation of histone H3 and H4 at the PPAR-gamma promoter. Hypoxic condition attenuates adipocyte differentiation by inhibition of PPAR-gamma expression in a histone deacetylase-independent manner (Kim et al. 2005). Hypoxic saturated free fatty acid-treated macrophages promote IL-6 and MCP-1 expression in primary human adipocytes (Snodgrass et al. 2016). The elevated chemokine MCP-1 may contribute to increased macrophage migration towards adipose tissue (Yu et al. 2011). Hypoxia inhibits adipocyte differentiation from preadipocytes (Trayhurn et al. 2008). In this case, inhibition of the preadipocytes differentiation is controlled by leptin signaling (Wood et al. 2009). Actually, the expression of proinflammatory adipokines is considerably higher in preadipocytes than in adipocytes. One percent hypoxia significantly enhances the release of VEGF, IL-6, and PAI-1 from preadipocytes, whereas the secretion from the hypoxic adipocytes achieves approximately one-half of this enhancement (Mack et al. 2009). PPAR gamma and aP2 mRNA levels, markers of adipocyte differentiation, are reduced by hypoxia in both preadipocytes and adipocytes (Wang et al. 2008). PI3K is required for adipogenesis. Regulatory subunit 1 of PI3K (PIK3R1) is a critical component of the PI3K signaling pathway. PPAR-gamma interacts with the two peroxisome proliferator response elements regions of the PIK3R1 promoter in mature adipocytes (Kim et al. 2014).

Adipose tissue growth and expansion is primarily determined by adipocyte hypertrophy and hyperplasia in obesity. Triglyceride accumulation is responsible for adipocyte hypertrophy while increased adipocyte differentiation contributes to hyperplasia. The total adipocyte number is greatest in hyperplasia and smallest in hypertrophy. Obese individuals with hypertrophy generate 70% less adipocytes per year than those with hyperplasia. An inverse quantitative relationship between the residual value for adipocyte volume and adipocyte production rate is observed

(Arner et al. 2010). Adipocyte hypertrophy may impair adipose tissue function by inducing local inflammation, mechanical stress. Additionally, adipocyte size is an important determinant of adipokine secretion (Monteiro et al. 2006; Skurk et al. 2007). In response to hypoxia, free fatty acid uptake is reduced and lipolysis is increased. The molecular mechanism of decreased fatty acid uptake may be related to inhibition of FATP1, CD36 and PPAR-gamma and C/EBPalpha by hypoxia. Adipose tissue hypoxia promotes free fatty acid release and inhibit glucose uptake in adipocytes by inhibition of the insulin-signaling pathway and induction of cell death (Yin et al. 2009). Low generation rates of adipocytes associate with adipose tissue hypertrophy, whereas high generation rates associate with adipose hyperplasia. The relative death rate is approximately 10% per year and mean age of adipocytes is approximately 10 years (Arner et al. 2010). New adipocytes are generated from pre-adipocytes or adipose-derived stromal/stem cells to replace the dead adipocytes and to increase total adipocyte numbers in the fat depots. However, both inflammation and hypoxia inhibit new adipocyte generation from pre-adipocyte differentiation (Yin et al. 2009).

10 Adipose Tissue Hypoxia and Fibrosis

The expansion of adipose tissue in obesity with inappropriate angiogenesis results in reduced oxygen supply and hypoxia development. Subsequent activation of HIF-1 inhibits preadipocyte differentiation and initiates adipose tissue fibrosis. In addition to stressed adipocytes, hypoxia contributes to immune cell immigration and activation which further aggravates adipose tissue fibrosis (Buechler et al. 2015). Exposure of white adipocytes to hypoxic conditions changes the expression of more than 1000 genes. Thereby hypoxia is a key factor in adipose tissue dysfunction in obesity and leads to the development of adipose tissue fibrosis by inducing insulin resistance in fat cells (Trayhurn 2014). Hypoxia also stimulates the inflammatory response of macrophages and inhibits adipocyte differentiation from preadipocytes (Trayhurn et al. 2008). Loss of the potent angiogenic factor VEGF in myeloid cells leads to excessive collagen deposition and fibrosis (Stockmann et al. 2010). At first transforming growth factor beta (TGF-beta) upregulates connective tissue growth factor (CTGF). Later CTGF stimulates binding of TGF-beta to its receptor and enhances TGF-beta activity. Expression of CTGF mRNA is two-fold higher in the central fat depots compared with subcutaneous fat. At the same time CTGF inhibits adipogenesis in adipocytes (Tan et al. 2008).

In animals which are exposed to hypoxia, collagen, type I, alpha 1 (COL1A1), COL3A1 and the enzyme lysyl oxidase with a central role in collagen cross-linking are increased (Buechler et al. 2015). In omental white adipose tissue, fibrosis could contribute to limit adipocyte hypertrophy and is associated with a better lipid profile (Divoux et al. 2010). However, immature mast cells that infiltrate into adipose tissue at the non-obese stage gradually mature with the progression of obesity and that MCP-6 secreted from mature mast cells induces COL5 expression in obese adipose tissue. COL5 may contribute to the process of adipose tissue fibrosis and inhibits adipogenesis (Hirai et al. 2014). Excessive triglyceride load expands adipose tissue through both adipocyte hypertrophy and hyperplasia. This expansion is furthermore associated with hypoxia, fibrosis, local inflammation, and concomitant insulin resistance. Local adipose tissue hypoxia may be the most important driving force for the downstream events associated with adipose tissue dysfunction. Adipose tissue hypoxia serves as an early upstream initiator for adipose tissue dysfunction by inducing a local state of fibrosis (Halberg et al. 2009). High fat diet induces the expression of IL-13 from non-Th2 CD4+ T cells and may mediate the deposition of collagen to induced adipose tissue fibrosis even though IFN-gamma expression is enhanced due to increased Th1 CD4+ T cells (Pessin and Kwon 2012). Actually, in adipose tissue, fibrosis appears to be initiated in response to adipocyte hypertrophy, which occurs as the initial step toward fat pad expansion through enlargement of the lipid droplet size in existing adipocytes.

Mechanical stress on the hypertrophic adipocyte membrane, triggered by the expanding lipid droplet, may also be the source of shear stress at the level of the plasma membrane (Khan et al. 2009). Increase in cell size, by modifying the relationships between cell and extracellular matrix, could turn on and increase the level of activation of the β1-integrin/ERK signaling pathway. This pathway is responsible for the adaptation of adipose functions to cell size (Farnier et al. 2003). The carboxy-terminal domain cleaved from COL-6A3 promotes adipose tissue fibrosis, angiogenesis and inflammation (Sun et al. 2014). A positive correlation of COL6A3 expression in abdominal subcutaneous fat with body mass index and fat mass have been described. Elevated COL6A3 mRNA levels are found in patients with greater visceral fat mass and higher inflammation (Pasarica et al. 2009a). Shear stress in large adipocytes is the trigger for adipocyte death and the impending inflammation. In particular COL6 has an essential role in the fibrotic component of obesity and directly affects the ability of adipocytes to expand (Khan et al. 2009).

11 Conclusion

Although there is very scanty evidence about the influence of hypoxia in human adipose tissue, increased level of HIF-1alpha is found in the visceral adipose tissue of morbidly obese subjects. Decline in adipose tissue blood flow reflects a failure in compensatory angiogenesis or vasodilation. Reversing hypoxia might reduce adipose tissue inflammation, improve insulin action, and reduce cardiovascular disease risk in obesity.

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Adipocyte-Macrophage Cross-Talk in Obesity

14

Ayse Basak Engin

Abstract

Obesity is characterized by the chronic low-grade activation of the innate immune system. In this respect, macrophage-elicited metabolic inflammation and adipocyte-macrophage interaction has a primary importance in obesity. Large amounts of macrophages are accumulated by different mechanisms in obese adipose tissue. Hypertrophic adipocyte-derived chemotactic monocyte chemoattractant protein-1 (MCP-1)/C-C chemokine receptor 2 (CCR2) pathway also promotes more macrophage accumulation into the obese adipose tissue. However, increased local extracellular lipid concentrations is a final mechanism for adipose tissue macrophage accumulation. A paracrine loop involving free fatty acids and tumor necrosis factor-alpha (TNF-alpha) between adipocytes and macrophages establishes a vicious cycle that aggravates inflammatory changes in the adipose tissue. Adipocyte-specific caspase-1 and production of interleukin-1beta (IL-1beta) by macrophages; both adipocyte and macrophage induction by toll like receptor-4 (TLR4) through nuclear factor-kappaB (NF-kappaB) activation; free fatty acid-induced and TLR-mediated activation of c-Jun N-terminal kinase (JNK)-related pro-inflammatory pathways in CD11c+ immune cells; are effective in macrophage accumulation and in the development of adipose tissue inflammation. Old adipocytes are removed by macrophages through trogocytosis or sending an "eat me" signal. The obesity-induced changes in adipose tissue macrophage numbers are mainly due to increases in the triple-positive CD11b+ F4/80+ CD11c+ adipose tissue macrophage subpopulation. The ratio of M1-to-M2 macrophages is increased in obesity. Furthermore, hypoxia along with higher concentrations of free fatty acids exacerbates macrophage-mediated

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inflammation in obesity. The metabolic status of adipocytes is a major determinant of macrophage inflammatory output. Macrophage/adipocyte fatty-acid-binding proteins act at the interface of metabolic and inflammatory pathways. Both macrophages and adipocytes are the sites for active lipid metabolism and signaling.

Keywords

Obesity • Monocyte chemoattractant protein-1 (MCP-1) • M1 macrophages • M2 macrophages • Visceral adipose tissue • Free fatty acids • Interleukin-6 (IL-6) • Tumor necrosis factor-alpha (TNF-alpha) • NOD-like receptor (NLR) family protein (NLRP3) • C-C chemokine receptor 2 (CCR2) • Toll like receptor 4 (TLR4) • Chemokine (C-C motif) ligand 2 (CCL2) • Insulin-like growth factor-1 (IGF1) • Hypoxia-inducible factor-1 alpha (HIF-1alpha)

1 Introduction

The chronic low-grade activation of the innate immune system is evident even in childhood obesity. Thus, inflammatory markers are elevated in obese children as young as 3 years old. Furthermore, individuals are at risk for life-long meta-inflammation (Skinner et al. 2010). Adipose tissue macrophage accumulation is directly proportional to adiposity in humans. Furthermore, adipocyte size is a strong predictor of the percentage of CD68-expressing macrophages in human subcutaneous adipose tissue. (Weisberg et al. 2003). The ratio of the macrophages is 5% in lean adipose tissue, whereas, during obesity this ratio rises up to 50% (Cinti et al. 2005). Although macrophages comprise 10–15% of stromal vascular cells (SVCs) in visceral adipose tissue of lean subjects, their numbers are increased to 40–50% of the SVCs of visceral adipose tissue in obese humans (Weisberg et al. 2003). During obesity immune cell population differs, not only in number, but also in inflammatory phenotypes. In this context, macrophage-elicited metabolic inflammation and adipocyte-macrophage interaction has the key importance in obesity (McNelis and Olefsky 2014).

2 Macrophage Infiltration into Adipose Tissue

Actually the initiation of macrophage infiltration into adipose tissue occurs with four different mechanisms (Sun et al. 2011). First of all, necrosis of adipocytes driven by hypertrophy is a prominent phagocytic stimulus that regulates adipose tissue macrophage infiltration which is gradually increased by obesity (Cinti et al. 2005). In the late stages of adipose tissue obesity, macrophages form crown-like structures surrounding necrotic adipocytes (Nishimura et al. 2007; Strissel et al. 2007). In this way, white adipose tissue macrophages are selectively localized to dead adipocytes. Clearance of free lipid appears to be an important function of galectin-3 (MAC-2)-expressing macrophages in white adipose tissue (Cinti et al. 2005). Galectin-3 is one of the major factors involved in the influx of macrophages to inflammatory sites (Sano et al. 2000). At first, galectin-3 expression at sites of adipocyte necrosis may be functionally linked to macrophage aggregation and crown-like structures formation (Cinti et al. 2005). Furthermore, galectin-3 possesses anti-apoptotic activity intracellularly and that is implicated in macrophage survival at sites of inflammation (Hsu and Liu 2004). Secondly,

hypertrophic adipocyte-derived chemotactic monocyte chemoattractant protein-1 (MCP-1)/CC chemokine receptor 2 (CCR2) pathway also promotes more macrophage accumulation into the obese adipose tissue. Secretion of MCP-1 from adipocytes directly triggers the recruitment of macrophages to adipose tissue. The infiltrated macrophages may in turn secrete a variety of chemokines and other cytokines that further promote a local inflammatory response and affect gene expression in adipocytes, resulting in systemic insulin resistance (Kanda et al. 2006). Adiponectin expression may occur indirectly through interactions of adipocytes with CCR2-expressing adipose tissue macrophages and the alterations of obesity-induced inflammatory gene expression (Weisberg et al. 2006) (Fig. 14.1).

The inhibitor of differentiation or inhibitor of DNA binding (Id) proteins are negative regulators of cell differentiation and play key roles in the regulation of cell fate decisions, and in the timing of differentiation (Norton 2000). There are several type of Id proteins. In particular, Id3 attenuates visceral fat expansion by inhibiting

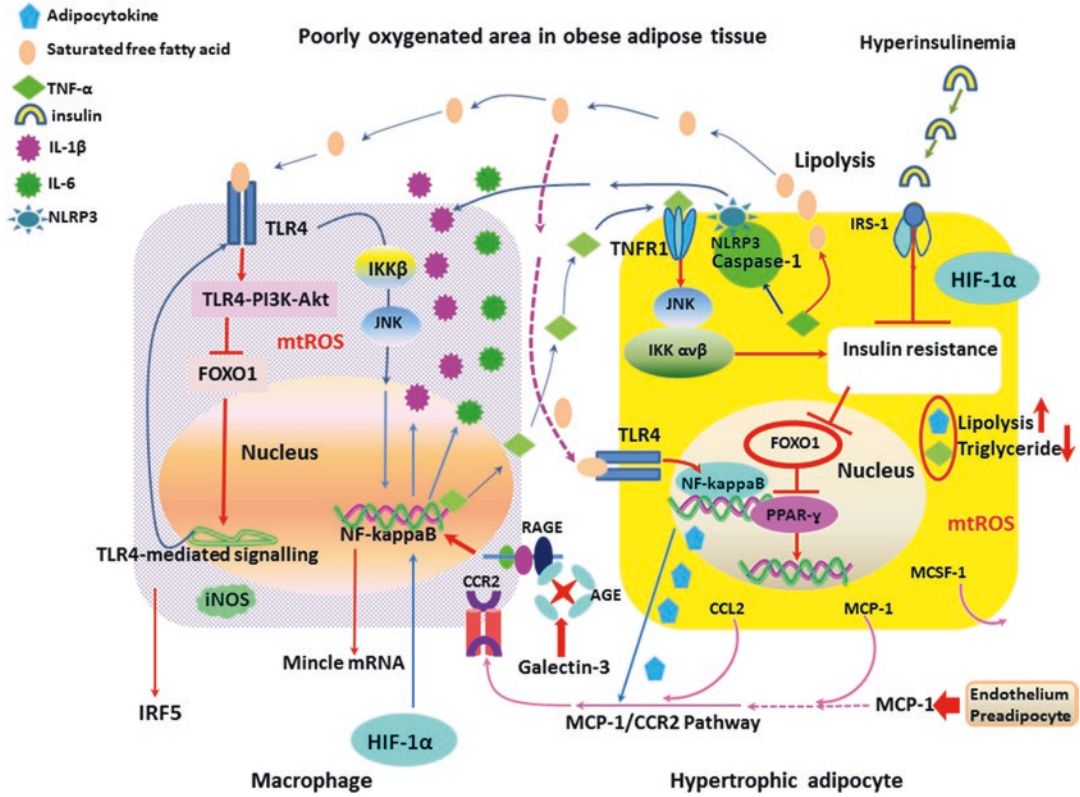

Fig. 14.1 Macrophage-elicited metabolic inflammation and adipocyte-macrophage interaction has a primary importance in obesity. Large amounts of macrophages are accumulated by different mechanisms in obese adipose tissue (*TLR4* Toll-like receptor 4, *Akt* Protein kinase-B, *FOXO1* Forkhead box protein O1, *NF-kappaB* Nuclear factor-kappa B, *JNK* c-Jun N-terminal kinase, *IKK* Inhibitor kappa B kinase, *PPAR-ɣ* Peroxisome proliferator-activated receptor-gamma, *IRS* Insulin receptor substrate, *MCP-1* Monocyte chemoattractant protein-1, *MCSF* Macrophage colony stimulating factor, *CCL2: CCR2* Chemokine (C-C motif) receptor ligand 2, *IRF5* Interferon regulatory factor-5, *TNF-α* Tumor necrosis factor-alpha, *IL-1β* Interleukin-1 beta, *IL-6* Interleukin-6, *NLRP3*, *NLRP* NOD-like receptor (NLR) family protein; *PI3K* Phosphatidylinositol 3-kinase, *mtROS* Mitochondrial reactive oxygen species, *iNOS* Inducible nitric oxide synthase, *Mincle mRNA* Macrophage-inducible C-type lectin mRNA, *HIF-1* hypoxia-inducible factor-1, *RAGE* Receptor for advanced glycation end product, *AGE* Advanced glycation end product, *TNFR* Tumor necrosis factor receptor)

high fat diet-induced visceral fat vascular endothelial growth factor (VEGF), and regulated upon activation normal T-cell expressed and secreted (RANTES) expression. Eventually, increase in capillary density is restricted (Cutchins et al. 2012). The helix-loop-helix transcription regulator Id3 promotes high fat diet-induced adipocyte progenitor cell (AdPC) accumulation in visceral adipose tissue. Id3-dependent AdPC expansion is responsible for high fat diet-induced MCP-1 production, M1 macrophage accumulation, and metabolic dysfunction during obesity. Adipose depot-specific differences in inflammation may be due to depot-specific differences in AdPCs (Kaplan et al. 2015). Hence MCP-1 expression is up-regulated in white adipose tissue of high fat diet-induced obesity. The 7.2-fold increase in MCP-1 as compared with normal subjects may alter adipocyte function by decreasing insulin-stimulated glucose uptake and the expression of lipoprotein lipase, adipsin, glucose transporter type 4 (GLUT-4), fatty-acid-binding protein (aP2), beta3-adrenergic receptor, and peroxisome proliferator-activated receptor-gamma (PPAR-gamma) (Sartipy and Loskutoff 2003; Takahashi et al. 2003). Furthermore, adipocyte hypertrophy creates poorly oxygenated areas in the human obese adipose tissue. As the third mechanism, hypoxic cells secrete chemokines, which attract macrophages, presumably to clear out necrotic cells (Murdoch et al. 2004). Eventually, decreased adipose tissue partial oxygen concentration is paralleled by an increase in the expression and secretion of the chemokine and markers of macrophage infiltration (Pasarica et al. 2009). Hypoxia-induced fibrosis in adipose tissue is a key factor that ultimately stimulates the local inflammatory responses (Halberg et al. 2009). Moreover, increased local extracellular lipid concentrations, which drive adipose tissue macrophage accumulation occurs as a final mechanism for macrophage infiltration. Thus the majority of free fatty acids released from necrotic adipocytes are transported across the plasma membrane of adjacent adipocytes and locally re-esterified (Thompson et al. 2010). In this context, free fatty acids are esterified with glycerol 3-phosphate. Stimulation of glyceroneogenesis and re-esterification of free fatty acids by increasing in phosphoenolpyruvate carboxykinase (PEPCK) activity leads to ultimate rise in adipocyte size and body weight (Franckhauser et al. 2002). The close relationship between adipocyte size and the abundance of macrophages in adipose tissue indicates that the relationship of adipocyte size with adipocyte function may be regulated through a paracrine pathway involving adipose tissue macrophages (Weisberg et al. 2003).

A paracrine loop involving free fatty acids and tumor necrosis factor-alpha (TNF-alpha) between adipocytes and macrophages establishes a vicious cycle that aggravates inflammatory changes in the adipose tissue. Initially TNF-alpha increases the release of free fatty acids from adipocytes. By contrast, high amount of saturated fatty acids which are released from adipocytes can induce inflammatory changes via increasing TNF-alpha production (Suganami et al. 2005). TNF-alpha-induced activation of caspase-1 is accompanied by induction of NOD-like receptor (NLR) family protein-3 (NLRP3) in adipocytes. But caspase-1 activation does not depend on the NLRP3 inflammasome (Furuoka et al. 2016). Caspase-1 activation in adipose tissue of obese animals is partly independent of macrophage infiltration. However, caspase-1 induces the release of IL-1beta, and finally leads to the development of insulin resistance. These results indicate a crosstalk between adipocyte-specific caspase-1 and IL-1beta produced by macrophages in adipose tissue (Stienstra et al. 2010). Additionally, saturated fatty acid also serves as a ligand for toll like receptor-4 (TLR4). Thereby the inflammatory changes in both adipocytes and macrophages are induced by TLR4 through nuclear factor-kappaB (NF-kappaB) activation (Suganami et al. 2007). Free fatty acid-induced and TLR-mediated activation of c-Jun N-terminal kinase (JNK)-related pro-inflammatory pathways in CD11c+ immune cells plays a central role in the development of adipose tissue inflammation and insulin resistance (Nguyen et al. 2007). Although the adipocyte is the key player controlling local changes in the microenvironment, macrophages have pivotal role in remodeling events. Nevertheless, resident

macrophages display remarkable heterogeneity in their activities and functions (Gordon and Taylor 2005) (Fig. 14.1).

On the other hand, adipocyte-derived microparticles (MPs) are critical "find-me" signals for recruitment of monocytes and macrophages. Adipocytes exposed to saturated fatty acids show marked release of MPs, which are enriched in perilipin A. The release of MPs is highly dependent on caspase 3 and Rho-associated kinase. Thus hypertrophied and stressed adipocytes generate chemotactic signals that induce macrophage migration in a caspase 3 dependent manner (Eguchi et al. 2015). White adipose tissue is characterized by a continuous turnover of the adipocytes with approximately 10% of annual renewal (Spalding et al. 2008). In this context, old adipocytes undergoing programmed cell death are removed by macrophages (Duvall et al. 1985; Keuper et al. 2011). Removal of apoptotic adipocytes by macrophages is a process in which one cell takes bites out of another (trogocytosis). Therefore, adipocyte-derived DNA is not detected in the phagocytes (Sárvári et al. 2015). Apoptotic cells send an “eat me” signal to macrophages, triggering their own engulfment. Among the various molecules proposed to be involved in this process, in particular phosphatidylserine (PtdSer) is a strong candidate for the “eat me” signal (Krahling et al. 1999). PtdSer is transferred caspase-dependently from the inner leaflet to the outer leaflet of the plasma membrane (Martin et al. 1996). The specific signals of apoptotic cells are well recognized by the phagocytes. Thus milk fat globule-EGF-factor 8 (MFG-E8)-secreted active macrophages recognize aminophospholipids and specifically bind to apoptotic cells for engulfment (Hanayama et al. 2002). Actually the glycerophospholipid, PtdSer is perhaps the best-characterized “eat me” signal. In healthy cells, PtdSer is abundant in the inner leaflet of the plasmalemma, but during apoptosis it is quickly redistributed and exposed on the exofacial leaflet. It is recognized by specific phagocytic receptors such as T-cell immunoglobulin mucin receptor 4 (TIM4), which enhances engulfment (Miyanishi et al. 2007). Actually, MFG-E8 binds to the integrin alphavbeta3 complex in macrophages via the Rho and Rab family guaosine triphosphatases (GTPases) in MFG-E8’s EGF domain, hence bridging apoptotic cells and macrophages. TIM4 binds to PtdSer via the immunoglobulin-like domain in its extracellular region. The engulfment of apoptotic cells proceeds in two steps: TIM4 tethers apoptotic cells, and the integrin alphavbeta3 complex mediates engulfment in coordination with MFG-E8 (Toda et al. 2012). Thus, the expression of MFGE-8 and the alphavbeta5 integrin subunits are increased in adipose tissue of obese humans (Henegar et al. 2008).

3 Macrophage Phenotype in Adipose Tissue

When the visceral adipose tissue is excess, compared to subcutaneous adipose tissue, the absolute number of CD14+ adipose tissue macrophage is increased in visceral adipose tissue relative to subcutaneous adipose tissue. The absolute number of CD3+ T cells is increased in visceral adipose tissue relative to subcutaneous adipose tissue. An increase in adipose tissue macrophage predisposes to an M1 phenotype in visceral adipose tissue relative to subcutaneous adipose tissue in human obesity. Furthermore, the CD14-enriched cell population has a macrophage phenotype and is a dominant source of inflammatory cytokines in stromo-vascular cells (O’Rourke et al. 2009).

CD11c+ adipose tissue macrophages significantly increase in obesity. A novel F4/80+ CD11c+ population of macrophages in adipose tissue of obese mice is not found in lean mice (Lumeng et al. 2007a). CD11c is considered to be a classical cell-surface marker of dendritic cells (DCs) and F4/80 as substitute for function, have led to confusion on the exact contribution of DCs versus macrophages to tissue immunity in obesity. Indeed, the CD11c integrin can be expressed on macrophages. Therefore, the role of CD11c+ cells does not always reflect the role of DCs. Similarly, F4/80 is also expressed by eosinophils and neutrophils and is not a specific marker of macrophages (Hashimoto et al. 2011). However,

in obese adipose tissue, the CD11c+ adipose tissue macrophages also express CD11b and F4/80 and are very different from the adipose tissue DCs, which express CD11c, but not CD11b. The obesity-induced changes in adipose tissue macrophage numbers are mainly due to increases in the triple-positive CD11b+ F4/80+ CD11c+ adipose tissue macrophage subpopulation (Lee and Lee 2014).

As mentioned above, two different populations of F4/80+CD11b+ macrophages are defined in adipose tissue; one of which also express CD11c. Obesity largely increases CD11c+ macrophage number in adipose tissue. F4/80+CD11b+CD11c+ cells are a specific population of adipose tissue macrophages recruited to adipose tissue upon high fat diet exposure (Patsouris et al. 2008). Moreover, F4/80 and CD11c are primarily regarded as cell surface markers of macrophages and dendritic cells, which are immune-phenotypically defined as F4/80+CD11c− and F4/80−CD11c+, respectively. In obese conditions, all these cells have not simultaneously completed their process of transitioning from a F4/80+CD11c− to a F4/80−CD11c+ state or cells that have transitioned from a CD11c− to CD11c+ phenotype. Some of them may show their original phenotypical characteristics and may have gradually acquired a CD11c+ phenotype (Nguyen et al. 2007). Adipose tissue macrophages from lean mice express many genes, which display characteristic of M2 or "alternatively activated" macrophages, including Ym1, arginase 1, and interleukin-10 (IL-10). Diet-induced obesity decreases expression of these genes in adipose tissue macrophages while increasing expression of genes such as those encoding TNF-alpha and inducible nitric oxide synthase (iNOS) that are characteristic of M1 or "classically activated" macrophages (Lumeng et al. 2007a). Indeed, TNF-alpha is a major macrophage-derived mediator of inflammation in adipocytes, whereas free fatty acids is an important adipocyte-derived mediators of inflammation in macrophages. TNF-alpha acts on TNF-alpha receptor of hypertrophied adipocytes. Briefly, a paracrine loop involving free fatty acids and TNF-alpha between adipocytes and macrophages establishes a vicious cycle that aggravates inflammatory changes in the adipose tissue (Suganami et al. 2005). Macrophages express some adipocyte-specific gene products such as ap2, meanwhile adipocytes secrete macrophage-specific gene products such as IL-6 or TNF-alpha. Lipid accumulation by macrophages in atherosclerotic lesions or by phagocytic capacities exhibited by adipocytes reveal an apparent coordinated activity between these two cell-types during the course of an innate immune response (Wolowczuk et al. 2008). An additional similarity between adipocytes and macrophages is further observed in the expression of TLR4 (Lin et al. 2000). The presence of functional TLR2 and TLR4 is demonstrated on human adipocytes isolated from subcutaneous fat tissue (Bès-Houtmann et al. 2007). "Adipocyte-macrophage-TLR4" pathway might be involved in the inflammatory process occurring in obesity (Wolowczuk et al. 2008). As mentioned above, diet-induced obesity is associated with the loss of tissue homeostasis and development of type 1 inflammatory responses in visceral adipose tissue. A key event is a shift of adipose tissue macrophages toward an M1 phenotype. In fact, obesity-induced adipocyte hypertrophy results in upregulated surface expression of stress markers. Adipose stress is detected by the phenotype of natural killer (NK) cells and CD8+ T cells, which produce interferon-gamma (IFN-gamma), driving M1 macrophage polarization (Wensveen et al. 2015). The nuclear hormone receptor, PPAR-gamma is the first adipocyte-specific transcription factor, which is expressed in a highest level in adipose tissue (Chawla et al. 1994; Tontonoz et al. 1994). Further, PPAR-gamma is a critical signaling molecule in determining macrophage phenotype in adipose tissue (Charo 2007). The deficiency of PPAR gamma in immune cells favors expression of M1 and impairs M2 macrophage marker expression in adipose tissue (Bassaganya-Riera et al. 2009). Contrarily, PPAR-gamma activation induces monocyte differentiation into M2 macrophages (Bouhlel et al. 2007). The decreased PPAR-gamma protein level is associated with increased macrophage infiltration in visceral adipose tissue of obese subjects. In this case, pro-inflammatory

macrophages suppress PPAR-gamma activity in adipocytes via S-nitrosylation (Yin et al. 2015). Actually, adipocytes are the source of Th2 cytokines. In response to adipocyte-derived Th2 cytokines, the expression of macrophage PPAR-beta/delta is up-regulated. Adipose tissue macrophage polarization ultimately could be modulated by adipocyte derived Th2 cytokines in a paracrine manner. PPAR-delta is induced by Th2 cytokines to control the transcriptional program of alternative activation in the macrophages. PPAR-delta may act as a factor for Th2 cytokine-induced M2 gene expression (Kang et al. 2008). In fact, obesity is accompanied by a transformation in the polarized states of macrophages from an anti-inflammatory "alternatively activated" M2 form (Kosteli et al. 2010), to a more pro-inflammatory "classically activated" M1 form (Lumeng et al. 2007a). The primary trigger for the recruitment of M1 macrophages is thought to be the secretion of TNF-alpha from hypertrophied adipocytes (Wellen and Hotamisligil 2003). The obesity-driven phenotypic adipose tissue macrophages can be characterized in these two broad classes based on the expression of particular antigens. Thus, diet-induced obesity leads to a shift in the activation state of adipose tissue macrophages from an M2-polarized state that may protect adipocytes from inflammation to an M1 pro-inflammatory state (Lumeng et al. 2007a).

TLR-dependent polarization mediators of M1 macrophages include different transcription factors such as NF-kappaB, activator protein-1 (AP-1), transcription factor PU.1 (PU.1), CCAAT/enhancer-binding protein alpha (C/EBP-alpha), signal transducer and activator of transcription 1 (STAT1) as well as interferon regulatory factor-5 (IRF5). AP-1 together with NF-kappaB in signal-dependent gene expression is necessary for innate immunity (Juhas et al. 2015). Among these transcription factors, IRF5 expression in macrophages is reversibly induced by inflammatory stimuli and contributes to the macrophage polarization. In this context, high expression of IRF5 is characteristic of M1 macrophages (Krausgruber et al. 2011). Thereby, IRF5 has been implicated in polarizing macrophages towards an inflammatory phenotype. In obese individuals, IRF5 expression is negatively associated with insulin sensitivity and collagen deposition in visceral adipose tissue (Dalmas et al. 2015), whereas another interferon regulatory factor, IRF4 is a key transcription factor that controls M2 macrophage polarization (Satoh et al. 2010). Consequently, differentiation of M1 macrophages is connected with the upregulation of IRF5 levels. The overexpression of IRF5 in M2 polarized macrophages leads to their phenotypic switch. It makes the M2 macrophages functionally similar to M1 (Juhas et al. 2015). However, Prieur et al. showed that early stages of adipose tissue expansion are characterized by M2-polarized adipose tissue macrophages and progressive lipid accumulation within adipose tissue macrophages (Prieur et al. 2011). The microenvironment in a lean adipose tissue is composed of a 4:1; M2:M1 ratio (Lumeng et al. 2008). Actually, a delicate balance of polarized populations of macrophages is necessary to maintain adequate adipocyte function. Inflammatory signals in adipose tissue of obese individuals is eliminated by retaining M2 polarization of adipose tissue macrophages or by triggering the phenotypic switch from M1 to M2 (Sun et al. 2011). Diet-induced obesity increases the number of M1 macrophages by 65-fold, whereas the number of M2 macrophages per weight basis is also increased by six-fold. Eventually, the ratio of M1-to-M2 macrophages is increased in obesity (Fujisaka et al. 2009). However, Fjeldborg et al. found a relatively higher expression of the anti-inflammatory markers, CD163 and IL-10, and a relative reduction of the pro-inflammatory markers, TNF-alpha and IL-6, in adipose tissue from obese subjects compared to lean individuals. Thus, human adipose tissue macrophages change polarization to a more anti-inflammatory profile in obesity than towards a pro-inflammatory profile (Fjeldborg et al. 2014). There is a shift towards a M2 phenotype in non-crown like structure macrophages in adipose tissue from obese subjects compared with lean ones. Macrophages in crown like structure are predominantly M1, but most other macrophages, particularly those in fibrotic areas, are M2 (Spencer et al. 2010). At initial stage the expression of IL-10 by M2 macrophages is

approximately 100-fold higher than that in adipocytes. Hence M2 macrophage-derived IL-10 constitutes the major portion of the IL-10 in adipose tissue. In this context, the phenotypic shift of adipose tissue macrophages toward an M1-dominant state cannot be explained simply by the conversion of M2 macrophages to M1. De novo accumulation of circulating monocytes to adipose tissue followed by their differentiation into M1 and M2 macrophages (Fujisaka et al. 2009). This situation can be explained as follows; first of all, transient accumulation of M2 macrophages could be essential for the control of tissue fatty acid levels during activation of lipolysis. M2 macrophage-borne signaling molecules could inhibit lipolysis and re-esterification of lipolyzed fatty acids form triacylglycerols (triacylglycerols/fatty acid cycle). Thus, M2 macrophages initially support the metabolically flexible adipocytes, which have a high capacity of both triacylglycerols/fatty acid cycling and oxidative phosphorylation in obese white adipose tissue (Masoodi et al. 2015). The M1 adipose tissue macrophage polarization in obesity is regarded as the optimization of the fat deposition and repartitioning toward adipocytes. It prevents adipose tissue macrophage lipotoxicity (Prieur et al. 2011). However, the expression of carnitine palmitoyltransferase 1A (CPT1A), the rate-limiting enzyme in mitochondrial fatty acid oxidation, is higher in human adipose tissue macrophages than in adipocytes. It is differentially expressed in visceral versus subcutaneous adipose tissue in obese individuals. Enhanced fatty acid oxidation in saturated fatty acid-incubated adipocytes and macrophages reduces triglyceride content and inflammation. While insulin sensitivity increases in adipocytes, endoplasmic reticulum (ER) stress and reactive oxygen species (ROS) damage decreases in macrophages (Malandrino et al. 2015). M2 macrophages sustain insulin sensitivity by secreting IL-4 and IL-10, while M1 macrophages induce insulin resistance through the secretion of pro-inflammatory cytokines, such as TNF-alpha (Tateya et al. 2013). M2 macrophages secrete anti-inflammatory cytokines and utilize oxidative metabolism to maintain adipose tissue homeostasis (Castoldi et al. 2015). M1 macrophages provide their energy from aerobic glycolysis. In this condition, glucose uptake as well as the conversion of pyruvate to lactate is increased. Meanwhile, the activities of the respiratory chain are attenuated, allowing for ROS production. However, M2 macrophages obtain much of their energy from fatty acid oxidation and oxidative metabolism (Galván-Peña and O'Neill 2014). In response to IL-4, STAT6 and PPARgamma-coactivator-1beta (PGC-1beta) induce the fatty acid oxidation and mitochondrial biogenesis in macrophages (Vats et al. 2006). IL-4 activates the transcriptional responses of STAT6. Active STAT6 can induce the coactivator PGC-1beta. PGC-1beta can induce mitochondrial respiration as well as mitochondrial biogenesis (Galván-Peña and O'Neill 2014; St-Pierre et al. 2003). In fact, PPAR-gamma is required for IL-4-induced increase in beta-oxidation of fatty acids. Despite IL-4 stimulation, the rate of fatty acid oxidation is reduced by approximately 70% in PPAR-gamma null macrophages. By contrast, increase in PPAR-gamma expression doubles the rate of beta-oxidation (Odegaard et al. 2007).

Progression of obesity increasingly induces a phenotypic switch from the M2 macrophages, to the M1 macrophages. Hence pro-inflammatory CD11c+ M1 macrophages is markers of insulin resistance in human obesity (Wentworth et al. 2010). Thus the enhanced macrophage-adipocyte crosstalk in obesity disrupts insulin action in adipocytes. Macrophage-secreted factors block insulin action in adipocytes via downregulation of GLUT4 and insulin receptor substrate-1 (IRS-1), leading to a decrease in Akt phosphorylation and impaired insulin-stimulated GLUT4 translocation to the plasma membrane (Lumeng et al. 2007c). The secretion of inflammatory cytokines and chemokines by adipose tissue macrophages includes TNF-alpha, IL-6, IL-1beta, MCP-1, CCL2, and macrophage inhibitory factor (MIF) (Olefsky and Glass 2010). In particular IL-1beta is produced largely by macrophages. It provokes the development of obesity-associated insulin resistance by inhibition of insulin signal transduction in adipocytes. Contrarily, blocking the activity of IL-1beta improves insulin signaling in human adipocytes. This is in parallel with a reduc-

tion in macrophage-stimulated pro-inflammatory profile and lipolysis (Bing 2015). Thus the reduction in protein expression of IRS-1, phosphatidylinositol 3-kinase (PI3K) p85alpha, and GLUT4 by macrophage-conditioned-medium is reversed when macrophage-derived IL-1beta production is inhibited. In contrast, macrophage-stimulated release of IL-6, IL-8, and chemokine (C-C motif) ligand 5 (RANTES) by adipocytes is also significantly reduced by blocking IL-1beta production (Gao et al. 2014). PGC-1beta plays a key role in saturated fatty acids metabolism and in the regulation of inflammatory signaling. PGC-1beta expression is significantly decreased in response to saturated fatty acid in macrophages in a dose dependent manner. PGC-1beta inhibits saturated fatty acid-induced TNF-alpha, MCP-1, and IL-1beta mRNA and protein expressions. Furthermore, PGC-1beta significantly antagonizes saturated fatty acid-induced macrophage NF-kappaB-p65 and JNK activation. PGC-1beta overexpression in saturated fatty acid treated macrophages improves adipocytes PI3K-Akt insulin signaling in a paracrine fashion (Chen et al. 2016). In response to high-fat diet the tumor necrosis factor receptor (TNFR)-associated factor-3 (TRAF-3), binds to and activates TGF-beta-kinase 1 (TAK1). Thereby the activation of downstream inhibitor of nuclear factor kappa-B kinase subunit beta (IKKbeta)–NF-κB and mitogen-activated protein kinase kinase (MAPKK)–JNK–IRS1 signaling cascades are enhanced, while disrupting AKT–glycogen synthase kinase 3 beta (GSK3beta)/forkhead box protein O1 (FOXO1) phosphorylation cascade. Eventually insulin resistance and inflammatory response is facilitated (Wang et al. 2016) (Fig. 14.1).

The release of obesity-related danger signals such as ROS, lysosomes, and other obesity-induced danger signals result in the oligomerization of NLRP3 in adipose tissue (Gurung et al. 2015; Zhou et al. 2011). Activation of inflammasomes by damaged mitochondria results in caspase-1-dependent secretion of the inflammatory cytokines IL-1beta and IL-18, and leads to an inflammatory form of cell death. In addition, regulation of mitochondria-induced inflammasome activation centrally contributes to the inflammatory process that is responsible for obesity (Gurung et al. 2015). Actually NLRP3 with macrophage marker F4/80 co-localizes in crown-like structures. Hence, obesity is associated with significant increase in serum IL-18 concentrations which is blocked upon ablation of NLRP3. Indeed, NLRP3 inflammasome is activated in response to high fat diet and controls the production of IL-1beta in adipose tissue and IL-18 in obesity. Induction of high-fat diet induced obesity causes marked caspase-1 activation in adipose tissue. Ceramides are lipid metabolites that accumulates in tissues in response to obesity, and induce caspase-1 activation in an NLRP3-dependent mechanism. In contrast, elimination of NLRP3 inflammasome reduces M1-like macrophage gene expression and increases M2-like expressed cytokines (Youm et al. 2011). Partial depletion of macrophages from adipose tissue of obese subjects decreases the expression of the macrophage marker CD68, with no significant alteration in the expression of caspase-1. This indicates that the effects of the NLRP3-apoptosis-associated speck-like protein (ASC)-caspase-1 protein complex on adipose tissue are not exerted though infiltrating macrophages (Benetti et al. 2013; Stienstra et al. 2011). Actually, innate pattern recognition receptors inflammasomes (NLRs) are cytosolic sensors that detect endogenous metabolic stress and activates caspase enzymes. Activated caspase-1 processes the cytosolic precursors of the related cytokines IL-1beta and IL-18 (Li et al. 2014; Stienstra et al. 2011).

One important molecule regulating glycolysis and macrophage activation is hypoxia-inducible factor 1-alpha (HIF-1alpha). However, hypoxia induces the inflammatory phenotypes of macrophages via HIF-1alpha-dependent and -independent mechanisms (Fujisaka et al. 2013). PI3K/Akt contributes hypoxic stress-induced TLR4 expression through the regulation of HIF-1 activation (Kim et al. 2012). Moreover, hypoxia increases the activation of JNK and p38 mitogen-activated protein kinase signaling in saturated fatty acid-treated macrophages. Inhibition of JNK blocks the hypoxic induction of pro-inflammatory cytokine expression. In any case,

inhibition of hypoxia-induced transcription factors fails to reduce the expression of cytokines by saturated fatty acid-treated hypoxic macrophages. Enhanced pro-inflammatory cytokine production and JNK activity under hypoxia are prevented by inhibiting ROS generation. It is well known that hypoxia along with higher concentrations of free fatty acids exacerbates macrophage-mediated inflammation in obesity (Snodgrass et al. 2016). In white adipose tissue, increased oxidative stress due to the mRNA overexpression of NADPH oxidase subunits in ectopic fat leads to enhanced plasminogen activator inhibitor-1 (PAI-1), IL-6, and MCP-1 mRNA expression. Furthermore, the selective increase in ROS production in obesity eventually leads to elevation of systemic oxidative stress (Furukawa et al. 2004). In M2 macrophages, overexpression of HIF-2alpha decreases nitric oxide production and suppresses expression of pro-inflammatory cytokines through induction of arginase-1. HIF-2alpha-overexpressing macrophages alleviate pro-inflammatory responses and improve the insulin resistance in adipocytes (Choe et al. 2014). Additionally, recruited adipose tissue macrophages overexpress IL-6, iNOS, and CCR2 (Lumeng et al. 2007b). In particular, CCR2 is expressed by obese adipose tissue macrophages, which are derived from bone marrow cells (Ito et al. 2008). A high level of de novo IL-6 secretion by macrophages is a result of engulfment of the lipid content of adipocytes by macrophages. Thus, IL-6 secretion during interaction of adipocytes and macrophages might have an anti-inflammatory role in the inflamed adipose tissue by downregulating the induction and release of pro-inflammatory cytokines (Sárvári et al. 2015). In total, production of these inflammatory factors in obesity is under the transcriptional control of two key intracellular inflammatory pathways; JNK-activator protein 1 (AP1) and IKKbeta. Extracellular free fatty acids activate JNK and IKKbeta via TLR signaling pathway. Elevation of the pro-inflammatory cytokines in obesity activates JNK and IKKbeta by a specific signaling pathway (Solinas and Karin 2010). Endogenous fatty acids, which are released from adipocytes via the beta3-adrenergic stimulation, result in the activation of the TLR4/NF-kappaB pathway. In this process, saturated fatty acids, which are released in large quantities from hypertrophied adipocytes via the macrophage-induced adipocyte lipolysis, serve as a natural ligand for TLR4. Thereby the inflammatory changes in both adipocytes and macrophages are induced through NF-kappaB activation (Suganami et al. 2007). Macrophage-inducible C-type lectin (Mincle; also called Clec4e and Clecsf9), is a type II transmembrane C-type lectin. It is induced selectively in macrophages during the interaction between adipocytes and macrophages. Saturated fatty acid released from adipocytes induces Mincle mRNA expression in macrophages through the TLR4/NF-kappaB pathway. Macrophage-induced adipocyte lipolysis aggravates obesity-induced adipose tissue inflammation by this way (Ichioka et al. 2011). Engagement of either TLR4 or TLR2 with long-chain saturated fatty acids leads to recruitment of the myeloid differentiation primary response gene 88 (MyD88) adapter protein and formation of signaling complexes containing the IL-1 receptor associated serine/threonine kinase (IRAK1) and the TRAF family members, TRAF3 and TRAF6. The latter involves the activation of the MAP kinase kinase kinase (MAP3K) TAK1, which is required for JNK and IKKbeta activation (Kawai and Akira 2007). In addition to these pathways, IRAK-1 is also a tissue marker of meta-inflammation in obesity and a key component of the TLR2/IL-1receptor/MyD88 signaling pathway. Enhanced IRAK-1 gene expression correlates with adipose tissue infiltration by macrophages in obesity-associated chronic low-grade metabolic inflammation (Ahmad et al. 2015) (Fig. 14.1).

Inflammation leads to an increased number of microRNAs (miRNAs) detectable in both adipocytes and M1 macrophages. Indeed, under inflammatory conditions, adipocytes and M1 macrophages share the expression of 147 miRNAs, and 100 common miRNAs. Additionally, miR-221 by 2-fold, miR-222 by 2.5-fold, and miR-155 by 5-fold increased in inflamed adipocytes (Ortega et al. 2015). Although there is no significant change in the level of chemokine (C-C motif) ligand 2 (CCL2) either in adipose tissue or

in circulation, the cellularity of adipose tissue macrophages (ATMs) is dramatically elevated at the early stage of obesity. Thus alternatively activated macrophages at the early stages of obesity are the major population of resident macrophages, with more specialized lipid catabolism than that of immigrant macrophages (Zheng et al. 2016). In subcutaneous white adipose tissue obtained from 56 subjects, 11 miRNAs are present in all subjects and downregulated in obesity. Of these, ten affect adipocyte CCL2 secretion for miR-126 and miR-193b. The levels of miR-193b in subcutaneous white adipose tissue are significantly associated with CCL2 secretion, whereas expression of integrin-alpha-X, an inflammatory macrophage marker, is associated with miR-193b and miR-126. In this context, miRNAs may be important regulators of adipose inflammation through their effects on CCL2 release from human adipocytes and macrophages (Arner et al. 2012). Overexpression of miR-126/-193b/-92a in different pairwise combinations reduce CCL2 secretion more efficiently than either miRNA alone. However, although effects on CCL2 secretion by co-overexpression of miR-92a/-193b and miR-92a/-126 are additive in adipocytes, the combination of miR-126/-193b is primarily additive in macrophages. Furthermore signals for miR-92a and -193b converge on the NFkappaB pathway (Kulyté et al. 2014).

The expression of insulin-like growth factor-1 (IGF1) by adipose tissue is derived from adipocytes and macrophages. In lean animals, adipocytes are the primary source of IGF1, but in obesity expression by adipocytes is reduced and by macrophages increased, as to maintain overall adipose tissue IGF1 expression (Chang et al. 2016). IGF1 reduces free fatty acid-induced JNK1 activation and TNF-alpha expression in human subcutaneous but not omental preadipocytes. Impaired anti-inflammatory action of IGF1 in omental preadipocytes is a result of the chronic inflammation in visceral adipose tissue (Neacsu et al. 2013). Actually, preadipocytes could function as macrophage-like cells and raise the possibility of a potential direct involvement of adipose tissue in inflammatory processes (Cousin et al. 1999). Moreover, preadipocyte and macrophage phenotypes are very similar and that preadipocytes have the potential to be very efficiently and rapidly converted into macrophages (Charrière et al. 2003). In this respect, both macrophages and adipocytes are the sites for active lipid metabolism and signaling. Macrophage/adipocyte aP2 expression is controlled with the same promoter or enhancer elements that confer expression in adipocytes. Hence, there is a striking overlap between the biology of adipocytes and macrophages (Makowski et al. 2001). The macrophage/adipocyte aP2 has also been expressed exclusively by differentiated adipocytes. aP2 plays a vital role in the local macrophage responses and binds a number of hydrophobic ligands which are known to influence macrophage function (Makowski et al. 2005). As lipid chaperones, fatty acid-binding proteins (FABPs) may actively facilitate the transport of lipids to specific compartments in the cell. Adipocyte/macrophage aP2 and FABP5, act at the interface of metabolic and inflammatory pathways. Although being the minor isoform in adipocytes, FABP5 protein levels are comparable to aP2 in normal macrophages (Makowski and Hotamisligil 2005). PPAR-gamma induces the expression of the adipocyte/macrophage aP2 and increases aP2 mRNA of primary human monocytes in time- and dose-dependent manner (Pelton et al. 1999). FABP-mediated lipid metabolism is closely linked to both metabolic and inflammatory processes through modulating critical lipid-sensitive pathways in adipocytes and macrophages (Furuhashi and Hotamisligil 2008). Deletion of FABPs in adipocytes results in reduced expression of inflammatory cytokines in macrophages, whereas the same deletion in macrophages led to enhanced insulin signaling and glucose uptake in adipocytes. The metabolic status of adipocytes is a major determinant of macrophage inflammatory output. Neither macrophages nor adipocytes individually could account for the total impact of FABPs on systemic metabolism (Furuhashi et al. 2008).

In adipose tissue, eosinophils are responsible for 90% of IL-4 expression and accelerate M2 macrophage polarization by secreting Th2 type cytokines such as IL-4 and IL-13. Hence, eosino-

phils might act as anti-inflammatory immune cells in obesity-induced adipose tissue inflammation (Huh et al. 2014). Th1 cells primarily secret IFN-gamma which stimulates monocyte differentiation into M1 type macrophages. IFN-gamma exacerbates adipose tissue inflammation in obesity (Rocha et al. 2008). IFN-gamma might also play an important role in growing adipose tissue, where adipocytes respond to inflammatory products derived from infiltrating macrophages (Wellen and Hotamisligil 2003).

Adipose tissue synthesizes complement proteins and is a target of complement activation. C3a-desArg/acylation-stimulating protein induces lipogenesis and affects lipid metabolism. The C3a receptor and C5a receptor are involved in the development of insulin resistance in adipocytes through macrophage infiltration and the activation of adipose tissue (Vlaicu et al. 2016). C5a receptor-like receptor 2 (C5L2) has been identified as a receptor for acylation-stimulating protein (ASP) and the inflammatory factor C5a. While adipocyte-conditioned medium increases C5L2-C5a receptor co-localization in macrophages, this is blocked by C5a. Induction of ASP increases Akt phosphorylation in both cell types. However, C5a induces Akt phosphorylation in adipocytes with less effect in macrophages (Poursharifi et al. 2013). ASP may induce specific inflammatory cytokines in adipocytes through PI3K- and NF-kappaB-dependent pathways, thus further promoting macrophage infiltration and local inflammation in obese adipose tissue (Tom et al. 2013).

4 Conclusion

Adipocyte death defines macrophage localization and function in adipose tissue of obese humans. However, mechanistic details of the macrophage accumulation surrounding the adipocytes and other parts of the adipose tissue is still vague. In addition, the source of TNF-alpha and the other cytokines requires a detailed analysis by taking the inflammatory alterations in obesity into account. The triggering mechanisms of lipid mediators in adipose tissue has been described by indirect evidences. Therefore, further investigations are necessary in order to clarify the molecular nature of the macrophage-adipocytes interactions in obesity-related diseases.

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Endothelial Dysfunction in Obesity

15

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Abstract

Chronic inflammatory state in obesity causes dysregulation of the endocrine and paracrine actions of adipocyte-derived factors, which disrupt vascular homeostasis and contribute to endothelial vasodilator dysfunction and subsequent hypertension. While normal healthy perivascular adipose tissue (PVAT) ensures the dilation of blood vessels, obesity-associated PVAT leads to a change in profile of the released adipo-cytokines, resulting in a decreased vasorelaxing effect. Adipose tissue inflammation, nitric oxide (NO)-bioavailability, insulin resistance and oxidized low-density lipoprotein (oxLDL) are main participating factors in endothelial dysfunction of obesity. In this chapter, disruption of inter-endothelial junctions between endothelial cells, significant increase in the production of reactive oxygen species (ROS), inflammation mediators, which are originated from inflamed endothelial cells, the balance between NO synthesis and ROS, insulin signaling and NO production, and decrease in L-arginine/endogenous asymmetric dimethyl-L-arginine (ADMA) ratio are discussed in connection with endothelial dysfunction in obesity.

Keywords

Obesity • Asymmetric dimethyl-L-arginine (ADMA) • Nitric oxide (NO) • Reactive oxygen species (ROS) • Cyclic guanosine monophosphate (cGMP) • Tumor necrosis factor-alpha (TNF-alpha) • Endothelin-1 (ET-1) • Endothelial nitric oxide synthase (eNOS) • Endothelial nitric oxide synthase (eNOS) uncoupling • Peroxynitrite • Super oxide dismutase (SOD) • Plasminogen-activator inhibitor-1 (PAI-1) • Endothelium • Saturated fatty acids • Vasodilatory-stimulated phosphoprotein (VASP) • Protein kinase

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C (PKC) • Intercellular adhesion molecule-1 (ICAM-1) • Pyrin domain-containing 3 (NLRP3) inflammasome • Vascular cell adhesion molecule-1 (VCAM-1) • Low-density lipoproteins (LDL) • Oxidized low-density lipoprotein (OxLDL) • Vascular endothelial growth factor (VEGF) • Nuclear factor kappa-B (NF-kappaB) • Inducible nitric oxide synthase (iNOS)

1 Introduction

Besides lipid-filled mature adipocytes, the adipose tissue is also composed of various stromal cells, including fibroblasts, endothelial cells, and various immune cells (Huh et al. 2014). Adipose tissue expansion during obesity leads to immune cell infiltration and a homeostatic process that promotes inflammation in adipose tissue. The release of proinflammatory cytokines stimulates lipolysis and causes insulin resistance, leading to adipocyte dysfunction (Grant and Stephens 2015). Actually, human adipocytes express many cytokines and chemokines that are biologically functional. Nevertheless, the primary event in the sequence leading to chronic inflammation in adipose tissue is the metabolic dysfunction of adipocytes, followed by the recruitment and activation of adipose tissue macrophages (Meijer et al. 2011). Evidences show that the adipose tissue secretes more than 50 hormones and signaling molecules, collectively called adipokines, which exert their biological roles in an autocrine, paracrine, or systemic manner and influence several physiological processes (Waki and Tontonoz 2007).

Recently, genome-wide expression analyses showed that of the 156 human perivascular adipose tissue upregulated genes, 59 associate with angiogenesis, vascular biology, or inflammation whereas of the 166 downregulated genes, 21 associate with vascular biology and inflammation (Chatterjee et al. 2013). Consistently, obese humans show perivascular adipose tissue-derived tumor necrosis factor-alpha (TNF-alpha) excess, and an increased vascular expression of endothelin-1 (ET-1). Latter contributes to the ET-1/nitric oxide (NO) system imbalance by impairing NO release. In addition, reactive oxygen species (ROS) excess, via nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase2 (NOX2) activation, induces the endothelial nitric oxide synthase (eNOS) uncoupling, which in turn generates superoxide and impairs NO production (Virdis et al. 2015). Indeed, in addition to chronic inflammatory state, dysregulation of the endocrine and paracrine actions of adipocyte-derived factors disrupt vascular homeostasis by causing an imbalance between the NO pathway and the ET-1 system (Campia et al. 2012). Thus, obesity is associated with enhanced ET-1-mediated vasoconstriction that contributes to endothelial vasodilator dysfunction and subsequent hypertension (Weil et al. 2011). Actually, the net release of ET-1 from adipose tissue is 2.5-fold higher in obesity. ET-1 attenuates the antilipolytic effect of insulin in visceral adipocytes by decreasing the expression of insulin receptor, insulin receptor substrate-1 (IRS-1) and phosphodiesterase-3B and by increasing the expression of endothelin receptor-B (ETBR) (van Harmelen et al. 2008). Consequently, fatty acid release from the visceral adipose tissue may occur more than any other fat depots during insulin resistance (Arner 2005). The protein expression of ETAR is also significantly higher in obese subjects. Thus, ET-1 causes significant increase in lipolysis through the activation of ETAR of adipose tissue. However, there is no difference in ETBR expression between the subcutaneous adipose tissues of obese and lean subjects (Eriksson et al. 2009). In obese animals, basal vascular hydroxyl radical formation and ROS activity are reduced by three-fold and five-fold, respectively. Although the vascular formation of ROS, including ET-1 mediated hydroxyl radical formation is lower in obesity, the sensitivity to exogenous ROS and hydroxyl radicals is increased in obese animals (Mundy et al. 2007). Eventually, endothelial dysfunction

is aggravated due to the imbalance between pro-oxidant and anti-oxidant mechanisms in perivascular adipose tissue after long-term high-fat diets. In diet-induced obesity, the deleterious effect of perivascular adipose tissue might be the result of the down-regulation of both eNOS expression and NO production in addition to the down-regulation of extracellular super oxide dismutase (ecSOD) and total super oxide dismutase (SOD) activity in endothelial cells. Finally, high superoxide radicals attack due to an increased NOX2 and a reduced SOD activity, and contribute to endothelial dysfunction by consuming NO (Gil-Ortega et al. 2014). In this respect, the contributors to endothelial dysfunction in obesity, such as the function of perivascular adipose tissue, fatty acid-related adipose tissue inflammation, NO-bioavailability, insulin resistance, oxidized low-density lipoprotein (oxLDL) and adipose tissue hypoxia are discussed.

2 Perivascular Adipose Tissue and Endothelium

Blood vessels are composed of three layers: intima, media and adventitia. Adventitial fat is also called perivascular adipose tissue (PVAT); it surrounds almost all blood vessels. It has a dual regulatory role in modulating vessel function. Firstly, PVAT attenuates vasoconstriction by perivascular adipocyte-derived relaxation factors and secondly, promotes constriction in response to perivascular nerve excitation by perivascular adipocyte-derived constricting factors (Gao 2007). Perivascular fat differs substantially from typical visceral adipose tissue both in the states of health and disease. This difference results not only from its location adjacent to vascular wall but also from most likely a differential release of adipocytokines and cytokines (Guzik et al. 2007). Additionally, PVAT composes of both brown adipose tissue and white adipose tissue, with different ratios depending on the location (Cinti 2011). Actually PVAT exerts its anti-contractile effects by releasing a transferable relaxing factor which induces endothelium-dependent relaxation through NO release and subsequent calcium-dependent K^+ channel activation. However, endothelium-independent relaxation mechanism involves hydrogen peroxide and subsequent activation of soluble guanylyl cyclase (Gao et al. 2007). PVAT secretes a number of bioactive substances including vascular endothelial growth factor (VEGF), TNF-alpha, leptin, adiponectin, insulin-like growth factor (IGF), interleukin-6 (IL-6), plasminogen activator substance, resistin and angiotensinogen (Oriowo 2015). Thus, normal healthy PVAT ensures the dilation of blood vessels. However, obesity is associated with an increased adipocyte mass in PVAT. In this case adipose tissue with excessive adipocyte hypertrophy becomes a more inflammatory phenotype by attracting macrophages into PVAT. This leads to a change in profile of the released adipocytokines, resulting in a decreased vasorelaxing effect of PVAT (Guzik et al. 2007). Obesity results in PVAT inflammation, characterized by imbalance between pro- and anti-inflammatory cells as well as adipokines. PVAT inflammation promotes insulin resistance in the vasculature. This process results in impaired insulin-mediated vasodilatory responses and vascular remodeling (Lastra and Manrique 2015). Eventually, in obese PVAT, increased release of TNF-alpha and free fatty acids modify the inflamed adipose tissue to a more pro-inflammatory vasoconstrictive phenotype (Almabrouk et al. 2014). Indeed, PVAT releases a wide range of biologically active molecules. The infiltrating macrophages and T lymphocytes present in PVAT or recruited in response to chemokine release by adipocytes, not only release inflammatory mediators, but also maintain a balance amongst these factors. Additionally, PVAT expresses a complex ROS/ reactive nitrogen species (RNS) machinery which contains NOX2, eNOS and SOD isoforms (Szasz and Webb 2012). Although PVAT of obese aorta displays an impaired endothelium-dependent vasodilation, endothelium-independent vasodilatation is unaltered. PVAT promotes endothelial dysfunction in diet-induced obesity via mechanisms that are linked to increased NOX2-derived oxidative stress and increased production of pro-inflammatory cytokines (Ketonen et al. 2010). Thus, long-term high-fat diet-induced PVAT dys-

function is characterized by a substantial reduction in eNOS and NO production with the increased NOX2 activity and superoxide anion release. Furthermore, reduction in ecSOD expression and total SOD activity indicates the imbalance between antioxidant and pro-oxidant mechanisms in PVAT. Eventually the ratio of oxidized glutathione/(glutathione+oxidized glutathione) [GSSG]/([GSH]+[GSSG]) is two-fold higher in the mesenteric PVAT from obese animals (Gil-Ortega et al. 2014). Indeed, PVAT impacts obesity-related vascular dysfunction and remodeling through impairment of eNOS-mediated vasodilatation and the 5′-adenosine monophosphate (AMP)-activated protein kinase (AMPK)/mammalian target of rapamycin (mTOR) pathway (Ma et al. 2010). Hence, pericardial fat, perivascular fat, pericoronary fat, and myocardial fat may exert their harmful effects on the heart and blood vessels by direct lipotoxicity and by indirect cytokine secretion (Lim and Meigs 2014). In addition to release of adipokines, adipocytes in PVAT also produce classical chemokines (or cytokines) including IL-6, IL-8, monocyte chemoattractant peptide-1 (CCL2 or MCP-1) and plasminogen-activator inhibitor-1 (PAI-1) (Rajsheker et al. 2010; Thalmann and Meier 2007). In particular ectopic fat accumulation around the heart and coronary arteries is associated with oxidative stress, activation of the coagulation cascade, disturbances in the renin-angiotensin system, and enhanced lipid oxidation, which generates oxidized low density lipoprotein (McGavock et al. 2006). Perivascular adipocytes surrounding human coronary arteries exhibit a reduced state of adipogenic differentiation and a heightened proinflammatory state by secreting more than 50-fold higher levels of the proinflammatory cytokine and MCP-1 compared with adipocytes from other regional depots (Omar et al. 2014). The decreased levels of adiponectin and increased levels of leptin or TNF-alpha in PVAT increase the quantity of adipose tissue, inflammation cell proliferation and endothelial dysfunction in obesity (Ozen et al. 2015). Indeed, expression and secretion of adiponectin by perivascular adipocytes is markedly reduced, while secretion of pro-inflammatory IL-6, IL-8 and MCP-1 is increased (Braunersreuther et al. 2007). As mentioned above, perivascular adipocytes are not separated functionally from the blood vessel wall, thereby PVAT-derived mediators readily access to the blood vessels and transduce metabolic signals (Rajsheker et al. 2010). On the other hand, PVAT-derived visfatin protein expression is the highest, 3.7-fold and 1.8-fold higher than that in subcutaneous and visceral adipose tissue respectively (Wang et al. 2009b). In obesity, visfatin may be increased in systemic circulation and local PVAT. Aortic and coronary atherosclerosis is positively correlated with visfatin expression in the corresponding PVAT (Spiroglou et al. 2010).

The pro-renin receptor, angiotensin-converting enzyme-2 (ACE2) and of three angiotensin II type 1a receptor (AT1a) receptor isoforms are expressed by perivascular adipose tissue. However, the functional role of the renin–angiotensin system components in perivascular adipose tissue does not depend on local renin synthesis but rather on renin or pro-renin uptake from the circulation. Levels of expression of angiotensinogen, ACE1, and ACE2 are similar between periaortic and mesenteric adipose tissues. Renin receptor expression is five times higher, whereas both expression of AT1a and type 2 (AT2) receptors are significantly lower in periaortic adipose tissue compared with mesenteric adipose tissue (Gálvez-Prieto et al. 2008). In this manner, PVAT-derived angiotensin II is critically involved in the potentiation of vasoconstriction to perivascular neuronal stimulation in rat mesenteric arteries (Lu et al. 2010), whereas angiotensin 1-7 are transferable perivascular-derived relaxation factors that induces endothelium-dependent relaxation through NO release and activation of voltage-dependent potassium channels (Lu et al. 2011). Adaptative NO overproduction occurs in PVAT during early diet-induced obesity which might be aimed at preserving vascular function (Gil-Ortega et al. 2010). Additionally, high levels of free fatty acids could attenuate the anti-contractile properties of PVAT by an endothelium-dependent mechanism (Sun et al. 2013).

3 Fatty Acid-Related Adipose Tissue Inflammation and Endothelial Dysfunction

The endothelium is a semi-permeable barrier. Transport across the endothelium can occur either through the endothelial cell or through interendothelial junctions. However, the permeability of the vascular barrier can be modified in response to specific stimuli acting on endothelial cells (Vandenbroucke et al. 2008). In this regard, endothelial cells are directly and continuously exposed to a variety of hemodynamic forces that are created by blood flow and the cardiac pulse waves. It is defined as shear stress which acts at the interface between blood flow and the vessel wall. These signals are detected and recognized by endothelial cells. Eventually, shear stress determines the shape and orientation of endothelial cells (Ando and Yamamoto 2011). In obesity, continuous exposure of the endothelial layer to hyperlipidaemia causes the internalization of lipids. Subsequent to endothelial activation, progressive accumulation of lipids may primarily occur within the intima. These events enhance the permeability of the endothelial layer by increasing the expression of cytokines, chemokines and adhesion molecules (Badimon et al. 2011). Endothelial junctional complexes include tight junctions, adherens junctions, and gap junctions (Bazzoni and Dejana 2004). These interendothelial junctions can be disassembled or assembled to either increase or decrease paracellular permeability (Vandenbroucke et al. 2008). Several signaling complexes have been identified to associate with tight junctions. Expression of tight junction components suppresses proliferation of endothelial cells to allow differentiation in a coordinated manner with adherens junctions and extracellular matrix adhesion (Balda and Matter 2009). In particular, endothelial cell-to-cell junctions are vital for the formation and integrity of blood vessels. In fact, adherens junctions and tight junctions are formed by transmembrane adhesive proteins that are linked intracellular signalling partners (Nyqvist et al. 2008). Although more than 40 different proteins have been discovered to be located at the tight junctions of different cells, among these, occludin and claudins are capable of interacting with complementary molecules on adjacent cells (González-Mariscal et al. 2003). Actually, the typical tight junction proteins in endothelial cells are zona occludens proteins-1/2 (ZO-1/2) and occludin (Hernandez et al. 2007). Of these, ZO-2 shuttles between the plasma membrane and the nucleus. Thus, ZO-2 contains several protein binding sites that allow it to function as a scaffold for the clusters, adaptor and signaling proteins (Hernandez et al. 2007). Endothelial permeability is partially regulated by the dynamic opening and closure of cell-cell adherens junctions. In endothelial cells, adherens junctions are largely composed of vascular endothelial cadherin. Vascular endothelial growth factor induces the tyrosine phosphorylation of cadherin. Phosphorylated cadherin is accompanied with an increase in vascular permeability (Dejana et al. 2008). In fact, vascular endothelial cadherin is exclusively expressed by endothelial cells and is the most important transmembrane component of endothelial adherens junctions. Increased levels of soluble vascular endothelial-cadherin, as well as auto-antibodies to human vascular endothelial-cadherin may be an indicator of endothelial dysfunction in humans (Blaise et al. 2015). Disruption of interendothelial junctions between endothelial cells occurs in the early stages of endothelial barrier dysfunction (Chen et al. 2015). Lipolysis products generated from lipoprotein lipase-mediated hydrolysis of triglyceride-rich lipoproteins on the endothelial cell surface can promote the accumulation of these products in the subendothelial space. This process rearranges the tight junction proteins, ZO-1 and occludin, adherens junctional cadherin, and cytoskeletal F-actin (Eiselein et al. 2007). Meanwhile, triglyceride-rich lipoprotein lipolysis significantly increases the production of ROS in endothelial cells by activating NOX2. Thereby free fatty acid release during triglyceride-rich lipoprotein lipolysis may contribute to the triglyceride-rich lipoprotein lipolysis-induced endothelial cell dysfunction (Wang et al. 2008). Epidemiological studies implicate that elevated triglyceride-rich lipoprotein levels are risk factor for vascular inflammation. However, the contribution of triglyceride-rich lipoprotein lipolysis to inflammation depends less on the triglyceride-rich lipoprotein concentration than on the balance

between partially lipolyzed remnant particles or saturated fatty acids and unsaturated fatty acids released by lipolysis. While remnant particles enhancing foam cell formation, saturated fatty acids and oxidized fatty acids induce inflammation, via oxidative stress, whereas unsaturated fatty acids released by lipolysis have anti-inflammatory effects (Schwartz and Reaven 2012). Furthermore, triglyceride-rich lipoprotein and low density remnant particles are promptly internalized by endothelial cells via endocytosis through LDL family receptors (Wang et al. 2011). In this manner, the free fatty acid fractions show pro-inflammatory responses, which induce TNF-alpha and intracellular adhesion molecule expression and ROS production in human aortic endothelial cells (Wang et al. 2009a). Picchi et al. suggested that overexpression of TNF-alpha induces activation of NOX2 and production of ROS leading to endothelial dysfunction in the metabolic syndrome. In this case TNF-alpha reduces NO-bioavailability and impairs NO-dependent dilation (Picchi et al. 2006) (Fig. 15.1).

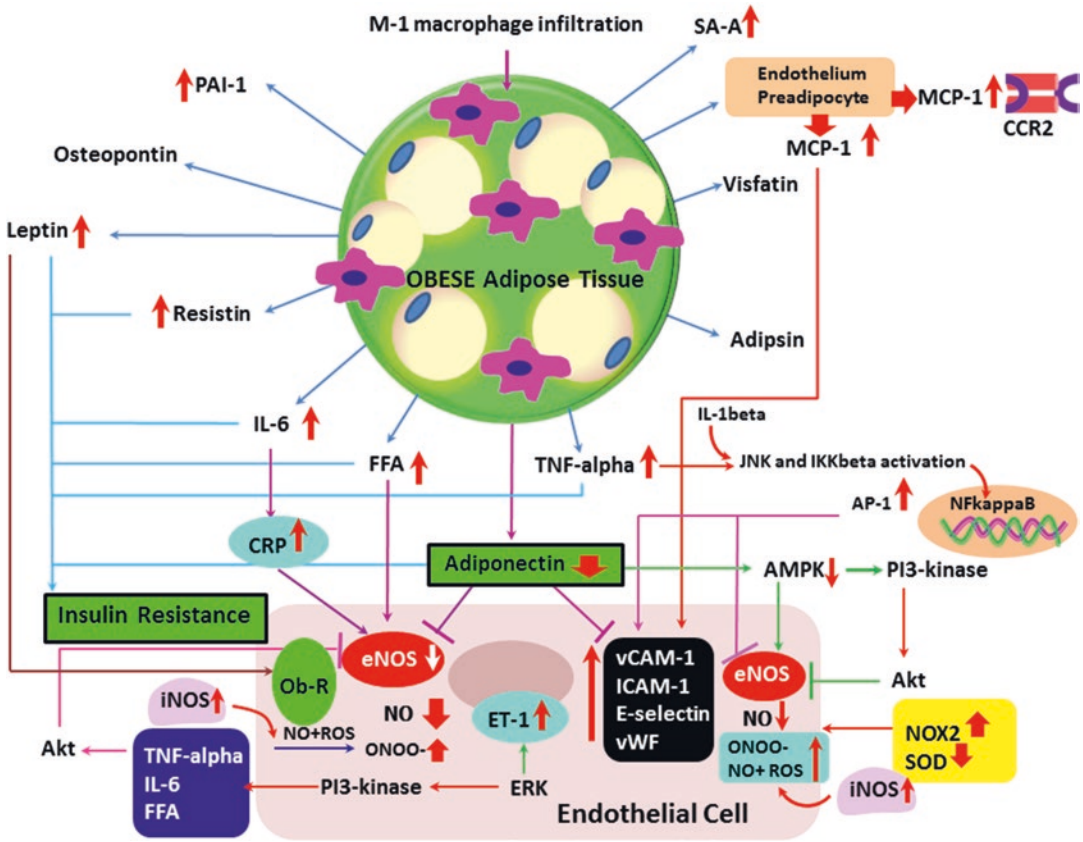

Fig. 15.1 Several highly active molecules released by adipocytes as well as by inflammatory cells infiltrate into adipose tissue. While all of these molecules may cause low grade chronic inflammation, they may also affect vascular endothelial function by modulating the balance between NO synthesis and reactive oxygen radicals (*PAI-1* plasminogen activator inhibitor-1, *SA-A* serum amyloid A, *MCP-1* monocyte chemoatractant protein-1, *CCR2* chemokine(C-C motif) receptor 2, *IL-6* interleukin-6, *TNF-alpha* tumor necrosis factor-alpha, *CRP* C-reactive protein, *FFA* free fatty acid, *AMPK* 5′ adenosine monophosphate-activated protein kinase, *PI3-kinase* phosphatidylinositol 3-kinase, *Akt* protein kinase B, *NO* nitric oxide, *eNOS* endothelial nitric oxide synthase, *VCAM-1* vascular cell adhesion molecule-1, *ICAM-1* intercellular adhesion molecule-1, *vWF* von Willebrand factor, *ET-1* endothelin-1, *ERK* extracellular signal-regulated kinase, *ONOO–* peroxynitrite, *ROS* reactive oxygen species, *Ob-R* leptin receptor, *NF-kappaB* nuclear factor-kappa B, *IL-1beta* Interleukin-1 beta, *JNK* c-Jun N-terminal kinase, *IKKbeta* inhibitor kappa B kinase-beta, *AP-1* activating protein-1, *NOX2* nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase, *SOD* Superoxide dismutase, *iNOS* inducible nitric oxide synthase)

On the other hand, lipolysis of triglyceride-rich lipoprotein initiates c-Jun N-terminal kinase (JNK)-mediated signaling that drives activating transcription factor 3 (ATF3) complexes towards pro-apoptotic and pro-inflammatory responses through formation of a phospho-c-Jun/ATF3/activating protein-1 (AP-1) binding complex in endothelial cells. Furthermore, a 30-fold induction of the SOD3 gene and 10-fold induction of prostaglandin-endoperoxide synthase 2 occurs during the oxidative stress response. Actually, lipolysis of triglyceride-rich lipoprotein results in an imbalance between mitogen-activated protein-kinase (MAPK)-JNK-nuclear factor-kappaB (NF-kappaB) pathway that favors ATF3/AP-1 mediated inflammation and apoptosis (Aung et al. 2013). Post-prandial triglyceride-rich lipoproteins also induce the phosphorylation of p38 MAPK, cyclic AMP (cAMP) response element-binding protein (CREB) and an endogenous inhibitor of NF-kappaB-alpha (IKB-alpha) and increase the DNA binding activity of CREB, calcineurin-nuclear factor of activated T-lymphocytes (NFAT) and NF-kappaB in endothelial cells from type IV hyperlipidemic patients. Furthermore, these lipoproteins upregulate the expression of several pro-inflammatory genes including vascular cell adhesion molecule-1 (VCAM-1), platelet endothelial cell adhesion molecule-1 (PECAM-1), endothelial cell leukocyte adhesion molecule-1 (ELAM-1), intercellular adhesion molecule-1 (ICAM-1), P-selectin, MCP-1, IL-6, toll-like receptor 4 (TLR-4), CD40, a disintegrin and metalloproteinase with thrombospondin motifs 1 enzyme gene (ADAMTS1) and PAI-1 during the post-prandial period (Norata et al. 2007). Additionally, nucleotide-binding oligomerization domain (NOD)-like receptor family pyrin domain-containing 3 (NLRP3) inflammasome is a multiprotein complex that activates caspase 1, leading to the processing and secretion of the pro-inflammatory cytokines, IL-1beta and IL-18 (Tschopp and Schroder 2010). Moreover, adipocyte-derived visfatin significantly increases caspase-1 activity and IL-1beta release from microvascular endothelial cells, subsequent to the activation of NLRP3 inflammasomes. The formation and activation of NLRP3 inflammasomes by visfatin may be an important initiating mechanism to turn on the endothelial inflammatory response leading to endothelial dysfunction during the early stage of obesity (Xia et al. 2014). Virtually, the NLRP3 inflammasome senses obesity-associated danger signals and contributes to obesity-induced inflammation and insulin resistance. Thus elimination of NLRP3 in obese animals reduces IL-18 and adipose tissue interferon-gamma (IFN-gamma) expression in addition to the decrease in the effector T cell numbers in adipose tissue (Vandanmagsar et al. 2011). NLRP3 inflammasome activation and a Th-1 shift in the T cell population of abdominal subcutaneous adipose tissue from obese subjects is related to insulin resistance and impaired glucose homeostasis, which is attributed to the adipose tissue inflammatory processes (Goossens et al. 2012). High fat diet caused disruption of tight and adherens junction proteins in coronary arterial endothelium is accompanied by the activation of endothelial NLRP3 inflammasome, which enhances expression of high mobility group box protein 1 (HMGB1), and T cell infiltration and adhesion in the coronary arterial walls (Chen et al. 2015). Confocal microscopic analysis demonstrated that hypercholesterolemia markedly increases caspase-1 activity and HMGB1 expression in coronary arterial endothelium of NLRP3 (+/+) animals. Thereby, in addition to inflammatory actions, activation of endothelial NLRP3 inflammasome directly impairs endothelial function (Zhang et al. 2015).

Actually, the increased accumulation of lipid components in obesity causes mild inflammatory state in adipose tissue. Indeed, human adipose tissue is a potent source of classic inflammatory cytokines like PAI-1, MCP-1, IL-8, IL-6, macrophage migration inhibitory factor (MIF), VEGF, transforming growth factor beta-1 (TGF-beta1), prostaglandin E2 (PGE2), TNF-alpha, IL-1beta, IL-10 and C-reactive protein (CRP), however the majority of these cell signaling proteins are released from non-fat cells (Fain 2006).

In physiological conditions, endothelial cells resist to leukocyte adhesion. When the endothelial monolayer becomes inflamed, endothelial

cells begin to express on their surface selective adhesion molecules that mediate the attachment of circulating various classes of leukocytes to endothelial cell membrane (Libby et al. 2002). Transcription of E-selectin or ELAM-1, VCAM-1, and ICAM-1 is induced by the inflammatory cytokines, IL-1beta and TNF-alpha (Collins et al. 1995). Some of the inflammation mediators, which are originated from inflamed endothelial cells such as NO, PGE1 or PGE2, fibronectin, ICAM-1, P-selectin, E-selectin, integrin and von Willebrand factor (vWF) promote platelet adhesion and activation (Badimon et al. 2011). These stimuli induce platelet deposition that mostly intervenes in the progression of atherosclerosis rather than thrombotic complications. On the other hand, endothelial secretion of these chemotactic substances and subsequent expression of adhesion receptors favour monocyte and T-cell recruitment, adhesion, and transmigration into the arterial wall (Badimon et al. 2011).

Adipose tissue macrophages participate in inflammatory pathways that are activated in adipose tissues of obese individuals. The levels of macrophage-derived MCP-1 are positively related to the levels of CRP or IL-6 and homeostasis model assessment of insulin resistance (HOMA-IR) index, and negatively related to the levels of high density lipoprotein (HDL)-cholesterol in obese subject (Kim et al. 2006b). Actually MCP-1 is a pro-inflammatory chemokine and mainly is produced by macrophages and endothelial cells in response to modified lipoproteins (Packard et al. 2009). Meanwhile, MCP-1 is also produced by human adipocytes and may be involved in obesity-related health complications (Christiansen et al. 2005). In obese patients, intensity of macrophage infiltration is in proportion to adipocyte size or to roughly body mass index (Compher and Badellino 2008). MCP-1 exerts its effect by binding to C-C chemokine receptor type 2 (CCR2) on the surface of migrating monocytes and directs the migration and diapedesis of adherent monocytes like other chemoattractant factors (Packard et al. 2009). Further, it is associated with a low-grade systemic inflammatory reaction which is often found in the metabolic syndrome (Kim et al. 2011). Peroxisome proliferator-activated receptor-gamma (PPAR-gamma) agonist improves adipocyte function, restores insulin sensitivity, and inhibits atherosclerosis by decreasing the number of lipid-loaded macrophages. In this case, increased interleukin-1 receptor-associated kinase-3 (IRAK3) and decreased MCP-1 expressions indicate a switch from M1 to M2 macrophages and provide a protection against atherosclerosis (Hulsmans et al. 2013). A positive association has been estimated between the extent of coronary artery disease and visceral fat areas. Increased visceral adiposity is significantly associated with higher circulating levels of lipocalin-2 and MCP-1 (Lee et al. 2010). Furthermore, serum lipocalin-2 levels are positively correlated with body fat content and independently associated with visceral fat area (Luo et al. 2016). When the genetically C receptor-deficient and MCP-1-deficient animals are fed with a high cholesterol diet, genetic absence of MCP-1 in addition to the LDL-receptor-deficiency causes dramatic decrease in atherosclerotic disease with the marked inhibition of monocyte recruitment (Gu et al. 1998). Virtually, adipose tissue macrophages are responsible for almost all adipose tissue TNF-alpha expression as well as significant amounts of inducible nitric oxide synthase (iNOS) and IL-6 expressions (Fain et al. 2004; Weisberg et al. 2003). In particular, TNF-alpha not only enhances the monocyte adhesion to the vessel wall by increasing MCP-1 expression, but also transforms the monocytes to macrophages by stimulating macrophage colony stimulating factor (M-CSF) (Lyon et al. 2003). M-CSF stimulation also leads to increased expression of scavenger receptors by macrophages. These receptors are pattern-recognition units involved in innate immunity and engulf modified lipoproteins and apoptotic bodies through receptor-mediated endocytosis (Packard et al. 2009). It is thought that developmental modifications of perivascular adipose tissue by maternal high-fat diet exposure promotes atherosclerosis in adult offsprings. Markedly elevated mRNA expression and protein levels of M-CSF in offsprings of high fat diet-fed dams demonstrate that increased

M-CSF expression contributes to the augmented accumulation of macrophages and exaggerates atherosclerosis development. These processes are followed by the enhanced perivascular adipose tissue proinflammatory response (Wakana et al. 2015).

Furthermore, TNF-alpha also stimulates expression of ICAM-1 and VCAM-1 on the surface of the endothelial cells by activating NF-kappaB (Chudek and Wiecek 2006; Lyon et al. 2003). Compared with non-obese women, obese women have increased basal concentrations of TNF-alpha, IL-6, P-selectin, ICAM-1 and VCAM-1 (Ziccardi et al. 2002). Upregulated expression of adhesion molecules in macrophages and endothelial cells in obese visceral adipose tissue suggest that interactions between these cells contribute to local activation of inflammatory processes. Thus, increased vascular permeability in obese visceral fat could be normalized by anti-ICAM-1 treatment (Nishimura et al. 2008) (Fig. 15.1). Similarly, anti-TNF-alpha antibody pre-treatment blocks the loss of uncoupling protein 2 (UCP-2) expression within the aorta and relieves vascular damage in animals under high-fat diet. In addition, the pretreatment with iNOS inhibitors prevents TNF-alpha-induced UCP-2 reduction in vascular cells and attenuates vascular damage (Gómez-Hernández et al. 2014). Actually, chronic inflammation in obese adipose tissue is linked to endoplasmic reticulum stress and systemic insulin resistance. Fatty acid binding protein directly regulates intracellular free fatty acid levels and indirectly controls macrophage inflammation and endoplasmic reticulum stress by regulating the expression of UCP2 (Xu et al. 2015).

On the other hand, stimulation of endothelial cells with TNF-alpha results in nuclear accumulation of the p50 and p65 components of NFkappaB. Endothelial cells also express an inhibitor of NFkappaB inhibitory protein, IkappaB-alpha. Actually, IkappaB is functionally convenient in cytokine-induced E-selectin expression. TNF-alpha and p65-mediated expression of an E-selectin promoter-reporter gene is blocked by the overexpression of IkappaB-alpha in endothelial cells. This autoregulatory mechanism of NF-kappaB-I kappa B loop ensures a continuous maintenance of cytoplasmic NF-kappaB complexes in endothelial cells (Read et al. 1994). Eventually, activation of NF-kappaB plays an important role in mediating endothelial dysfunction, in part via stimulation of oxidative stress, in peripheral arteries of non-diabetic adults with elevated total and abdominal body fatness, and chronic low-grade inflammation (Pierce et al. 2009). In particular, obese adults demonstrate increased evidences of endothelial oxidative stress. Subsequent ET-1 and NFkappaB protein expression also elevates in obese compared with lean adults (Silver et al. 2007). Consequently, NF-kappaB and its inhibitory protein regulates the vascular endothelial cell function in obese individuals by creating a tightly controled system.

White adipose tissue is the most well-known type of fat in which triglyceride is stored. These stored lipids are mobilized for systemic utilization when other tissues require energy (Shoelson et al. 2007). Originally, even considered to be a passive depot for energy storage, white adipose tissue is known to secrete a variety of substances that regulate metabolic homeostasis (Fried et al. 1998). Several highly active molecules released abundantly by adipocytes like leptin, resistin, adiponectin or visfatin, as well as some more classical cytokines are released by inflammatory cells infiltrating into the adipose tissue. While all of these molecules may act on immune cells leading to local and generalized inflammation, they may also affect vascular endothelial function by modulating vascular NO and superoxide release in obesity-related vascular disorders (Guzik et al. 2006).

At large, classical cytokines; IL-6 and TNF-alpha are expressed and secreted by human adipose tissue. However, visceral adipose tissue releases 2–3 times more IL-6 than the subcutaneous adipose tissue. The most consistent relationship between cytokine expression and obesity-related insulin resistance involves increased TNF-alpha secretion from adipose tissue and increased plasma IL-6 levels (Fried et al. 1998; Kern et al. 2001). Basal release of IL-6 is greater for preadipocytes than differentiated

adipocytes, either derived from subcutaneous or omental fat depots (Antunes et al. 2006). Increase in low-grade inflammation markers, CRP, IL-6, serum amyloid A (SAA), soluble intercellular adhesion molecule-1 (sICAM-1), soluble vascular cell adhesion molecule-1 (sVCAM-1) indicate that the obesity and metabolic syndrome is a risk factor for both the severity of coronary artery disease and of peripheral arterial diseases (Jacobs et al. 2009). Human visceral adipose tissue is an important contributor to the elevated plasma PAI-1 levels in central obesity (Alessi et al. 1997). PAI-1 is one of the serine protease inhibitors and is a key regulator of fibrinolysis. Abdominal accumulation of visceral fat is an independent predictor of plasma PAI-1 activity (Cigolini et al. 1996). Increased PAI-1 levels are associated with insulin resistance and atherothrombosis. Thus, higher PAI-1 increases both atherosclerotic risk and atherothrombotic risk (Kohler 2002). Virtually, moderate hyperglycemia can result in increases of proinflammatory markers, ICAM, VCAM, IL-6, E-selectin, P-selectin activation, reduced fibrinolytic balance or increased PAI-1 levels, and endothelial dysfunction in obese individuals (Perkins et al. 2015). PAI-1 is positively associated with body mass index, waist circumference, waist/hip ratio, total cholesterol, triglyceride, LDL, fasting plasma glucose, fasting insulin, systolic blood pressure and diastolic blood pressure. However, PAI-1 is negatively associated with HDL levels in obese patients (Wei et al. 2013).

CRP is a well-known acute phase reactant and considered as a marker of IL-6 action. It has been convincingly shown that CRP causes a decrease in eNOS expression and bioactivity by decreasing stability of eNOS mRNA, especially at higher concentrations. On the other hand, it exerts a direct pro-inflammatory effect by enhancing monocyte adhesion to human endothelial cells (Venugopal et al. 2002). A combination of increased CRP and PAI-1 levels seem to be a strengthening possibility of atherothrombosis in the metabolic syndrome (Devaraj et al. 2003) (Fig. 15.1).

Lipotoxicity may decrease eNOS gene expression and eNOS catalytic activity to ultimately result in endothelial cell dysfunction (Symons and Abel 2013). It has been proposed that the elevated levels of 18-carbon free fatty acids observed in obesity may inhibit eNOS by increasing protein kinase C (PKC) activity in endothelial cells (Davda et al. 1995). Despite normal VEGFR2 phosphorylation, saturated free fatty acid-pretreated endothelial cells have markedly diminished Akt, eNOS, and ERK activation in response to VEGF. Akt-inactivating protein phosphatase 2A (PP2A) activity is eNOS-associated in these cells (Mehra et al. 2014). Thus, ceramide accumulation in diet-induced obesity precipitates vascular dysfunction via increased association between PP2A and eNOS at the plasma membrane. Hence, PP2A inhibitors not only prevent disruption of the Akt-Heat shock protein90 (Hsp90)-eNOS complex in the vasculature, but also preserve arterial function and maintain normal blood pressure in obese mice by attenuating PP2A activation (Bharath et al. 2015). Meanwhile, ceramide-mediated eNOS phosphorylation at Ser1177 and Ser617 impairs nitric oxide-bioavailability, and leads to endothelial dysfunction. The actual underlying mechanism of ceramide mediated-endothelial dysfunction in diet-induced obesity is the PP2A-related disruption of the eNOS/Akt/Hsp90 signaling complex. Indeed, PP2A association with the eNOS/Akt/Hsp90 complex attenuates eNOS phosphorylation by preventing phosphorylation of Akt (Zhang et al. 2012).

Total plasma adiponectin levels and secretion of total subcutaneous adipose tissue adiponectin decrease in obese humans (Kovacova et al. 2012). Actually, adiponectin inhibits lipolysis in adipocytes by suppressing hormone-sensitive lipase activation without altering adipose triglyceride lipase expression. In this case, adiponectin suppresses triglyceride hydrolysis by inhibiting protein kinase A (PKA)-induced hormone-sensitive lipase activation (Qiao et al. 2011). Otherwise, aortic eNOS expression and activity are significantly reduced in adipose triglyceride lipase deficiency (Schrammel et al. 2014). Adiponectin regulates the vascular homeostasis by affecting signaling pathways in endothelial cells and modulating inflammatory responses in the subendo-

thelial space. The effects of adiponectin on the cardiovascular system are partially mediated by the activation of AMPK and cyclooxygenase-2 (COX-2) pathways, thereby endothelial cell apoptosis and TNF-alpha synthesis are reduced, whereas NO production is promoted (Rojas et al. 2014). Furthermore, adiponectin reduces oxidative stress via increasing NO synthesis through both the phosphatidylinositol 3-kinase (PI3K)/AKT and AMPK signaling pathways (Xiao et al. 2011). Conversely, lack of NO production is responsible for decreased adiponectin synthesis, decreased mitochondrial biogenesis, and oxidative mitochondrial damage in adipocytes, in addition, endothelial dysfunction (Koh et al. 2010). Indeed, adiponectin improves endothelial dysfunction through increasing NO production by eNOS phosphorylation, and decreasing NO inactivation by blocking superoxide production in high-fat diet fed animals (Deng et al. 2010). Furthermore, adiponectin protects the endothelium against hyperlipidemic injury by multiple mechanisms, including promoting eNOS activity, inhibiting iNOS activity, preserving bioactive NO, and attenuating oxidative/nitrative stress (Li et al. 2007). Consequently, in addition to the inhibition of lipolysis in adipocytes, adiponectin prevents human endothelial cell apoptosis through the stimulation of endothelial NO production (Chen et al. 2003; Lin et al. 2004). On the one hand, the adiponectin receptor 1 (AdipoR1) decreases the saturated fatty acid-induced apoptosis by enhancing fatty acid metabolism. On the other hand, AdipoR1 expression increases the gene expression of cytochrome C oxidase, PPAR-alpha, and decreases the gene expression of PGC1alpha and AMPKalpha in hepatocytes under palmitate treatment (Chou et al. 2014). In contrast, saturated fatty acid-induced endoplasmic reticulum stress leads to adiponectin resistance. However, saturated fatty acid induces autophagy subsequent to endoplasmic reticulum stress and this confers a prosurvival effect against lipotoxicity-induced cell death (Park et al. 2015).

The beta3-adrenergic receptor (beta3-AR) gene, which is predominantly expressed in adipose tissue, involves in lipolysis and thermogenesis, so its impairment may lead to visceral fat accumulation, obesity and the metabolic syndrome (Emorine et al. 1994; Walston et al. 2003). In the rat thoracic aorta, beta3-ARs are mainly located on endothelial cells, and act in conjunction with beta1- and beta2-adrenoceptors to mediate relaxation through activation of NOS pathway (Trochu et al. 1999). Furthermore, follow-up study in 496 obese individuals indicated that the prevalence rate of metabolic syndrome is much higher in Arg64 carrier group than that in Trp64 differences in the adiposity. It is concluded that the mutation of beta3-AR gene is the independent risk factor for the prevalence of metabolic syndrome in Arg64 carrier subjects (Zhu et al. 2010). Diet-induced obesity leads to brown adipose tissue inflammation and insuln resistance. In this manner calorie consuming capacity of brown adipose tissue by beta3-AR-mediated thermogenesis and regulation of energy balance are markedly impaired (Roberts-Toler et al. 2015).

4 Nitric Oxide and Endothelial Dysfunction in Obesity

Actually impaired NO-dependent vascular function of endothelium due to biochemical injury is called endothelial dysfunction (Anderssohn et al. 2010). Thus, high concentration of LDL modifies the antithrombotic properties of the vascular endothelium and change vessel contractility by reducing the availability of endothelial NO and activating pro-inflammatory signaling pathways (Badimón et al. 2009). NO synthesis and nitrosative stress are increased in severely obese subjects and are correlated with abdominal obesity, oxidative stress and inflammatory markers (Codoñer-Franch et al. 2011a). Indeed, obesity is an independent risk factor for the development of endothelial dysfunction which is primarily depended on the reduced bioavailability of the signaling molecule, NO. The balance between NO synthesis and ROS is the major determining factor for the effectiveness of NO on the endothelial cells (Williams et al. 2002). In any way, the endothelial dysfunction in obesity could mainly be attributed to decrease in NO availability. In

this respect, the major cause of the endothelial dysfunction in obesity is reduced NO-bioavailability due to excess ROS generation. Therefore, endothelial dysfunction has been defined as impairment of endothelium-dependent vasodilation caused by a loss of NO activity on the vessel wall (Davignon and Ganz 2004). On the other hand, besides the eNOS, AMPK and mTOR participate in the regulation of vascular function. A high-fat diet led to a downregulation of AMPK and eNOS in the periaortic adipocytes with a concurrent upregulation of mTOR. Thus, perivascular adipose tissue impacts obesity-related vascular dysfunction and remodeling through impairment of eNOS-mediated vasodilatation and the AMPK/mTOR pathway (Ma et al. 2010). Vascular AMPK, as a dual sensor for energy and redox status, plays a critical role in the regulation of blood flow and vascular tone by stimulating NO release in endothelial cells. Thereby, obesity leads to endothelial damage via AMPK dysregulation (García-Prieto et al. 2015). In fact, baseline NO production of the obese patients is not different from that of the healthy controls. However, in the extremely obese subjects, over-expression of iNOS may be one of the possible mechanisms for the peroxynitrite formation (Lin et al. 2007). In reality, obesity has been strongly associated with increased synthesis of ROS and reactive nitrogen species (RNS). The reaction of superoxide with NO produces peroxynitrite. Eventually, enzymes, nucleic acids, proteins, and carbohydrates are destroyed by excess free radicals (Codoñer-Franch et al. 2011b). In fact, NO is produced in high enough concentrations by iNOS. Excess NO contributes to formation of peroxynitrite by out-competing the SOD. Hence, the direct toxicity of excess NO is greatly enhanced by reacting with superoxide to form peroxynitrite (Beckman and Koppenol 1996). In general, NO is synthesized by three different isoforms of NOS (Kone et al. 2003). In addition to direct production via NOS enzymes, NO can be produced endogenously from the more oxidized nitrogen oxide, nitrite. Xanthine oxidoreductase-mediated one-electron reduction of nitrite to NO is increased under hypoxic conditions in the presence of NADH (Millar et al. 1998). eNOS is predominantly expressed in endothelial cells and is also subject to rapid regulation by Ca^{2+}/calmodulin, whereas iNOS is expressed by macrophages and cells of macrophage/monocyte lineage subjected to cytokines or other proinflammatory stimuli. Despite the structural similarity of the three NOS isozymes, the influence of L-Arginine and tetrahydrobiopterin (BH4) on their formation and stability differ markedly (Kone et al. 2003; Rizzo et al. 2010). Activation of the transcription factors; NFkappaB and signal transducer and activator of transcription (STAT)-1alpha are followed by the activation of iNOS promoter, which is an essential step in the regulation of iNOS expression in most cells (Kleinert et al. 2004). The TNF-alpha and iNOS expressions are enhanced in the vascular wall of obese patients, together with the increased vascular superoxide and NO generation. Increased TNF-alpha production reduces NO-availability by promoting superoxide generation via NOX2 and iNOS activation (Virdis et al. 2011). Additionally, early feature of obesity is a blunting of acetylcholine-mediated vasodilatation. This effect is compensated by ROS-mediated vasodilatation. As obesity progressed, there is an increase in vascular NO synthesis due partly to iNOS activity. Since inflammation plays a key role in the development and progression of atherosclerosis, the expression of iNOS may be considered as one of the obesity-related inflammatory changes. As mentioned above, simultaneously produced ROS and NO may lead to peroxynitrite overproduction. Eventually, obesity associated endothelial dysfunction is developed by the newly formed peroxynitrite (Noronha et al. 2005). Indeed, endothelium-dependent vasodilation is reduced by 40% in obese individuals (Steinberg et al. 1996). The improper response of the endothelial cells in endothelial dysfunction is dependent on either decreased bioavailability of the vasodilator NO or in some cases an increase in the production of constrictor factors by the endothelium. NO-bioavailability represents the balance between the amounts of NO produced by eNOS and the amount of active NO scavenged by ROS (Belin de Chantemele and Stepp 2012). Production of ROS from the NOX2

leads to oxidation of BH4 and uncoupling of eNOS. This promotes the oxidative inactivation of NO with subsequent formation of peroxynitrite (Bitar et al. 2005). eNOS is dually acylated by the saturated fatty acids and is directed to the endothelial cell membrane and caveolae (Michel and Feron 1997). Peroxynitrite selectively targets and disrupts endothelial caveolae, which contributes to eNOS uncoupling, and, hence, reduced NO-mediated vasodilation (Cassuto et al. 2014). Given a high-fat diet, while the body weight is increased, eNOS protein-mRNA expression and ROS decrease urinary nitrites excretion and plasma BH4 concentration (Gámez-Méndez et al. 2014). Inactivation of eNOS and the subsequent decrease in NO bioavailability is related to the sequestration of NO by ROS. During obesity, the uncoupling of eNOS is developed by reduction in BH4 availability (Gamez-Mendez et al. 2015). Consequently, NOX2 and uncoupled eNOS are responsible for the increase in vascular oxidative stress, which is likely to be involved in the endothelial dysfunction (Haruna et al. 2006).

As a matter of fact, insulin resistance and insulin-related NO production is the subject of debate in endothelial dysfunction. The endothelial NO is produced by the eNOS from the semi-essential amino acid L-Arginine in the presence of oxygen and multiple co-factors such as NADPH, flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), and BH4 (Yang and Ming 2013). However, the NO-producing activity of eNOS is diminished in some metabolic diseases. In particular, the early onset of vascular inflammation in diet-induced obesity is accompanied by reduced NO production and impaired insulin-induced phosphorylation of Akt and eNOS (Kim et al. 2008). In these subjects, inhibited insulin signal transduction and blocked insulin-stimulated NO production are dependent on both TLR4 and NF-kappaB activation (Kim et al. 2007). Lower arterial eNOS phosphorylation and vascular dysfunction following high fat diet are the results of free fatty acid-mediated impairment of eNOS phosphorylation rather than defective upstream signaling via Akt (Symons et al. 2009). In contrast, animals overexpressing eNOS are resistant to diet-induced obesity and hyperinsulinemia. Adipose tissue from these animals shows higher levels of PPAR-alpha and PPAR-gamma gene expression, elevated abundance of mitochondrial proteins, and a higher rate of oxygen consumption (Sansbury et al. 2012). As it is seen from the above findings, NO production is reduced as a consequence of eNOS uncoupling which depends on the pre-existing oxidative stress in diet-induced obesity (Li and Förstermann 2013). Virtually the mechanisms of eNOS-uncoupling are multifactorial (Kietadisorn et al. 2012). In addition to intracellular Ca^{2+} concentration, eNOS primarily requires co-factor BH4 for enzyme activity. Deficiency in BH4 or inactivation of BH4 by oxidative stress has been shown to destabilize eNOS dimer and decreases NO production. Loss or oxidation of BH4 to 7,8-dihydrobiopterin (BH2) is associated with eNOS uncoupling, resulting in the production of superoxide rather than NO. Dihydrofolate reductase (DHFR) can recycle BH2, and thus regenerate BH4. It is therefore likely that net BH4 cellular bioavailability reflects the balance between de novo BH4 synthesis, loss of BH4 by oxidation to BH2, and the regeneration of BH4 by DHFR (Crabtree and Channon 2011).

Extracellular L-Arginine, but not intracellular L-Arginine, is the major determinant of NO production in endothelial cells. At once it is transported inside the cell, L-Arginine can no longer gain access to the membrane-bound eNOS. In this respect, intracellular L-Arginine concentration should not be considered as a reference point for "L-Arginine paradox" (Shin et al. 2011). In this manner, exogenous L-Arginine is channeled to eNOS to produce NO. If there is an excessive arginase activity in the vicinity of eNOS, insufficient NO may be produced due to relative L-Arginine deficiency (Topal et al. 2006). In humans and mammals, there are two isoforms of arginases: arginase-1 and arginase-2 (Yang and Ming 2013). Endothelial NO synthesis depends on the activity of cell membrane L-Arginine carriers and mitochondrial arginase-2 through two types of L-Arginine pools. The freely exchangeable pools of L-Arginine are caveolae, whereas the non-exchangeable ones are mitochondria. The arginase activity is associated with significant

constitutive expression of both mRNA and protein of only arginase-2. This means that the mitochondria participate in intracellular L-Arginine compartmentalization (Topal et al. 2006). The fraction of eNOS is localized in caveolae. Thereby, eNOS can interact with the caveolae coat protein, caveolin-1. Caveolin-1 is necessary for negative regulation of eNOS function (Sowa et al. 2001). Consumption of L-Arginine due to increased expression of arginase competitively reduces eNOS-derived NO. Cytosolic arginase-1 and mitochondrial arginase-2 reduce eNOS activity equally regardless of where in the cell eNOS is expressed (Elms et al. 2013). In addition to increased arginase activity, competition of excess endogenous asymmetric dimethyl-L-arginine (ADMA) with L-Arginine for eNOS binding is evident. Moreover, S-glutathionylation of eNOS are the other mechanisms of eNOS uncoupling (Förstermann and Sessa 2012). Coincidental plasma concentrations of ADMA are associated with impaired endothelium-dependent, NO-mediated vasodilation (Böger et al. 1998). Virtually, elevations of all three methylarginines; ADMA, symmetrical dimethylarginine, *N*-monomethyl-L-arginine may amplify insulin resistance and endothelial dysfunction in obesity (Marliss et al. 2006). Particularly, higher plasma ADMA levels and resultant decrease in L-Arginine/ADMA ratio are risk factors for endothelial dysfunction in humans (Böger et al. 1998). Consistent with the metabolic syndrome, ADMA levels are also positively correlated with fasting triglyceride levels. Increase in plasma ADMA concentration may contribute to the endothelial dysfunction, which is also observed in insulin-resistant patients (Stühlinger et al. 2002).

As mentioned above, oxidative stress has been shown to convert eNOS from a NO-producing enzyme to an enzyme that generates superoxide radicals. Additionally, S-glutathionylation of eNOS reversibly decreases eNOS activity with an increase in superoxide radical generation primarily from the reductase. Actually two glutathionylated cysteine residues are identified within the reductase domain. Under oxidative stress, S-glutathionylation occurs through thiol-disulphide exchange with oxidized glutathione or reaction of oxidant-induced protein thiyl radicals with reduced glutathione. In this manner, cysteine residues are critical for the maintenance of eNOS function (Chen et al. 2010).

Actually ROS-mediated eNOS uncoupling is a major source of NO reduction, however, ROS can also directly affect NO release by inhibiting the dimethyl-arginine dimethylaminohydrolase (DDAH) activity, which metabolizes ADMA to L-citrulline and dimethylamine. Thus, inhibition of DDAH causes ADMA accumulation and subsequent suppression of NO synthesis (Lin et al. 2002). Furthermore, serum ADMA levels are found to be associated with the endothelial function and vascular oxidative stress in patients with coronary artery disease. In these cases, serum ADMA is correlated with, and is an independent predictor of, vascular superoxide generation. Although ADMA has no effect on NADPH-stimulated superoxide in intact human vessels, it induces eNOS uncoupling, either through substrate reduction or through a direct effect on eNOS catalysis (Antoniades et al. 2009). ADMA or native LDL cholesterol increases endothelial oxidative stress to the same degree. This effect of ADMA is simultaneous with the activation of the redox-sensitive transcription factor NFkappaB (Böger et al. 2000a). Virtually, ADMA is formed in human endothelial cells by redox-sensitive methylating enzymes, *S*-adenosylmethionine-dependent protein-arginine *N*-methyltransferases. Oxidative stress induced by oxLDL or by TNF-alpha reduces DDAH activity. Finally, oxidative stress is increased during the atherogenesis and thus may contribute to the elevation of ADMA concentration and subsequently reduced biological activity of endothelium-derived NO (Böger et al. 2000b; Ito et al. 1999). Indeed, the dose-dependent TNFalpha-induced ADMA accumulation is significantly inhibited when co-stimulated with insulin or adiponectin in endothelial cells. In this manner, considerable increase in DDAH activity occurs. This result indicated that the TNFalpha-induced accumulation of ADMA is achieved through the DDAH pathway (Eid et al. 2007).

Cationic amino acid transporter-1 (CAT-1) is a novel interendothelial cell adhesion molecule.

CAT-1 proteins are distributed on the entire surface of cells and function as L-Arginine transporters, but most of the CAT-1 are localized at interendothelial junctions and serve as cell adhesion molecules. Extracellular L-Arginine exposure stabilizes endothelial integrity via decreasing the cell junction disassembly of CAT-1 and blocking the cellular membrane CAT-1 internalization. This mechanism is defined as L-Arginine paradox. Eventually, CAT-1 directly regulates endothelial integrity and mediates the protective actions of L-Arginine to endothelium via a NO-independent mechanism (Guo et al. 2015). High-fat diet enhances arginase-2 activity and p38MAPK activity, which is associated with eNOS-uncoupling. Obese animals show decreased endothelium-dependent relaxations to acetylcholine despite of the higher eNOS protein level. Furthermore, overexpression of Arg-2 in human endothelial cells cause eNOS-uncoupling and augmented p38MAPK activation (Yu et al. 2014).

In brief, increased vascular endothelial expression of NOX2-p47(phox) and ultimate endothelial oxidative stress with selective compensatory upregulation of antioxidant enzymes and Ser1177-phosphorylated eNOS may occur in overweight and obese adults (Silver et al. 2007). Activation of TLR4 promotes the transcription of NOX2 1 and 4, resulting in elevated reactive oxidative stress. In endothelial cells, the increased level of ROS reduces eNOS coupling leading to a reduced NO-production and bioavailability, impairs endothelium-derived hyperpolarization-mediated responses, and increases the activity of COX-1 with augmented endothelium-derived contracting factors (EDCF)-mediated contractions. All these effects contribute to the initiation of endothelial dysfunction in arteries of obese and diabetic animals (Liang et al. 2013).

Endothelial dysfunction is observed in morbidly obese individuals only when the insulin resistance exists. Thus in obese-insulin resistant cases, elevated serum IL-6, and TNF-alpha levels enhance mitochondrial superoxide generation in addition to the systemic inflammation (El Assar et al. 2013). Indeed, potential enzymatic sources of ROS are the mitochondrial electron transport chain, xanthine oxidase, cyclooxygenase, lipoxygenase, NOS, heme oxygenase, peroxidases and NADH oxidases. However, in the endothelium, the NOX2s represent the primary source of ROS. NOX2 isoforms are expressed in the endoplasmic reticulum of endothelial cells. In the perinuclear membranes, ROS modulates redox sensitive signaling pathways (Cave et al. 2006). NOX2 activity occurs in response to saturated fatty acid overload. Fatty acid-induced NOX2 activation is dependent on the activation of classical PKC. Autophagic turnover is impaired by a lipotoxicity-linked mechanism via PKC-NOX2 pathway (Jaishy et al. 2015). NOX2s have important role in eNOS uncoupling, and PKC signaling in mediating increased vascular superoxide production and endothelial dysfunction (Guzik et al. 2002). However, in obesity NADPH overexpression is not the only triggering factor of endothelial dysfunction but also insulin resistance. Its consequent metabolic disturbances are the underlying risk factor driving vascular dysfunction. Despite persistent obesity, correction of peripheral insulin resistance improves NO functions and reduced NADPH subunits expression (Ali et al. 2009).

5 Insulin Resistance and Endothelial Dysfunction in Obesity

Microvascular insulin resistance is an early event in diet-induced obesity (Zhao et al. 2015). Regression analysis revealed a significant negative correlation between body mass index, insulin resistance estimated by HOMA and percent peak increase in lower forearm blood flow during acetylcholine infusion in obese individuals. In contrast, endothelium-independent vasodilation is unaffected from acetylcholine infusion. This test confirms the presence of an endothelial dysfunction in obese subjects who have a corresponding decrease in NO production (Perticone et al. 2001). In insulin-resistant states, activation of PKC by high concentrations of glucose, non-esterified fatty acids, intracellular lipid diacylglycerol (DAG) and increased superoxide production from NOX2 may result in downregu-

lation of eNOS expression and alter vascular cell signaling (Rask-Madsen and King 2005). Actually, the activation of PKC in the vascular tissues of insulin resistant subjects may inhibit the PI3K activity and eNOS expression, thereby decrease of NO production leads to endothelial dysfunction (Kuboki et al. 2000). On the other hand, insulin receptor tyrosine kinase, IRS-1 and phosphoinositide-dependent kinase-1 (PDK-1) plays necessary role in insulin-signaling pathways leading to activation of eNOS. The classical Ca^{2+}-mediated pathways for activation of eNOS are separable from IRS-1- and PDK-1-dependent insulin-signaling pathways (Montagnani et al. 2002b). Insulin-induced tyrosine phosphorylation of IRS-1/2, PI3K activity, and serine phosphorylation of Akt/PKB are impaired in the vasculature of the insulin-resistant obese animals. In these subjects, vasodilatory and anti-atherogenic functions of insulin are diminished, whereas pro-atherogenic actions mediated through MAPK cascade may be increased due to hyperinsulinemia (Jiang et al. 1999). PI3K-dependent insulin signaling pathway-specific impairment in insulin action contributes to reciprocal relationships between endothelial dysfunction and insulin resistance (Kim et al. 2006a). Furthermore, metabolic impairment of PI3K-dependent signaling in endothelium may cause imbalance between production of NO and secretion of ET-1. This derangement leads to decreased blood flow that worsens insulin resistance (Fig. 15.1).

Protein tyrosine phosphatase 1B (PTP1B) is a negative regulator of the leptin and insulin signaling pathways. The importance of PTP1B related to obesity is confirmed by a deletion of PTP1B gene (Cho 2013). It is predominantly located on the cystosolic surface of the endoplasmic reticulum. PTP1B not only dephosphorylates the insulin receptor that has been activated by insulin, but also regulates the insulin receptor precursor during its biosynthesis (Boubekeur et al. 2011). Increases in the activity and/or expression of PTP1B correlate with blunted insulin signaling in obese animals. The expression levels of NOX1 and its regulatory enzymes NOXa1/NOXo1 in the vasculature correlate with obesity-induced insulin resistance. In obese mice, the expression of NOX1, NOXa1, and NOXo1 are markedly increased (Ali et al. 2009). In fact, PTP1B is stimulated in the muscle cells by obesity-induced endoplasmic reticulum stress via the activation of the ROS-NFkappaB axis which causes unfolded protein response and mediates insulin resistance (Panzhinskiy et al. 2013). Deletion of insulin-desensitizing enzyme PTP1B enhances insulin sensitivity without the weight gain due to a second action of PTP1B as a negative regulator of leptin signaling (Koren and Fantus 2007). In contrast, adipocyte-specific PTP1B deletion reduces leptin sensitivity and increases basal lipogenesis, decreases insulin receptor and Akt/PKB phosphorylation, increases lipogenic gene expression and increases hypoxia-induced factor-1-alpha (HIF-1alpha) expression in high-fat diet (Owen et al. 2012). High-fat diet feeding significantly impairs endothelium-dependent vasorelaxation in response to acetylcholine in aortas from control mice. By contrast, liver-specific PTP1B-deficiency fully protects against high-fat diet-induced endothelial dysfunction. This is associated with alterations in eNOS phosphorylation and enhanced levels of prostacylin, a vasorelaxant metabolite (Agouni et al. 2014). Deletion of PTP1B improves both endothelium dependent and independent NO-mediated dilation and reduces superoxide generation in morbidly obese mice (Ali et al. 2009). Since in the endothelium, insulin promotes NO production, through the insulin receptor/IRS-1/PI3K/Akt/eNOS signaling pathway, an inhibitor of insulin action, a mammalian tribbles homolog (TRIB3), affects insulin action by binding to and inhibiting Akt phosphorylation. The TRIB3 R84 variant impairs insulin signaling and NO production in human endothelial cells (Andreozzi et al. 2008). Akt phosphorylation/activation outside the plasma membrane further requires the adaptor protein containing PH domain, PTB domain and Leucine zipper motif (APPL1). APPL1 interacts with Akt and blocks the association of Akt with its endogenous inhibitor TRIB3 through direct competition (Cheng et al. 2009). PI3Ks cause the accumulation of two lipid-second messengers on the plasma membrane: phosphatidylinositol-

3,4,5-trisphosphate and phosphatidylinositol-3,4-bisphosphate. These two molecules act as binding sites for serine/threonine kinase Akt (Braccini et al. 2015; Vanhaesebroeck et al. 2001). Regulatory subunit of PI3K is a lipid kinase and PI3K consists of two different subunits; while regulatory subunit is responsible for binding to insulin receptor substrates and a key element in the pathway leading to metabolic effects of insulin, catalytic subunit is responsible for phosphorylation of phosphatidylinositols and it is found in cellular membranes (Virkamäki et al. 1999).

In addition to the increase in PKC activity, hyperinsulinemia causes G protein-coupled receptor (GPCR) kinase 2 (GRK2) activation and translocation to the membranes in type 2 diabetic obese animals. Subsequently, GRK2 negatively regulates insulin-induced vascular relaxation via the Akt/eNOS pathway. In this case, the expression of beta-arrestin2 in vessel wall under insulin stimulation decreases. In brief the PKC/GRK2/Beta-arrestin2 pathway underlies in the impairment of the insulin-induced vascular relaxation in type 2 diabetic obese animals (Taguchi et al. 2011). Actually, GRKs participate, together with the beta-arrestins, in the regulation of multiple GPCRs. Beta-arrestin2 acts as a scaffold for the translocation of Akt and Src to the insulin receptor, even though the insulin receptor is not a GPCR (Luan et al. 2009). Insulin-induced relaxations and NOX-/cyclic guanosine monophosphate (cGMP) production are all greatly attenuated in insulin resistant animals. The relaxation responses to insulin and NO production mediated via the PI3K/Akt pathway are also decreased. These alterations seem to be the major causes of endothelial dysfunction (Kobayashi et al. 2004). Reasonably insulin-induced Akt/eNOS signaling is evidently enhanced by pretreatment with GRK2 inhibitor or PKC inhibitor. Eventually, the relaxation response to insulin via the Akt/eNOS signaling pathway is impaired in obesity. In this case, the activities of PKC and/or GRK2 simultaneously increases. In the obese animals, insulin-stimulated Akt and eNOS phosphorylations are reduced, but they are improved by GRK2 inhibitors (Taguchi et al. 2011). In fact, GPCRs are key regulators of cell physiology and control metabolic processes (Thal et al. 2011). The GRK-catalyzed phosphorylation and binding of beta-arrestin 2 to the receptors lead to the functional uncoupling of G proteins and receptors (Jurado-Pueyo et al. 2008). GRKs and beta-arrestins families have a central and coordinated role in the "desensitization" of G protein activation (Reiter and Lefkowitz 2006). In diabetic states, GRK2 is activated and translocated to the membrane in spite of non-GPCR stimulation. However, cytosolic beta-arrestin 2 is not translocated to the membrane even under insulin stimulation. Thereby GRK2 antagonizes the action of beta-arrestin 2 (Taguchi et al. 2015). Luan et al. showed that insulin stimulates the formation of beta-arrestin2 signal complex. Deficiency of this signal complex due to loss or dysfunction of beta-arrestin2 and a consequent disturbances of insulin signaling result in insulin resistance (Luan et al. 2009). In this context, GRK2 levels correlate with endothelial dysfunction. In a state of insulin resistance, vasculature expresses high levels of GRK2 which may induce endothelial dysfunction by reducing intracellular NO. GRK2 activation changes the subcellular localization of GRK2 itself and also of beta-arrestin 2. Alteration in GRK2 activity also leads to the modulation of insulin signals through a GRK2-IRS1 complex (Taguchi et al. 2015). The upregulation of GRK2 and a decrease in beta-arrestin 2 inhibit the insulin-induced stimulation of Akt/eNOS signaling, and that GRK2 overactivation may result from an increase in PKC activity in aortas from diabetic mice with hyperinsulinemia (Taguchi et al. 2011, 2012). In fact, as generated by iNOS, excess NO is cytotoxic, relative to the much lower levels produced by eNOS that activates the soluble guanylyl cyclase (sGC)/cGMP/protein kinase G (PKG) pathway which are cytoprotective (Handa et al. 2011). The effect of the high-fat diet is associated with the early onset of vascular inflammation, which is accompanied by biochemical evidences of endothelial dysfunction, reduced NO production, induction of ICAM-1, VCAM-1 and insulin resistance with the impaired insulin-induced phosphorylation of Akt and eNOS (Kim et al. 2008). Contrarily, overexpres-

sion of vasodilatory-stimulated phosphoprotein (VASP) in endothelial cells blocks inflammation and insulin resistance induced by saturated fatty acids (Cheng et al. 2014). NO/cGMP signaling plays a physiological role to attenuate vascular inflammation induced by nutrient excess, whereas reduced vascular NO-cGMP signaling due to diet-induced obesity is the mechanism underlying vascular inflammation and insulin resistance (Rizzo et al. 2010). VASP is a downstream mediator of the NO/cGMP pathway that is necessary and sufficient to protect against vascular inflammation and insulin resistance. Actually obesity is a state of chronic inflammation and VASP deficiency, thereby the mechanism underlying the anti-inflammatory action of VASP may involve antagonism of the palmitate/TLR4/IKKbeta/NF-kappaB pathway (Cheng et al. 2014).

An inverse correlation has been found between the predominant adducts of circulating advanced glycation end products (AGEs) and adiponectin levels in overweight patients (Del Turco et al. 2011). Elevated free fatty acids, high glucose levels and ROS generated by both NOX2 and the mitochondrial electron transport system are involved in AGE signaling through receptor for advanced glycation endproduct (RAGE) (Basta et al. 2005). Furthermore, the stimulation of endothelial cells by TNF-alpha evokes the following sequence of events in obesity: stimulation of NOX2, generation of ROS, activation of the mitochondrial respiratory chain, stimulation of NFkappaB activity and induction of RAGE expression (Mukherjee et al. 2005). Eventually, increases in TNF-alpha expression in low grade inflammatory process leads to endothelial dysfunction (Picchi et al. 2006). Nevertheless, impaired endothelial function in insulin-resistant obese individuals is secondary to the elevated free fatty acid concentrations (Steinberg et al. 1997). Obese patients with type 2 diabetes display increased plasma levels of free fatty acids bind TLR and activate NFkappaB through degradation of the inhibitory complex IkappaB alpha by IKKbeta-kinase (Kim et al. 2006a). Subsequent to nuclear translocation, NFkappaB triggers inflammation due to up-regulation of inflammatory genes, IL-6 and TNF-alpha. After binding the free fatty acids to TLR, IRS-1 is phosphorylated by JNK and PKC. This process results in down-regulation of IRS-1/Akt and glucose transporter insulin-responsive glucose transporter-4 (GLUT-4). Hence insulin resistance develops and inhibits PI3K and Akt (Paneni et al. 2013). Simply, down-regulation of PI3K/Akt pathway leads to eNOS inhibition and decreased NO production (Du et al. 2006). Impaired insulin sensitivity in the vascular endothelium primarily leads to increased free fatty acid oxidation and ROS formation, subsequently, detrimental biochemical pathways such as AGE synthesis, PKC activation, protein glycosylation as well as down-regulation of prostacyclin (PGI2) are enhanced. These events blunt eNOS activity thereby leading to endothelial dysfunction (Du et al. 2006; Giacco and Brownlee 2010). In this respect, ectopic fat accumulation combined with a low-grade chronic inflammatory state in obesity results in a abnormal glucose, fatty acid and lipoprotein metabolism, increased oxidative stress and endothelial dysfunction (Bhatia et al. 2012). Additionally, hyperglycemia-induced generation of superoxide anion decreases bioavailability of NO to form peroxynitrite which easily penetrates across phospholipid membranes and diminishes intracellular signal transduction (Creager et al. 2003). Protein nitrosylation blunts activity of antioxidant enzymes and eNOS. Hyperglycemia-induced ROS production triggers several major pathways including polyol pathway flux, increased formation of AGEs, increased expression of the receptor for AGEs and its activating ligands, activation of PKC isoforms, and overactivity of the hexosamine pathway (Giacco and Brownlee 2010). All of these pathways are in association with hyperglycemia-induced mitochondrial dysfunction and endoplasmic reticulum stress-promoted ROS accumulation (Fiorentino et al. 2013). Once activated, PKC isoforms have been associated with different structural and functional changes in the vasculature including alterations in cellular permeability, inflammation, angiogenesis, cell growth, extracellular matrix expansion, and apoptosis (Geraldes and King 2010). One of the important consequences of PKC activation is superoxide

production in vascular endothelial cells through NOX2-dependent pathway (Inoguchi et al. 2000). In fact, hyperglycemia, hyperlipidemia and hyperinsulinemia, are all associated with insulin resistance, hexosamine biosynthesis pathway flux and increased O-linked attachment of *N*-acetyl-glucosamine (O-GlcNAc) levels (Fülöp et al. 2007). In particular, increased O-GlcNAc levels in adipocytes decrease insulin stimulated GLUT-4 translocation, reduce insulin-stimulated phosphorylation of IRS-1 and Akt and increase O-glycosylation of GLUT-4, IRS-1 and Akt2 (Park et al. 2005). Phosphorylation of eNOS by Akt is also attenuated by both hyperglycemia and glucosamine. Activation of the hexosamine biosynthesis in human endothelial cells transduces the insulin effect from the membrane receptor to eNOS activation, by increased O-GlcNAcylation. In human endothelial cells, impairment of insulin signaling involves the PI3K/Akt/eNOS pathway, which is induced by high glucose or glucosamine (Federici et al. 2002).

6 Adipose Tissue Hyperoxia Versus Microcirculatory Dysfunction in Obesity

In obesity, excessive adipose tissue is attributed to hypertrophy and hyperplasia of adipocytes; however, their capillary density and function fail to meet the demand of adipose tissue growth. The barrier effect of large adipocytes most probably contributes to the changes in blood flow and oxygen tension in adipose tissue (Ye et al. 2007). Partial oxygen pressure (pO_2) in intercellular spaces between adipocytes of obese individuals is higher than that in lean individuals despite a reduced adipose tissue blood flow. Obese individuals exhibit adipose tissue hyperoxia despite lower adipose tissue blood flow. This paradox is explained by lower adipose tissue oxygen consumption accompanied by insulin resistance, impaired adipose tissue capillarization, and higher adipose tissue inflammation. Nevertheless, the net result of all these events in obese adipose tissue is local hypoxic response (Goossens et al. 2011). Chronic intermittent hypoxia decreases triglyceride rich lipoproteins clearance and inhibits lipoprotein lipase activity by five-fold in obese adipose tissue (Drager et al. 2012). The impaired blood perfusion provokes microcirculatory dysfunction. The hypoxia response in adipocytes and macrophages is one of the important causes of chronic inflammation in visceral obesity (Hosogai et al. 2007; Ye 2011). Hence, the increased HIF-1alpha activity is an indicator of chronic inflammation in adipose tissue during the development of obesity. For this reason, adipose tissue HIF-1alpha activity reflects multiple signals including adipogenesis, insulin and hypoxia in obesity (He et al. 2011). Basically, HIF is a heterodimer and composed of HIF-alpha and -beta, however, three HIF-alpha subunits, HIF-1alpha, -2alpha, and -3alpha, have been identified. Among these, HIF-1alpha subunit represses PPARgamma-2 gene expression and then inhibits adipogenesis. Conversely, HIF-2alpha is induced during adipocyte differentiation and positively regulate the adipogenesis (Hatanaka et al. 2009). Evidences outlined above indicate that adipogenesis leads to increase in VEGF expression through HIF-1alpha. Eventually adipocyte differentiation in obesity elevates the VEGF transcription (Claffey et al. 1992). At the same time, adipocyte hypoxia may lead to the gene expression by activation of NF-kappaB. All of the hypoxia genes are increased in mRNA level, except HIF-1-alpha. This result suggests that expression of inflammatory genes is induced by hypoxia in macrophages (Ye et al. 2007). Actually endothelial cell proliferation is primarily dependent on proangiogenic factor, VEGF which stimulates cell proliferation through VEGF receptor 2 in the endothelial cells. Moreover, activity of platelet-derived growth factor (PDGF) in angiogenesis is dependent on VEGF activity (Pang et al. 2008). Serum PDGF stimulates differentiation of endothelial cell and is secreted by many types of cells, including platelets, macrophages, fibroblasts, and endothelial cells. Particularly, macrophages are one of the major sources of PDGF activity (Mornex et al. 1986). PDGF is expressed in all types of cells which compose adipose tissue; however, their expression levels are different. Preadipocytes express more PDGF than the

mature adipocytes. In obesity, preadipocyte number is reduced, as most of them are differentiated into mature adipocytes. To meet the demand for PDGF, macrophage infiltration into adipose tissue is increased to compensate the loss of preadipocytes (Pang et al. 2008). Taken together all these evidences, during development of obesity macrophages may serve as a stimulator for angiogenesis in adipose tissue. However, PDGF, VEGF and endothelial cell are mandatory for capillary tube formation. Thus, HIF-1 is increased in both mRNA level and as protein expression, in adipose tissue as an activator of VEGF gene. Although HIF-1 is a major transcriptional activator for VEGF gene, it is not sufficient alone for the activation of VEGF gene expression in obesity. Recently, it is surprisingly shown that in adipose tissue, the increase in HIF-1 mRNA is likely a result of adipogenesis and hyperinsulinemia in obesity but does not depend on the adipose tissue hypoxia (He et al. 2011). Indeed, insulin induces HIF-1 mRNA and protein expression via insulin receptors in mature adipocytes. On the other hand, insulin-like growth factor (IGF) may also stimulate the HIF-1alpha activity as well, since it activates the insulin receptor (He et al. 2011).

7 Oxidized Low-Density Lipoprotein and Endothelium

Among the 1889 participants included in the longitudinal analyses, oxLDL positively associated with all metabolic syndrome components and CRP. OxLDL showed a graded relation to incident metabolic syndrome, amounting to an adjusted odds ratio of 3.5 (Holvoet et al. 2008b). Treatment of endothelial cells with oxLDL stimulates monocyte binding as well as the production of chemotactic factors for monocytes. Induction of the expression of granulocyte-macrophage colony-stimulating factor (GM-CSF), M-CSF and granulocyte CSF (G-CSF) affect the migration and proliferation of endothelial cells (Rajavashisth et al. 1990). Furthermore, oxLDL contributes to the hyperplasia and the hypertrophy of adipocytes by providing high proliferation rate, a low apoptosis level, and an impaired differentiation process with an increased pre-adipocyte factor-1 mRNA expression (Masella et al. 2006). A possible another explanation for the relationship between oxLDL and obesity is either directly or indirectly by increasing the infiltration of inflammatory monocytes/macrophages, by inducing the accumulation of fatty acids in adipocytes (Holvoet et al. 2008a). The modification of LDL not only enhances its uptake by macrophages, but also changes the natural structures of these molecules to generate a variety of modified lipids and proteins that represent highly immunogenic determinants. The immune responses to the variety of oxLDL and their association to atherosclerosis progression are very different processes (Shaw 2004).

Acute phase reactant, CRP specifically binds to oxLDL via coupling to Fc-gamma receptors on macrophages. Enhanced association of oxLDL to macrophage may cause an increase in the oxLDL uptake by macrophages. At elevated plasma LDL-cholesterol levels; this mechanism might promote endothelial inflammation, foam cell formation and accelerate atherosclerosis (van Tits et al. 2005). On the other hand, LDL also mediates the formation of foam cells through the binding to the scavenger receptors. Although the expression of these receptors is well documented in macrophages, they are undetectable or expressed at very low level in the endothelium. In 1997, Sawamura et al. described the presence of a lectin-like oxLDL receptor-1 (LOX-1) in endothelial cells. LOX-1 binds to oxLDL where it induces endothelial dysfunction (Sawamura et al. 1997). LOX-1 expression is rapidly induced in endothelial cells by oxLDL, angiotensin-2, TNF-alpha, and shear stress (Mehta et al. 2006). It is evident that oxLDL through the LOX-1 plays an important role by which the endothelial cell becomes dysfunctional. Actually, endothelial dysfunction occurs at distinct concentrations of oxLDL. At low concentrations of oxLDL, endothelial dysfunction may be caused by selective impairment of G-protein dependent pathways. At high concentrations of oxLDL, endothelial dys-

function may spread to other signal transduction processes (Flavahan 1992).

Although under physiological conditions, there is a strong correlation between decreases in NOS mRNA expression and reduction in NO synthase activity, in visceral adiposity, eNOS mRNA transcription and its degradation is independently regulated by oxLDL (Liao et al. 1995). While native LDL can inhibit NO production by either decreasing NOS protein expression or attenuating its enzymatic activity, exposure to non-cytotoxic concentrations of oxLDL causes a progressive decrease in NOS mRNA levels (Liao et al. 1995). Down-regulation of eNOS expression in response to atherogenic concentrations of native-LDL is a potential mechanism of endothelial function impairment (Vidal et al. 1998). OxLDL-induced redistribution of eNOS from caveolae by displacement of eNOS and caveolin-1 to the intracellular compartment causes the subsequent inability to activate eNOS with acetylcholine. Regarding to the mentioned mechanisms, elevations in oxLDL rapidly attenuate the capacity for NO production by the endothelium and cause endothelial dysfunction (Uittenbogaard et al. 2000).

Modified LDL particles also induce endothelial secretion of chemotactic substances and the expression of adhesion receptors that favour monocyte and T-cell recruitment, adhesion, and transmigration into the arterial wall (Badimon et al. 2011). Monocyte MCP-1/CCL2 complex interacts with monocyte receptor CCR2. Subsequent accumulation of the monocytes to the endothelial layer favours their entry by diapedesis (Libby 2002). Transmigration of monocytes occurs in areas where the basal lamina is enriched with modified LDL particles (Simionescu 2007). This takes place mainly through its junctional zone between endothelial cells. Junction adhesion molecule-A and -C have been shown to be involved in the control of vascular permeability and leukocyte transmigration across endothelial-cell surfaces (Weber et al. 2007). Thereby, LDLs modify the antithrombotic properties of the vascular endothelium and change vessel contractility by reducing the availability of endothelial NO and activating pro-inflammatory signaling pathways (Badimón et al. 2009).

It is well known that the subendothelial accumulation of macrophage-derived foam cells is one of the features of atherosclerosis (Han et al. 1998). Thus, incubation of cocultures of human aortic endothelial and smooth muscle cells with LDL resulted in a 7.2-fold induction of mRNA for MCP-1 and a 7.1-fold increase in the transmigration of monocytes into the subendothelial space of the cocultures (Navab et al. 1991). The accumulation of monocytes to the intima requires the interaction of locally produced chemokines with specific cell surface receptors, including the CCR2 for MCP-1. The increase in CCR2 expression and chemotaxis is promoted by native LDL but not by oxLDL. Virtually, oxLDL rapidly down regulates CCR2 expression. In contrast, elevated plasma LDL levels enhance monocyte CCR2 expression and chemotactic response and potentially contribute to increased monocyte recruitment to the vessel wall in chronic inflammation and atherogenesis (Han et al. 1998). Once monocytes reach the intimal space, colony-stimulating factors induce monocytes to phenotypically transform into macrophages and begin the uptake of modified LDL particles. Scavenger receptor class A (SRA)-I and SRA-II, CD36, LOX-1, or CXCL16 have been involved in oxLDL internalization (Collot-Teixeira et al. 2007). Actually, fully oxidized LDL induces adhesion of monocytic cells, which utilize at least two distinct adhesive receptors on endothelium. Enhanced endothelial adhesiveness is associated with an upregulation of ICAM-1 expression but not of VCAM-1 or E-selectin expressions. Therefore, adhesion of monocytic cells requires additional ligands, possibly endothelial proteoglycans (Erl et al. 1998). This mechanism could further explain the atherogenic potential of oxLDL.

8 Insulin Resistance in Obesity

Elevated energy intake and/or low expenditure due to insufficient physical activity leads to abnormal nutrient metabolism that increases the

accumulation of ectopic lipids contributing to lipotoxicity (Keane et al. 2015).

Increased plasma levels of free fatty acids provoke insulin resistance, and decrease Akt activation by insulin in obesity. As opposed to unsaturated free fatty acids, saturated free fatty acids impair mitochondrial function by decreasing the both mitochondrial hyperpolarization and ATP generation (Hirabara et al. 2010). The infiltration of immune cells into adipose tissue is significant in obese conditions. In this case, macrophage-derived TNF-alpha and IL-6 impair lipoprotein lipase activity and thus may increase blood triacylglycerol concentration. Furthermore, TNF-alpha can promote hormone-sensitive lipase activity in adipose tissue, which may result in release of excess non-esterified fatty acid into the blood. While TNF-alpha concomitantly decreases insulin sensitivity by inducing serine phosphorylation of IRS-1, it also converts IRS-1 into an inhibitor of the insulin receptor tyrosine kinase. Eventually, impaired signal transduction between insulin receptor and the downstream PI3K-Akt pathway leads to insulin resistance (Hotamisligil et al. 1996; Keane et al. 2015). Ceramide induces IL-1β secretion from macrophages in obese individuals while nonesterified fatty acids promote insulin resistance by activating the NLRP3 inflammasomes in macrophages (Vandanmagsar et al. 2011; Wen et al. 2011).

Insulin resistance is a state of impaired insulin action in which normal insulin levels are incapable of producing the corresponding normal insulin responses (Mustafa et al. 2009). Inflammatory adipokines-induced systemic low-grade inflammation may trigger hepatic inflammation and hepatic insulin resistance, which accompanies the accumulation of excess lipid in adipose tissue and liver and thereby predispose to nonalcoholic fatty liver disease (NAFLD) (Shoelson et al. 2007). NAFLD is one of the most frequent findings in subjects with the metabolic syndrome (Liangpunsakul and Chalasani 2005). Although insulin resistance is initially involved in the occurrence of hepatic steatosis, subsequent NAFLD may in turn contribute to further progression of insulin resistance (Loria et al. 2005). Glucose disposal effects of insulin are augmented by vascular actions of insulin in endothelium. Meanwhile, NO production is stimulated in endothelial cells. Thus, NO-dependent increases in blood flow to skeletal muscle are associated with 25–40% increase in glucose uptake in response to insulin stimulation (Kim et al. 2006a). Indeed, insulin may chronically modulate vascular tone by regulating the expression of eNOS gene in endothelial cells of microvessels. The activation of PKC in the vascular tissues as in insulin resistance may inhibit PI3K activity and eNOS expression and may lead to endothelial dysfunctions in pathological states (Kuboki et al. 2000). Interestingly, PI3K is able to act as a molecular switch to regulate the activity of serine/threonine-specific kinase cascades, which are important in mediating insulin's effects on endpoint responses. PI3K is also a key signaling molecule mediating metabolic actions of insulin in adipose tissue (Shepherd et al. 1998). Overexpression of inhibitory mutants of either PI3K or Akt result in nearly complete inhibition of insulin-stimulated production of NO (Zeng et al. 2000).

Insulin increases monocyte-endothelial interactions, therefore insulin resistant states may also be associated with augmentation of MAPK-dependent insulin signaling pathways. Insulin promotes VCAM-1 expression in endothelial cells through MAPK pathway, amplified by the PI3K blockage. This could contribute to increased atherosclerosis occurring in subjects with hyperinsulinaemia, or in states of insulin resistance (Madonna et al. 2004). Consistent with this hypothesis, selective inhibition of PI3K in human endothelial cells blocks the effects of insulin on eNOS expression while enhancing MAPK-dependent actions (Montagnani et al. 2002a).

Obesity-associated insulin resistance is also characterized by a state of chronic, low-grade inflammation. In obese individuals, there is increased macrophage infiltration and polarization in adipose tissue, as well as an increase in the number of "classically activated" (M1) macrophages. Furthermore, these macrophages have been identified as the primary source of many pro-inflammatory cytokines unlike M2 activation. Eventually, altered adipokine secretion and

excess of circulating non-esterified fatty acids contribute to insulin resistance that is detected in the obese state (Heilbronn and Campbell 2008). Thus, polarization of adipose tissue macrophages (ATMs) is associated with lipid accumulation and the consequent formation of foam cell-like cells in adipose tissue. Early stages of adipose tissue expansion are characterized by M2-polarized ATMs and that progressive lipid accumulation within ATMs. However later, the M1 polarization is associated with severe obesity and insulin resistance (Prieur et al. 2011). M1 and M2 macrophages exhibit completely different gene expression patterns. The number of M1 among the ATMs and M1-to-M2 ratio were closely associated with insulin resistance in high-fat diet plus IL-10 gene expression within macrophages. Due to this mechanism, IL-10 upregulation under atherogenic diet may be involved in M2 macrophage recruitment which contributes to the reduction of inflammation and improves insulin signal (Fujisaka et al. 2009).

Relationships between level of CRP and measure of obesity are consistent with the adipose tissue release of IL-6 and the levels of acute phase proteins and of proinflammatory cytokines. These are correlated not only with blood pressure and dyslipidemia, but with both the severity of insulin resistance and endothelial dysfunction (Yudkin et al. 1999). Both IL-6 and TNF-alpha are expressed and secreted by human adipose tissue. The most consistent relationship between cytokine expression and obesity-related insulin resistance involve increased TNF-alpha secretion from adipose tissue and increased plasma IL-6 levels (Kern et al. 2001). The initial insult in adipocyte inflammation and insulin resistance, mediated by macrophage recruitment and endogenous ligand activation of TLRs, is maintained through chemokine secretion, adipose retention of macrophages, and amplification of pro-inflammatory adipocytokines. Activation of various kinases modulates adipocyte transcription factors, including PPAR-gamma and NFkappaB. Metabolic results of this pathway are attenuating the insulin signaling and increasing adipocytokine and free fatty acid secretion (Shah et al. 2008).

9 Conclusion

Excessive circulating lipids due to over-nutrition are important independent causes of both endothelial dysfunction and insulin resistance. However, deficiency of endothelial cell-derived NO is the primary link between insulin resistance and endothelial dysfunction. The mechanisms of lipid-mediated toxicities include oxidative stress, inflammation, mitochondrial dysfunction, endoplasmic reticulum stress and ultimate cell death. Nevertheless, a very critical aspect of endothelial dysfunction is decreased NO-bioavailability. Additionally, systemic disturbances in obesity comprises hyperglycemia, oxidative stress, activation of the renin-angiotensin system, increased pro-inflammatory cytokines, inadequate vasodilation or paradoxical vasoconstriction in coronary and peripheral arteries. From the clinical aspect, therapeutic approaches that ameliorate insulin resistance, excessive circulating lipids and oxidative stress could eliminate the endothelial dysfunction and reduce the cardiovascular diseases-related mortality. In this context, changes in lifestyle in addition to pharmacological treatments, are useful tools at the face of lipotoxicity-associated endothelial dysfunction.

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Diet-Induced Obesity and the Mechanism of Leptin Resistance

16

Atilla Engin

Abstract

Leptin signaling blockade by chronic overstimulation of the leptin receptor or hypothalamic pro-inflammatory responses due to elevated levels of saturated fatty acid can induce leptin resistance by activating negative feedback pathways. Although, long form leptin receptor (Ob-Rb) initiates leptin signaling through more than seven different signal transduction pathways, excessive suppressor of cytokine signaling-3 (SOCS-3) activity is a potential mechanism for the leptin resistance that characterizes human obesity. Because the leptin-responsive metabolic pathways broadly integrate with other neurons to control energy balance, the methods used to counteract the leptin resistance has extremely limited effect. In this chapter, besides the impairment of central and peripheral leptin signaling pathways, limited access of leptin to central nervous system (CNS) through blood-brain barrier, mismatch between high leptin and the amount of leptin receptor expression, contradictory effects of cellular and circulating molecules on leptin signaling, the connection between leptin signaling and endoplasmic reticulum (ER) stress and self-regulation of leptin signaling has been discussed in terms of leptin resistance.

Keywords

Leptin resistance • Leptin receptor • Soluble leptin receptor • Leptin signaling • Suppressor of cytokine signaling 3 (SOCS3) • Anorexigenic pro-opiomelanocortin (POMC) neurons • Signal transducer and activator of transcription 3 (STAT3) • Signal transducer and activator of transcription 5 (STAT5) • Phosphodiesterase 3 (PDE3) • Endoplasmic reticulum stress

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1 Introduction

Leptin regulates energy expenditure and food intake by communicating with the central nervous system (CNS) (Harvey and Ashford 2003). Circulating leptin levels accurately reflect the amount of body lipid across a broad range of adipose tissue. Despite increased leptin levels in obesity, a high content of dietary fat changes the set point of leptin action (Frederich et al. 1995). Hypothalamic regulation of energy homeostasis is mediated by orexigenic agouti-related protein (AgRP) and anorexigenic pro-opiomelanocortin (POMC) neurons. Actually, leptin and insulin prevents obesity by maintaining energy balance. Elevated insulin and leptin levels in the cerebrospinal fluid of obese individuals indicate a chronic state of central insulin and leptin resistance (Zhang et al. 2008). Leptin signaling blockade by chronic overstimulation of the leptin receptor and activation of negative feedback pathways may cause leptin resistance. Alternatively, high-fat diet could either directly block leptin signaling or activate endoplasmic reticulum (ER) stress and inflammation (Knight et al. 2010). However, it is strongly thought that hypothalamic insulin resistance is the primary consequence of overfeeding, and this in turn causes leptin resistance later (Wang et al. 2001). Thus, after high-fat diet, hypothalamic inflammation occurs simultaneously with the increased serine phosphorylation of the insulin receptor and insulin receptor substrate-2 (IRS-2) (De Souza et al. 2005). However, endogenous hyperinsulinemia is associated with increased circulating leptin only in the absence of insulin resistance (Mantzoros et al. 1998). Elevated central saturated fatty acid level can induce hypothalamic pro-inflammatory responses and leptin resistance, which are associated with disorders of energy homeostasis due to diet-induced obesity (Cheng et al. 2015). Most of the studies have focused on the role of fatty acid-stimulated hypothalamic inflammation in leptin resistance. Thus, enhanced expressions of interleukin (IL)-6, tumor necrosis factor (TNF)-alpha, suppressor of cytokine signaling 3 (SOCS3), inhibitor kappa B kinase-beta (IKKbeta) and IKKepsilon is associated with increased accumulation of saturated fatty acids in the hypothalamus even before the onset of obesity or increased accumulation of adipose tissue (Thaler et al. 2012). Indeed, the activation of IKKbeta/nuclear factor-kappa B (NF-kappaB) may result in leptin and insulin resistance in the CNS via the expression of the SOCS3, a well-known inhibitor of insulin and leptin signaling. In this respect, hypothalamic inflammation induced by saturated fatty acids may be an initiator factor in obesity (Cesar and Pisani 2016). Although leptin has a vital role in body weight control by suppressing food intake, a high cereal fiber intake from oat or wheat bran improves leptin sensitivity by increasing the protein expressions of Janus kinase 2 (JAK2) and signal transducer and activator of transcription 3 (STAT3), and by decreasing the protein expression of SOCS3 (Zhang et al. 2016).

Molecular basis of leptin resistance is tried to be explained through the CNS-related mechanisms mostly (Howard et al. 2004; Myers 2004). By contrast, circadian disruption of peripheral endogenous adipose clock induces central leptin resistance. Thus, leptin resistance plays a key-role in circadian dysfunction-induced obesity. Although, leptin-feedback is controlled by coupling of the central and peripheral clocks, studies related to peripheral leptin resistance are very scanty (Kettner et al. 2015). Therefore, promising strategies to counteract leptin resistance have shown limited efficacy or even relevant adverse effects in preclinical and clinical studies (Santoro et al. 2015).

2 Leptin Signal Transduction Pathways in Diet-Induced Obesity

Although there are six different isoforms of leptin receptors, the long form leptin receptor (Ob-Rb) isoform is the important one for JAK2 and STAT3 binding. After binding to Ob-Rb, leptin activates JAK2/STAT3 signaling. By this process, Ob-Rb becomes phosphorylated. Leptin signaling through STAT3 is critical for maintaining normal energy homeostasis. Hence, hyperleptinemiae increases the STAT3 phosphorylation not only in

the hypothalamus but also in the brain. Phospho-STAT3 translocates to the nucleus and activates the SOCS3 (Hosoi et al. 2002; Hosoi and Ozawa 2016). Ob-Rb is highly expressed in the hypothalamus and cerebellum of the human brain. But the levels of Ob-Rb are significantly higher in cerebellum of both lean and obese individuals. Interestingly, obesity and hyperleptinemia is not associated with down-regulation of the Ob-Rb in the human brain routinely (Burguera et al. 2000). The signaling-form of the leptin receptor exhibits a somato-dendritic expression pattern in POMC and neuropeptide Y (NPY)/AgRP neurons (Ha et al. 2013). POMC neurons in diet-induced obesity are resistant to STAT3 activation by leptin. Furthermore, selectively over-expression of Ob-Rb in POMC neurons increases susceptibility to the development of diet-induced obesity (Gamber et al. 2012). Mainly, Ob-Rb initiates leptin signaling through both JAK2-dependent and JAK2-independent pathways, including the JAK2-STAT3 pathway, tyrosine phosphorylation sites of Ob-Rb-associated JAK2-IRS-phosphatidylinositol 3-kinase (PI3K) pathway, Ob-Rb-Tyr(985)-SH2-containing tyrosine phosphatase (SHP-2)-extracellular signal-regulated kinase (ERK) pathway, Ob-Rb-Tyr(1138)-STAT3-SOCS3-hypothalamic melanocortin pathway, PI3K-phosphodiesterase-3B (PDE3B)-cyclic adenosine monophosphate (cAMP) signaling pathway, PI3K/Akt/mechanistic (mammalian) target of rapamycin (mTOR)/S6 K pathway and alpha2-5′-adenosine monophosphate (AMP)-activated protein kinase (AMPK)-STAT3 signaling pathway (Martin et al. 2006; Morris and Rui 2009; Myers 2004) (Fig. 16.1). Although, it is proposed that leptin resistance of obese individuals may depend on insensitivity to peripheral leptin (Van Heek et al. 1997), all central leptin signaling pathways may be impaired during the development of diet-induced obesity.

The tyrosine phosphorylation of STAT proteins promotes their nuclear translocation. The phosphorylated STAT proteins including STAT3 are subjected to transcriptional regulation after binding to specific DNA elements (Darnell et al. 1994; Zhong et al. 1994). Upon the phosphorylation of JAK2 tyrosine kinase by Ob-Rb, the activated JAK2 then phosphorylates the intracellular domain of Ob-Rb on Tyr985, Tyr1077, and Tyr1138. While Ob-Rb Tyr1077 plays a dominant role for the acute phosphorylation of STAT5a, Ob-Rb Tyr1138 has a secondary role for the STAT5b phosphorylation. Leptin-induced phosphorylation of STAT5 and S6 in the hypothalamus exhibits the phosphorylation of Tyr1077 on Ob-Rb, and defines crucial roles for Tyr1077 and Tyr985 in the regulation of STAT5 and phosphorylation of the ribosomal S6 kinase (RSK)/S6(P)/7-methylguanosine cap (cap)-dependent translation, respectively (Gong et al. 2007) (Fig. 16.1). However, deletion of STAT5 in CNS may result in increased food intake and significantly more weight gain which is consistent with the increase in circulating leptin and insulin levels approximately 4.5- and 8-fold, respectively. In this regard, STAT5 deficiency may cause both leptin and insulin resistance (Lee et al. 2008). Deletion of both STAT5a and STAT5b in the brain causes leptin resistance, but to a lesser extent than STAT3 deletion (Lee et al. 2008). Tyr1138/STAT3-mediated feedback inhibition attenuates STAT5-dependent transcription during chronic Ob-Rb activation (Gong et al. 2007).

The activation of STAT3 by Tyr1138 on Ob-Rb is one of the major mechanisms initiating leptin signaling (Myers 2004). Inversely, Tyr1138 on Ob-Rb and SOCS3 represent major effector components for the feedback inhibition of Ob-Rb signaling (Dunn et al. 2005). Thus, leptin-stimulated SOCS3 acts as a feedback inhibitor in the JAK2-STAT3 pathway. Leptin also activates STAT3 in various hypothalamic nuclei. By contrast, loss of STAT3 effect on Ob-Rb abolishes leptin-induced STAT3 phosphorylation and causes hyperphagia and morbid obesity (Piper et al. 2008). In this context, another proposed mechanism for leptin resistance is a leptin-dependent increase of the SOCS-3 mRNA in hypothalamus. Indeed, SOCS-3 is a leptin-inducible inhibitor of leptin signaling and blocks leptin-induced signal transduction. Therefore, expression of SOCS-3 mRNA in the arcuate and dorsomedial hypothalamic nuclei is increased in leptin-resistant obesity (Bjørbaek et al. 1998a). As a negative regulator of leptin signaling, exces-

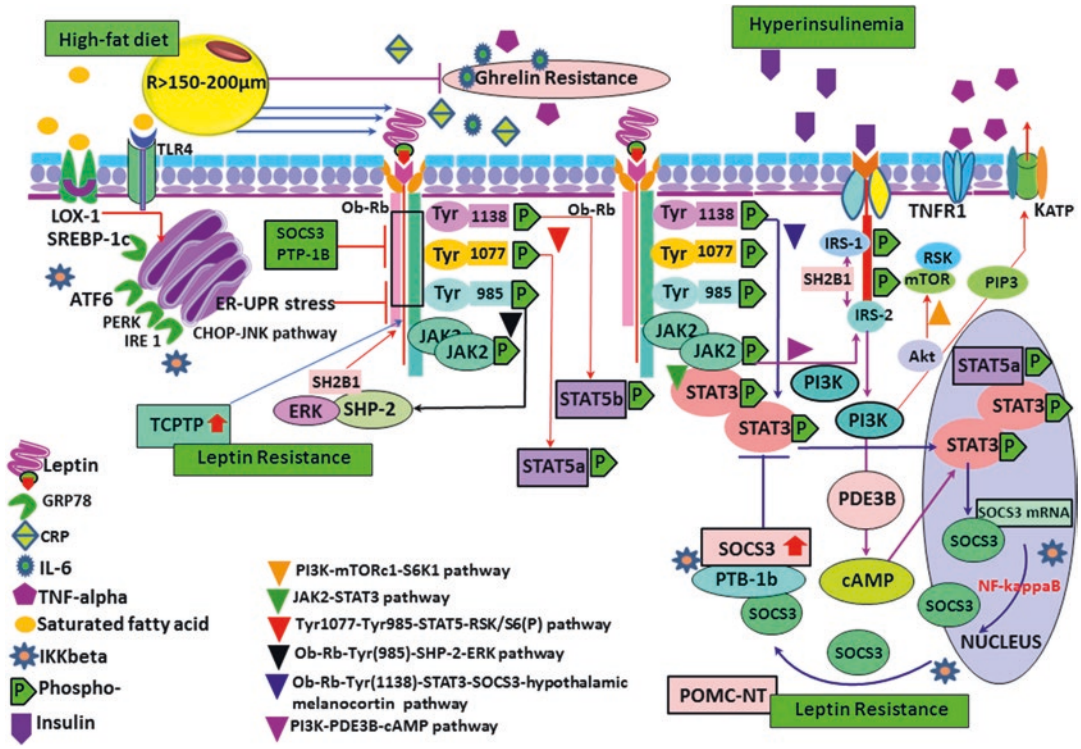

Fig. 16.1 The postulated mechanisms for the leptin signaling and leptin resistance. Leptin binds to Ob-Rb and activates leptin receptor-associated JAK2. Upon the phosphorylation of JAK2 tyrosine kinase by Ob-Rb, the activated JAK2 then phosphorylates the intracellular domain of Ob-Rb on Tyr985, Tyr1077, and Tyr1138. Ob-Rb initiates leptin signaling through both JAK2-dependent and JAK2-independent pathways. SOCS-3 as a major leptin-inducible inhibitor of leptin signaling blocks leptin-induced signal transduction. Excessive SOCS-3 activity associated with chronic low-grade inflammation in obesity is a potential mechanism for the leptin resistance that characterizes human obesity (*LOX* lectin-like oxidized low-density lipoprotein (ox-LDL) receptor, *SREBP-1c* sterol regulatory element-binding protein-1c, *ATF6* activating membrane-bound transcription factor 6, *PERK* PRKR-like endoplasmic reticulum kinase, *IRE1* inositol requiring enzyme 1, *TCPTP* T cell protein tyrosine phosphatase, *GRP78* 78-kD glucose-regulated/binding immunoglobulin protein, *CRP* C-reactive protein, *IL-6* interleukin 6, *TNF-alpha* tumor necrosis factor-alpha, *IKK* inhibitory-kappaB kinase, *TLR4* toll-like receptor 4, *ER* endoplasmic reticulum, *UPR* unfolded protein response, *CHOP* CCAAT/enhancer-binding protein homologous protein, *JNK2* c-Jun N-terminal kinase 2, *SOCS3* suppressor of cytokine signaling 3, *PTP1B* protein tyrosine phosphatase 1B, *SHP2B1* SH2-containing protein tyrosine phosphatase 2 B1, SH2 domain-containing transcription factor B1, *ERK* extracellular signal-regulated kinase, *Tyr* tyrosine, *Ob-Rb* long form of the leptin receptor, *STAT* signal transducer and activator of transcription, *pSTAT* phosphorylated signal transducer and activator transcription, *PI3K* phosphoinositide-3 kinase, *cAMP* 3′,5′-cyclic adenosine monophosphate, *PDE3B* PI3K-phosphodiesterase-3B, *Akt* protein kinase B, *mTOR* mammalian target of rapamycin, *NF-kappaB* nuclear factor-kappa B, *RSK* ribosomal S6 kinase, *IRS* insulin receptor substrate, *TNFR1* TNF receptor-1, K_{ATP} potassium-ATP channel, *PIP3* phosphatidylinositol 3,4,5-trisphosphate, *S6K1* S6 kinase 1)

sive SOCS-3 activity is a potential mechanism for the leptin resistance that characterizes human obesity (Bjørbaek et al. 1998a). (Fig. 16.1).

On the other hand, leptin signaling involves PI3K-phosphodiesterase-3B (PDE3B)-cAMP signaling pathway (Sahu et al. 2013). In addition to PI3K, leptin also increases PDE3B activities in the hypothalamus. Thus, PDE3 inhibition reverses the anorectic and body weight-reducing effects of leptin. In this case, PI3K-PDE3B-cAMP pathway interacts with the JAK2-STAT3 pathway (Zhao et al. 2002). In fact, diet-induced obesity is associated with hyperphagia, hyperleptinaemia, hyperinsulinaemia and increased hypothalamic

phosphorylated-Akt levels. During the development of diet-induced obesity, hypothalamic PDE3B-cAMP-Akt pathway of leptin signaling is impaired. This defect constitutes one of the principal mechanisms of central leptin resistance (Sahu et al. 2015). Furthermore, forkhead box protein O1 (FOXO1) stimulates the transcription of the orexigenic neuropeptides through the PI3K/Akt signaling pathway. On the other hand, it suppresses the transcription of anorexigenic POMC by antagonizing the STAT3 activity (Kim et al. 2006). Interestingly, PI3K may contribute to the activation of both AgRP and POMC neurons, but the direction of PI3K regulation by leptin depends on whether the action is mediated directly or indirectly. Approximately 60% of the POMC neurons have leptin-activated PI3K (Xu et al. 2005). Thus, POMC-specific leptin receptor deletion results in the development of hyperleptinemia and a mild obesity (Balthasar et al. 2004). In lean individuals, PI3K/3-phosphoinositide dependent protein kinase 1 (PDK1)/FOXO1-dependent signaling is required for hypothalamic POMC transcription. Impaired activation of this pathway also results in hyperphagia and increased body weight (Belgardt et al. 2008). Collectively, PI3K signaling plays an important role in transducing leptin action in the hypothalamus (Zhao et al. 2002).

Since NPY/AgRP neurons are important target of leptin signaling in the hypothalamus, NPY/AgRP neurons may be involved in the development of resistance to the satiety action of leptin in the hypothalamus (Sahu 2002). Leptin and insulin acts in parallel during the stimulation of PI3K in POMC neurons but they behave in opposite ways on NPY/AgRP neurons (Xu et al. 2005). In POMC neurons, insulin, via activation of adenosine triphosphate (ATP)-dependent potassium/ATP (K^+/ATP) channels, is strongly able to impede the effects of leptin. K^+/ATP channel activity in POMC neurons has a critical role in regulating the set point of hypothalamic POMC cell activity. Surprisingly, insulin-effect leads to accumulation of the PI3K product, phosphatidylinositol 3,4,5-trisphosphate (PIP3), in POMC neurons and opening of K^+/ATP channels (Plum et al. 2006). However, leptin is not able to overcome this insulin-induced hyperpolarization. Accordingly, chronic activation of PI3K by deletion of the PIP3 phosphatase leads to diet-induced hyperphagia, obesity and leptin resistance (Plum et al. 2006). In this manner, enhanced activation of PI3K signaling results in leptin resistance due to PIP3-deletion and K^+/ATP channel activation. Indeed, high-fat diet exhibits a significant reduction in POMC expression in an Akt activation, FOXO1-dependent manner (Belgardt et al. 2008). Nevertheless, the underlying mechanism behind the development of selective leptin resistance through the PI3K pathway is controversial. Substantially, high-fat feeding leads to impairment in PI3K pathway of leptin signaling at initial phase of diet-induced obesity. However, at longer period, both PI3K and STAT3 signaling is abolished. Ultimately, selective defect in the PI3K pathway of leptin signaling contributes to the central leptin resistance and refractory obesity (Metlakunta et al. 2008; Sahu 2011). Leptin utilizes both STAT3 and PI3K pathways, whereas insulin action is mediated by only PI3K-Akt pathway in the hypothalamus. However, insulin also increases the leptin-induced phosphorylation of STAT3 and its activation (Carvalheira et al. 2001). In this respect, interaction of PI3K-PDE3B-cAMP pathway with the JAK2-STAT3 pathway is one of the critical events of leptin signaling in the hypothalamus (Sahu 2011). Ob-Rb and other isoforms may also signal via mitogen activated protein kinases (MAPK), PI3K, and nitric oxide pathways (Bjørbaek et al. 1997). These evidences indicate that intracellular activation of PI3K and STAT3 signaling is crucial for the regulation of body weight by leptin (Bates and Myers 2003). In particular, the Ob-Rb signaling via STAT3 is the central component of energy expenditure control by leptin (Bates et al. 2004). Thereby, resistance to the appetite-suppressing effects of leptin is associated with obesity (Münzberg et al. 2005). While the orexigenic neuropeptides are downregulated by leptin, the anorexigenic neuropeptides are upregulated. In leptin-resistant state, despite having high plasma leptin concentrations related to the size of adipose tissue, obese humans fail to retain appetite-suppressing effect and could not reduce own

weight (Stanley et al. 2005). Although it is proposed that leptin-induced feeding behavior is blocked by the stomach-derived appetite-increasing peptide, ghrelin (Nakazato et al. 2001), high-fat diet blunts ghrelin signaling and causes ghrelin resistance via a mechanism involving in activation of inflammation in obesity (Naznin et al. 2015). Briqqs et al. claimed that the main reason of ghrelin resistance is diet-induced hyperleptinemia in obesity (Briggs et al. 2014). Moreover, increase in SOCS3 expression mediates inhibitory action of ghrelin on leptin-stimulated STAT3 phosphorylation, neuronal firing, and feeding behavior. Thereby, ghrelin induces leptin resistance by increasing SOCS3 expression by the adenylate cyclase-cAMP-the exchange protein activated signal transduction pathway, which impairs leptin-stimulated STAT3 phosphorylation and neuronal firing (Heldsinger et al. 2014).

Very rare genetic disorders have been reported in obese humans, which might be due to a sporadic mutation in leptin gene (Paz-Filho et al. 2010). Virtually, most of the obese patients are in state of leptin resistance. This means that obese individuals are resistant to the effects of endogenous and exogenous leptin (Jéquier 2002). During the development of obesity, skeletal muscle becomes resistant to the effects of leptin. In this context, reduced capacity for leptin to stimulate fatty acid oxidation, coupled with an increased potential for fatty acid uptake, may contribute to the accumulation of intramuscular triacylglycerol (Steinberg et al. 2002). Peripheral effect of leptin on skeletal muscle is mediated by the activation of the AMPK signaling pathway (Minokoshi et al. 2002). The activation of and changes in the subcellular localization of the alpha-2 catalytic subunit of alpha2 AMPK are required for leptin-induced stimulation of fatty acid oxidation and peroxisome proliferator-activated receptor-alpha (PPAR-alpha) gene expression in muscle cells (Suzuki et al. 2007). Blocking AMPK activation inhibits the phosphorylation of acetyl-CoA carboxylase (ACC), which is stimulated by circulating leptin. In this respect, AMPK is a critical mediator for leptin's effects on fatty acid oxidation (Minokoshi et al. 2002). Thus, diet-induced obesity alters alpha2-AMPK activity in muscle cells and hypothalamus. In obese individuals, effect of leptin on alpha2-AMPK-STAT3v signaling pathway is impaired. Defective responses of AMPK to leptin may contribute to resistance to leptin action on food intake and energy expenditure (Martin et al. 2006). AMPK stimulates fatty acid oxidation through phosphorylation of ACC. Reduced malonyl-CoA synthesis activates carnitine-palmitoyl-CoA transferase-1, while entry of fatty acid into mitochondria increased for oxidation (Kahn et al. 2005). Inhibition of AMPK-ACC pathway in hypothalamus is also necessary for the anorexigenic effects of leptin (Minokoshi et al. 2004). On the other hand, inhibition of ACC alone blocks leptin-mediated decreases in food intake, body weight, and mRNA level of the orexigenic AgRP (Gao et al. 2007). Thus, lack of responsiveness of AMPK-ACC pathway to leptin could provide a molecular mechanism of an early sign of leptin resistance in diet-induced obesity (Martin et al. 2006). SOCS3 suppresses basal and leptin-stimulated activity and phosphorylation of alpha2AMPK and its downstream target, ACC. SOCS3 within skeletal muscle is a critical regulator of leptin and insulin action and increased SOCS3 may mediate insulin and leptin resistance in obesity (Yang et al. 2012). A high-fat diet causes an inflammatory response in association with increased expression of hypothalamic IL-6, which is accompanied by increases in expression of several proinflammatory cytokines. Increases in proinflammatory cytokines is associated with the reduced leptin signaling (Thaler and Schwartz 2010). Thus, chronic low-grade inflammation in obesity is associated with increased circulating IL-6 and leptin levels, and ultimately increased SOCS3 expression. Increased SOCS3 can lead to the development of central and peripheral leptin resistance (Sarvas et al. 2013).

The consumption of a high-fat diet dampens hypothalamic ribosomal S6 kinase 1 (S6K1) phosphorylation. Consequently, reduced hypothalamic mTOR complex 1 (mTORC1)-S6K signaling contributes to the development of hyperphagia, weight gain and leptin resistance (Cota et al. 2008). By contrast, leptin stimulates

phosphorylation of S6K, which is a major physiological substrate of the mTOR kinase in the hypothalamus (Cota et al. 2006). Furthermore, deletion of S6K1 in the hypothalamus also abolishes leptin's effect (Blouet et al. 2008). Phospho-STAT3 (pSTAT3) and simultaneous mTOR-S6K signaling activity is inhibited by the SOCS3 upregulation in POMC neurons. The onset of leptin resistance and obesity follows this process (Reed et al. 2010). The PI3K/Akt pathway stimulates the mTOR/S6K pathway at least in POMC neurons; additionally, chronic activation of the PI3K/Akt/mTOR/S6K pathway in POMC neurons may alter synaptic transmission and/or neural wiring in the hypothalamus, resulting in leptin resistance (Morris and Rui 2009).

3 Limited Access of Leptin to Brain

Resistance to leptin's action may occur in a state of limited access of leptin to CNS through blood-brain barrier. Actually, leptin enters into the CNS via unidirectional system. Receptor-mediated transport of leptin across the blood-brain barrier is mediated by short leptin receptor isoform, which are intensely expressed in microvessels, choroid plexus, and leptomeninges (Bjørbaek et al. 1998b). The significant negative correlation between cerebrospinal-fluid leptin and POMC is secondary to leptin resistance and neuronal changes associated with obesity (Page-Wilson et al. 2015). In addition, leptin is transported from blood to brain by a saturable system (Banks et al. 1996). In other words, the transport of leptin across the blood-brain barrier is most efficient when serum levels are low. In this case, maximum leptin transport into brain is occurred at serum levels of 5–10 ng/mL (Banks 2012). Furthermore, the maximal hypothalamic leptin signaling capacity is diminished in leptin-resistant subjects (Scarpace et al. 2005). The leptin cerebrospinal-fluid/serum ratio in lean individuals is 4.3-fold higher than that in obese individuals. Thereupon, the capacity of leptin transport to brain is lower in obese individuals (Caro et al. 1996). This suggests that there is a saturable and active transporter of leptin from circulation into intrathecal space (Koistinen et al. 1998). Obese humans have lower levels of leptin in cerebrospinal fluid, even though their plasma leptin is higher when compared to non-obese humans. This finding indicates that obese individuals have either saturated or defective leptin transport system. Despite high plasma leptin levels, reduced efficiency of brain leptin transfer among obese individuals results in leptin resistance (Schwartz et al. 1996). Therefore, it is thought that the main cause of leptin resistance at least in part, is related to decreased transport of leptin across the blood-brain barrier in obese humans (Banks 2001). Substantially, peripheral leptin resistance may occur while retaining central leptin sensitivity in diet-induced obesity (Van Heek et al. 1997). In fact, leptin is a component of negative feedback loop between adipose tissue and brain (Banks 2008). Therefore, high serum leptin/adiponectin ratio with high levels of serum triglycerides may be markers of "at-risk" obesity (Labruna et al. 2011). Adipose tissue dysfunction in obesity leads to hypertriglyceridemia due to increased hepatic very-low-density lipoprotein (VLDL) production and decreased triglyceride hydrolysis (van de Woestijne et al. 2011). Triglycerides inhibit the transport of leptin across the blood-brain barrier. Decreasing leptin levels in the brain re-directs caloric use towards food-seeking activities. Thereby, hypertriglyceridemia is important in the onset of the peripheral leptin resistance and obesity (Banks et al. 2006; Banks 2012).

4 Leptin Receptor Sensitivity

Reductions in central leptin signaling and defects in leptin blood-brain barrier transport can develop independently and are likely to have different underlying mechanisms (Levin et al. 2004). In diet-induced obesity, increase in leptin levels is earlier when compared to the increased insulin levels. There is a mismatch between high leptin and the amount of leptin receptor expression. Thus, elevated leptin is associated with 10% lower leptin receptor mRNA expression in the

arcuate, dorsomedial, and ventromedial hypothalamic nuclei. In this case, reduced leptin signal might play a causal role in obesity (Levin et al. 2003). Reduced leptin-Ob-Rb binding on the plasma membrane causes defective signaling on multiple pathways such as JAK2-STAT3, PI3K-Akt-FOXO1, SHP2-ERK, AMPK-ACC and PI3K/Akt/mTOR/S6K signaling pathways. Furthermore, leptin signaling is regulated positively with SH2B1 and negatively with SOCS3, protein-tyrosine phosphatase 1B (PTP1B) and ER stress (Kwon et al. 2016; Morris and Rui 2009). Disruption of the STAT3 binding site in Ob-Rb, or deletion of neuronal STAT3, results in severe hyperphagia and morbid obesity (Cui et al. 2004). A soluble isoform (soluble Ob-Rb) of Ob-Rb can regulate serum leptin concentration and serve as a carrier protein for binding the leptin to Ob-Rb when transducing the signal into the cell (Gorska et al. 2010).

While increased soluble Ob-Rb concentrations directly inhibit leptin's effects, reduced amounts of soluble Ob-Rb may reflect decreased membrane expression of Ob-Rb. Alterations of leptin sensitivity in part are associated with the changes in soluble Ob-Rb intensity (Schaab et al. 2012). Thus, in contrast high leptin levels and increased soluble Ob-Rb concentrations block leptin action. However, decreased amounts of soluble Ob-Rb due to ER stress are accompanied by impaired leptin signaling and reduced leptin binding (Schaab et al. 2012).

5 Contradictory Effects of Cellular and Circulating Molecules on Leptin

PTP1B also plays a role in leptin resistance by inhibiting the effects of leptin via dephosphorylating JAK2 (Zabolotny et al. 2002). Indeed, JAK2/STAT3 signal transduction cascade is stimulated by binding leptin to leptin receptor. Thus, the negative regulatory role of PTP1B on leptin signaling is mediated through a direct and selective dephosphorylation of JAK2 and STAT3 (Lund et al. 2005). However, leptin-activated JAK2 is a substrate of PTP1B. Consequently, PTP1B negatively regulates leptin signaling through the dephosphorylation of JAK2 (Cheng et al. 2002). In this regard, PTP1B regulates adipocyte leptin production and is essential for the development of leptin resistance (Bence et al. 2006).

In human blood, there are several serum leptin-interacting proteins (SLIPs). One of the major SLIPs is C-reactive protein (CRP). Human CRP directly binds leptin in extra-cellular settings, thus impairs the biological actions of leptin. Furthermore, CRP correlates with increased adiposity and plasma leptin. Chronic elevation of CRP may worsen leptin resistance (Chen et al. 2006; Hribal et al. 2014). Low level of chronic inflammatory state in adipose tissue is reflected by high levels of IL-6, TNF-alpha, and CRP. Meanwhile, chronic inflammation-induced insulin resistance and endothelial dysfunction are linked with obesity (Yudkin et al. 1999). In AMPK-alpha1-Akt-endothelial nitric oxide synthase (eNOS) pathway, Akt functions downstream of AMPKalpha1. In addition, CRP impairs leptin-induced AMPK activation, eNOS-Ser1177 phosphorylation, eNOS activity, and intracellular cyclic guanosine monophosphate (cGMP) accumulation. In this case, interaction between leptin and CRP may have a role in impairing leptin's effect on eNOS activation (Procopio et al. 2009). At the blood-brain barrier, leptin activates the saturable transport system for urocortin. Urocortin in turn potentiates leptin-induced STAT3 activation (Pan et al. 2007).

6 The Connection of Overeating and Leptin Resistance Through Endoplasmic Reticulum Stress

Dietary-fat could either directly block leptin signaling or activate cellular processes, such as ER stress and inflammation, that impair leptin responsiveness of neuronal cells. The IKKbeta/NF-kappaB activation is stimulated by high-fat diet in the hypothalamus. Elevated ER stress in the hypothalamus, is reflected by increased levels

of both protein kinase R (PKR)-like ER-resident kinase (PERK) and eukaryotic translation initiation factor (eIF)-2alpha phosphorylation. IKKbeta expression increases SOCS3 promoter activity. SOCS3 is produced by STAT3 which can be activated through leptin. This process is controlled by NFkappaB. High-fat diet significantly induces SOCS3 in hypothalamus (Zhang et al. 2008). Saturated fatty acids act as ligands for toll-like receptor 4 (TLR4) and cause diet-induced leptin and insulin resistance (Lee et al. 2001b). Indeed, palmitate- and high-fat diet induced, leptin resistance emerges via TLR-activated MyD88-dependent signals. Palmitate-induced IKKbeta-activation and inhibitor kappaB-alpha degradation critically depend on MyD88. In this regard, neuronal MyD88-dependent signaling is a key regulator of diet-induced leptin resistance (Kleinridders et al. 2009). Thus, excessive intake of saturated fatty acids may cause ER stress and leptin resistance in obesity. In saturated fatty acid-treated pancreatic beta-cells, accumulation of misfolded proteins triggers the ER stress response (Karaskov et al. 2006). As mentioned above, long-chain saturated fatty acids activate predominantly TLR4 signaling, which determines not only the induction of local cytokine expression and inflammatory response but also promotes ER stress in the hypothalamus (Milanski et al. 2009). Eventually, forced activation of hypothalamic IKKbeta/NF-kappaB interrupts central insulin/leptin signaling and actions. SOCS3, which is a core inhibitor of insulin and leptin signaling is regulated by IKKbeta/NFkappaB (Zhang et al. 2008). While ER stress markedly inhibits leptin-induced STAT3 phosphorylation, in contrast, it cannot affect leptin-induced c-Jun NH(2)-terminal kinase (JNK) activation. These results suggest that ER stress induces leptin resistance through PTP1B (Hosoi et al. 2008). The increased expression of PTP1B and T cell protein tyrosine phosphatase (TCPTP) attenuate leptin and insulin signaling. By contrast, deficiencies in PTP1B and TCPTP promote insulin and leptin signaling and prevent diet-induced obesity by modulating ER stress (Zhang et al. 2015). TCPTP is a critical negative regulator of hypothalamic leptin signaling and elevated TCPTP contributes to cellular leptin resistance in obesity (Loh et al. 2011).

On the other hand, homocysteine-induced ER stress is one of the important factors involved in leptin resistance. Indeed, the plasma homocysteine level is increased in obese patients compared with non-obese controls. Moreover, the plasma homocysteine levels are positively associated with the serum leptin levels (Narin et al. 2005). Increased ER stress and accumulation of unfolded proteins in the hypothalamus of obese mice inhibits leptin receptor signaling (Ozcan et al. 2009). Unfolded protein response (UPR) is a feedback mechanism that prevents the accumulation of unfolded proteins in the lumen of the ER. UPR inhibits protein translation and increases the production of chaperone protein, glucose-regulated protein 78 (GRP78) (Schröder and Kaufman 2005). Homocysteine-induced GRP78 expression indicates the homocysteine-induced ER stress. Furthermore, homocysteine dose- and time-dependently inhibits leptin-induced STAT3 phosphorylation. Actually, STAT3 phosphorylation is an indicator of leptin resistance. Collectively, these findings suggest that homocysteine contributes to leptin resistance, which is mediated through PTP1B (Hosoi et al. 2008). In fact, GRP78 protects cells against ER stress-induced cell death by regulating the activation of the PERK, activating transcription factor 6 (ATF6), and inositol-requiring enzyme1 alpha (IRE1alpha) (Bertolotti et al. 2000; Harding et al. 2000). The leptin-induced expression of GRP78 is mediated through the PI3K-mTOR pathway. In the early phase of a ER stress, leptin can protect against ER stress-induced cell death. However, when ER stress is severe and prolonged, leptin resistance may develop (Thon et al. 2014). Likewise, insulin also could enhance GRP78 expression and block the cleavage of poly (ADP-ribose) polymerase (PARP) and caspase-3 activity through PI3K/Akt/mTOR pathway during the ER stress thereby GRP78 protects cell from ER stress-induced apoptosis (Liu et al. 2014).

ER stress-induced leptin resistance is mediated through PTP1B in obesity. In addition, a chemical chaperone, 4-phenyl butyric acid

(4-PBA) improves the protein folding capacity and reverses ER stress-induced leptin resistance (Hosoi et al. 2008). Furthermore, 4-PBA significantly improves central leptin resistance in diet-induced obese mice (Won et al. 2009).

Alpha-melanocyte stimulating hormone (alpha-MSH) inhibits feeding and stimulates energy expenditure (Wardlaw 2011). In high-fat diet-induced obese subjects, leptin resistance prevents the leptin-induced increase of alpha-MSH secretion while preventing a decrease in AgRP secretion. This leads to weakened central melanocortin activation. Despite normal Ob-Rb levels and increased SOCS-3 levels, leptin fails to induce the leptin signaling cascade (Enriori et al. 2007). During diet-induced obesity, along with the decrease in alpha-MSH, a significant reduction in pro-convertase 2 (PC2), which catalyzes the conversion of adrenocorticotropin to alpha-MSH arises (Cakir et al. 2013). Despite decreases in mitochondria number, their size increase in orexigenic AgRP neurons in the period of overfed state. Virtually, deletion of mitofusin 2 (MTF2) results in altered mitochondria size and density in these cells. Thus, deficiency in MTF2 destroys the signaling activity of AgRP neurons in condition of high-fat diet (Dietrich et al. 2013). Furthermore, lipotoxic stimulation induced by saturated fatty acid activates ER stress response and decreases MTF2 protein levels in arcuate nucleus of hypothalamus (Diaz et al. 2015). Obesity-induced ER stress abolishes the post-translational processing of POMC by decreasing PC2. Thereby alpha-MSH peptide production decreases (Cakir et al. 2013). Mitochondria-ER contacts in anorxigenic POMC neurons are decreased in diet-induced obesity. Eventually, POMC-specific ablation of MTF2 results in loss of mitochondria-ER contacts, defective POMC processing, ER stress-induced leptin resistance, hyperphagia, reduced energy expenditure, and obesity (Schneeberger et al. 2013). MTF2 deletion in POMC neurons elicits ER stress, thus altering alpha-MSH processing that leads to leptin resistance and obesity (Ramírez and Claret 2015).

The non-steroidal anti-inflammatory drugs and caffeine exhibit chemical chaperone activity and reduce the ER stress-induced leptin resistance via decreasing the accumulation of unfolded proteins (Hosoi et al. 2014a, b). Moreover, caffeine markedly improves ER stress-induced impairments in the leptin-induced phosphorylation of STAT3 (Hosoi et al. 2014a). Transforming growth factor beta (TGF-beta)-activated kinase 1 (TAK1) is an indispensable signaling intermediate in TNF-alpha, IL-1, and TLR signaling pathways (Broglie et al. 2010). CNS-specific TAK1 deletion prevents ER-stress-induced hypothalamic leptin resistance and hyperphagic obesity under a high-fat diet (Sai et al. 2016).

Caffeine inhibits ER stress-associated genes, PERK and IRE1alpha. Thereby, caffeine ameliorates leptin resistance by markedly reducing ER stress-induced impairments in the leptin-induced phosphorylation of STAT3 (Hosoi et al. 2014a). The carbon monoxide (CO)-releasing molecule, tricarbonyldichlororuthenium (II) dimer (CORM-2) blocks the ER stress-dependent inhibition of leptin-induced STAT3 phosphorylation. CORM-2 induces the phosphorylation of PERK, and eIF-2alpha during ER stress. Furthermore, CORM-2 inhibits IRE1alpha phosphorylation, which is induced by ER stress. IRE1alpha deficiency ameliorates leptin resistance. CO-dependent regulation of IRE1alpha reduces body weight in animals fed high-fat diets (Zheng et al. 2013).

7 Caloric Restriction

During caloric excess, leptin minimizes the accumulation of lipids in nonadipose tissues. Leptin concentration progressively increases as the overnutrition continues (Lee et al. 2001a). In self-regulation of leptin signaling, diminished Ob-Rb expression in diet-induced obesity is associated with attenuated maximal leptin-induced STAT3 phosphorylation capacity. However, caloric restriction results in a significant increase in leptin-stimulated STAT3 phosphorylation in obese subjects (Wilsey and Scarpace 2004). Dietary fats alone are insufficient to block the response to leptin. Hyperleptinemia itself can contribute to leptin resistance by downregu-

lating cellular response to leptin (Knight et al. 2010). POMC neurons that express Ob-Rb and leptin regulates POMC mRNA levels via activation of intracellular STAT3 proteins (Münzberg et al. 2003). Furthermore, leptin increases the frequency of action potentials in the anorexigenic POMC neurons either by depolarization or simultaneously reducing their inhibition by local orexigenic NPY (Cowley et al. 2001). On the other hand, essential nuclear protein for neuronal function of POMC, methyl-CpG-binding protein 2 (Mecp2), positively regulates POMC expression. Absence of Mecp2 in POMC and AgRP mRNA expression leads to obesity and leptin resistance (Wang et al. 2014). Deletion of Mecp2 increases the expression of sirtuin 1 (SIRT1). Thus, hypothalamic SIRT1 plays an important role in the regulation of food intake and body weight (Cakir et al. 2009). Expression of POMC is activated by pSTAT3 and is repressed by FOXO1. Decrease in FOXO1 phosphorylation followed by a decrease in phospho-Akt are key points in the transcriptional control of POMC. All these steps constitute leptin-induced signal transduction. Moreover, lack of Mecp2 disrupts body weight balance by altering post-translational modifications in leptin-signaling components that regulate POMC and AgRP expression. Last of all, the decreased POMC expression associates with the increased levels of leptin and leptin resistance due to the increase in FOXO1 expression (Torres-Andrade et al. 2014). Overexpression of hypothalamic FOXO1 increases body weight. However, simultaneous overexpression of SIRT1 suppresses this process (Sasaki and Kitamura 2010). Thus, SIRT1 improves both leptin and insulin sensitivity by eliminating several molecules that impair leptin and insulin signal transduction (Sasaki 2015). Collectively, POMC neurons regulate energy balance primarily by controlling feeding behavior and energy expenditure. Furthermore, hypothalamic POMC attenuates comorbidities such as hyperglycemia, hyperinsulinemia, and hepatic steatosis (Bumaschny et al. 2012). Expression of POMC requires a sensitive leptin signaling pathway to reduce body weight and fat accumulation (Chhabra et al. 2016).

8 Conclusion

Leptin resistance appears to be caused by multiple mechanisms that may vary considerably among different obese patients. Leptin resistance ultimately may be minimized by attenuating UPR activation and ER stress, however it will be challenging to develop diagnostic approaches for the different forms of leptin resistance. Leptin receptor containing neurons comprise approximately less than 5% of all hypothalamic neurons. Therefore, it is challenging to identify cell-autonomous changes in gene transcription for any subset of neurons. Clearly more work will be necessary to detect the causative pathway for leptin resistance and to identify the hypothalamic gene targets of Ob-Rb and STAT3 signaling. Collectively, POMC, STAT3, SOCS3, PI3K and Ob-Rb transcriptions seem to be responsible for much of the leptin action, thereby may represent potential targets for therapy, in addition to shedding light on the mechanisms of leptin action for each leptin resistant obese.

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Influence of Antioxidants on Leptin Metabolism and its Role in the Pathogenesis of Obesity

17

Harald Mangge, Christian Ciardi, Kathrin Becker, Barbara Strasser, Dietmar Fuchs, and Johanna M. Gostner

Abstract

Obesity is associated with low-grade inflammation. Leptin, a hormone made by fat cells regulates appetite and hunger and thus food intake behavior. Interestingly, , food preservatives like sodium sulfite and sodium benzoate and also natural colorant and spice compounds such as curcumin were found to decrease the release of leptin in murine 3T3-L1 adipocytes, after co-incubation with LPS, which was added to mimic the pro-inflammatory status in obesity. Several of these compounds are well known food antioxidants.

Whilst reducing oxidation events is beneficial in states of elevated oxidative stress, overexposure to food antioxidant can lead to adverse effects. There are hints from *in vivo* data, that antioxidant stress in younger age plays a role in the development of adiposity in later life. The insufficient exposure to oxidizing compounds like reactive oxygen species (ROS) cannot only cause an insufficient burning of calories but there is also a link to the regulation of food intake behavior. If the *in vitro* findings can be extrapolated to the *in vivo* situation, consumption of antioxidant

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supplemented food could lead to decreased leptin release and contribute to an obesogenic environment. This aspect sheds some new critical light on the potential role of an antioxidant-enriched nutrition in the obesity epidemic during the past few centuries. Doing sports could represent not only a proper strategy to initiate physiological ROS production and burning of calories, but also may shift the hormone milieu towards a reduction of hunger feelings and thus reduce appetite and food intake.

Keywords

Antioxidants • Leptin • Obesity • Reactive oxygen species • Inflammation • Physical exercise • Satiety regulation • Tryptophan • Serotonin

1 Introduction

The diabesity pandemic, which describes the strong association of obesity and diabetes, further escalates in Western countries, and latterly also in Asia, especially in China (Califf 2009; Zimmet et al. 2001; Yu et al. 2015). It is predicted that by 2030, 86.3% of the US adult population will be overweight with 51.1% of those being obese (Wang et al. 2008).

In parallel to the obesity epidemic, the availability of different types of food from all over the world has increased, which also enhanced the daily intake of food additives due to the extensive use of preservatives. Antioxidant preservatives are used to prolong the shelf life of food and ensure the nutritional adequacy, palatability and safety. In addition, there is a trend concerning "health beneficial" supplements. In the 1999–2000 National Health and Nutrition Examination Survey, 52% of respondents reported taking a dietary supplement in the previous month and 47% of those contained an antioxidant [http://nccam.nih.gov/health/antioxidants/introduction.htm]. Currently, ascorbic acid supplements are marketed at concentrations up to 1000 mg, which is half the upper intake level and ten times the recommended daily allowance (RDA) set by the Food and Nutrition Board [http://lpi.oregonstate.edu/infocenter/vitamins/vitaminC/].

It is now well established that overweight/obesity and metabolic syndrome are directly linked to a systemic low grade chronic immune-mediated inflammation associated with a disturbed adipokine balance (Mangge et al. 2004, 2008, 2010; Pilz et al. 2005; Stelzer et al. 2012; Arnold et al. 2011; Drogan et al. 2015). This process is especially active in the visceral adipose tissue, and has been brought into connection with the most serious consequences of obesogenic lifestyle, i.e. cardiovascular disease and cancer (Mangge et al. 2010; Drogan et al. 2015).

A reductive milieu can influence the polarization of the adaptive immune response towards Th2-type immunity, as Th1- and Th2-type immune responses cross-regulate each other (Romagnani 2004; Lucey et al. 1996). Suppressive effects on Th1-type response pathways were observed in cultures of mitogen-stimulated human peripheral blood mononuclear cells upon treatment with antioxidant food preservatives like sodium sulphite, benzoate, and colorant curcumin showed the same effect as ascorbic acid (Winkler et al. 2006). Such mechanisms could provide new link between antioxidant uptake and the development of allergies (Zaknun et al. 2012; Murr et al. 2005; Tan et al. 2005).

As obesity usually leads to the activation of Th1 weighted pro-inflammatory pathways, the implication of an antioxidative trigger to the obesity related inflammation remains to be fully clarified (Rocha et al. 2008). Superficially viewed, a skew towards Th2 may be beneficial for atherosclerotic driven inflammatory processes. Nevertheless, more recent data took also a pro-atherogenic potential of Th2 cells into consideration (Ait-Oufella et al. 2009). Essentially,

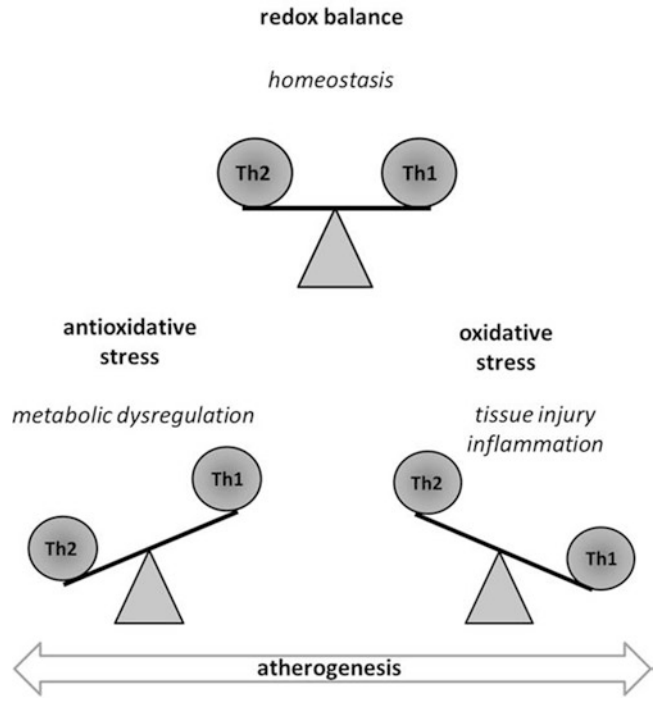

Fig. 17.1 Impact of the redox milieu on atherogenesis

the immunologic network of atherosclerosis is much more complex with regulatory T cells, Th17 cells and important components of the innate immunity, e.g. Toll-like receptors (TLR) 2, 4, 7, being centrally involved in the local immune responses of atherosclerotic vascular lesions (Salagianni et al. 2012; Brown et al. 2015; Liang et al. 2015; Lu et al. 2015).

Theoretically, antioxidants aid to relieve oxidative stress resulting from inflammation (Schroecksnadel et al. 2007; Mccall and Frei 1999). Nevertheless, research, generally or in association with weight loss studies, has not been able to demonstrate a clear benefit of antioxidant supplements and, furthermore, the concept of antioxidative stress was implemented (Poljsak and Milisav 2012). Bjelakovic et al. even proposed that supplements may raise overall morbidity (Bjelakovic et al. 2007). Antioxidants are reducing agents and neutralizers of reactive oxygen species (ROS) without molecular selectivity. Nevertheless, it is usually neglected that most antioxidant supplements are synthetic and some of them can even act as pro-oxidants under certain conditions (Sies 2014) e.g., when they reduce molecular oxygen to form the strongly reactive superoxide anion (O_2^-) (Bjelakovic and Gluud 2007).

Moreover, oxidative stress is not always an unfavorable effect. In fact, some consequences may act beneficial for physiological reactions in cells. Hence, potentially harmful effects of "antioxidative stress," especially in the cases of overconsumption of synthetic antioxidants have been proposed (Poljsak and Milisav 2012; Mangge et al. 2013). It has even been proposed that this could be a contributing factor for an increased risk of cancer (Bjelakovic et al. 2004a, b; Caraballoso et al. 2003) and cardiovascular diseases (Vivekananthan et al. 2003). Referring on this concerning attributes, clearly, further research of antioxidants is imperative.

Obesity has also been described as an addiction since many cerebral pathways of obesity/over-nutrition overlap with drug abuse (Volkow et al. 2012b). While central food intake regulation involves complex pathways and relationships between neuropeptides, monoamines

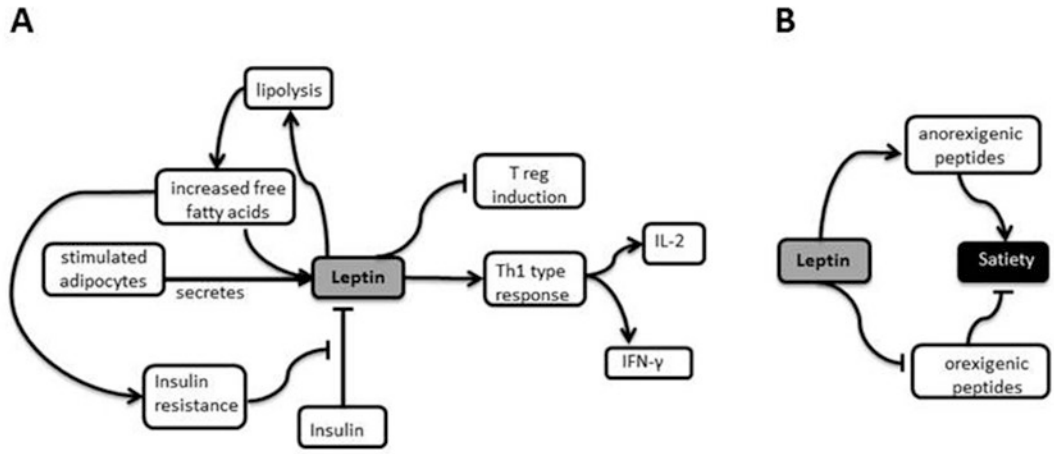

Fig. 17.2 Obesity-propagation via leptin via interaction with the immune system (**a**) satiety regulation (**b**)

and other brain messengers (Smith 2000), this chapter will focus on a specific pathway that links obesity-related chronic inflammation to the reward-deficiency syndrome, as well as to its main components: leptin, insulin, interferon-γ (IFN-γ) and tryptophan and its catabolites serotonin and melatonin. Furthermore, the potential interactions of antioxidant food additives in this pathway are discussed.

1.1 Over-Nutrition, Leptin, and the Adaptive Immune Response

The eating behaviour of the Western world is characterized by over-nutrition. Preferred foods like pastries, sweets, margarine, beverages, cheese and meat are supplemented with antioxidant preservatives like sodium sulfite (E221), benzoate (E211) and sorbate (E201) (Karasz et al. 1976; Luck 1990). It is well documented that obesity resulting from this lifestyle is associated with low-grade systemic inflammation and immune activation, which already starts in childhood (Mangge et al. 2004, 2010, 2013; Cottam et al. 2004). Thus, obesity may primarily represent an inflammatory disease and the chronic immune activation fundamentally contributes to the pathogenesis of co-morbidities such as atherosclerosis, diabetes mellitus, and coronary artery disease (Mangge et al. 2010, 2013).

Leptin, a pro-inflammatory adipokine that is produced in proportion to fat stores, plays a critical role in the early phase of obesity-related inflammation (Stelzer et al. 2012). Leptin drives $CD4^+$ T-cells towards a Th1-type phenotype, which secretes the pro-inflammatory cytokines interleukin-2 (IL-2) and IFN-γ (Fig. 17.2a). Of note, IFN-γ-deficient mice had decreased plasma leptin levels in comparison to the wild-type mice, indicating a possible positive feedback loop (Rocha et al. 2008). In addition, leptin inhibits regulatory T (Treg) cells. Moreover, it has been shown recently that leptin deficiency reduced the expression of dendritic cell (DC) maturation markers, and favoured the development of Treg and Th17 cells (Moraes-Vieira et al. 2014). All these facts identify leptin as a potent pro-atherogenic adipokine with reference to the immune-mediated facets of the atherosclerotic plaque associated inflammation.

Over-nutrition induces leptin mainly through increased plasma free fatty acid (FFA) concentrations and via stimulated adipocytes. The increase in FFA concentrations favours oxidative stress, inflammation, vascular endothelial dysfunction, and insulin resistance by blocking insulin signals (Cascio et al. 2012; Riccardi et al. 2004). Since insulin acts as a leptin suppressor, insulin resistance allows the production of leptin to continue (Dandona et al. 2005). This was further supported in a study by Pacifico et al. which showed that

insulin resistance was linked to a Th1-type cytokine profile in obese children (Pacifico et al. 2006). In addition to activating Th1-type cells, leptin amplifies lipolysis, and thus FFA concentrations (Dandona et al. 2005).

Serum leptin concentration increases with weight gain and corresponds to the size of adipose tissue (Considine et al. 1996; Saladin et al. 1995; Hamilton et al. 1995). The fact that leptin levels do not correlate with meal intake and related insulin peaks, and instead run in a circadian rhythm (Anubhuti and Arora 2008), argues against the hypothesis that leptin directly induces satiety and satiation (Considine et al. 1996; Jequier 2002). It has been shown that leptin only indirectly causes satiety by inducing the anorexigenic peptides α-melanocyte-stimulating hormone (α-MSH), cocaine- and amphetamine-related transcript (CART), corticotropin-releasing hormone (CRH), and suppressing the orexigenic neuropeptides agouti-related peptide (AGRP), orexin and neuropeptide Y (NPY)—one of the most potent orexigenic peptides in the hypothalamus (Jequier 2002; Ramos et al. 2005) (Fig. 17.2b). This indirect satiety effect, with either endogenous or exogenous leptin, is significantly lower in obese humans, which is referred to as leptin resistance (Flier 2012). Leptin resistance has been associated with insulin resistance and an increased pro-inflammatory state (Lago et al. 2008; Scarpace and Zhang 2009). Current hypotheses for the origin of leptin resistance discuss defects in the blood-brain barrier (BBB) transport system, mutated forms of the leptin receptor, and/or an inhibition of leptin signaling mechanism in the hypothalamus (Considine et al. 1996; Maffei et al. 1995; Heymsfield et al. 1999; Banks et al. 1996; Caro et al. 1996; Schwartz et al. 1996; Abhilash 2010; Vaisse et al. 1996; Bjorbak et al. 2000; Bjorbaek et al. 2001; Jequier 2002). Peruzzo et al. postulated that leptin can access the medial basal hypothalamus without using active transport to cross the BBB (Peruzzo et al. 2000). From this, leptin resistance would originate from a signaling defect (Bates and Myers 2003). Furthermore, a sessile lifestyle favors hypoxia and reduces the production of beneficial adipokines, like adiponectin. Pro-inflammatory adipokines like leptin increase during long-term exposure of fat cells to hypoxia (Netzer et al. 2015). This favors the chronic inflammation of obesity.

1.2 The Antioxidant Impact on Leptin and Th1-type Cells

Here, we describe the effect of antioxidants on leptin from the viewpoint that decreasing leptin plasma levels is beneficial to reduce the inflammation cascade that ensues as a result of increased leptin. However, we must also recognize that any beneficial antioxidant effect on leptin is an imperfect solution because leptin is probably increased in obese people to compensate for the diminished leptin satiety effect/ leptin resistance.

Many antioxidant food additives and colorants have been shown to decrease both leptin concentrations and Th1-type activity. Specifically, sodium benzoate, sodium sulphite, and curcumin decreased leptin production in a dose-dependent manner in lipopolysaccharide-stimulated murine adipocytes derived from NIH-3T3 cells (Ciardi et al. 2012). Moreover, like other antioxidant compounds such as vitamins –vitamin C (ascorbic acid) and E (tocopherol)- anti-inflammatory compounds like salicylic acid and acetyl salicylic acid (aspirin) and stilbene derivative resveratrol, common food preservatives such as sodium sulphite, sodium benzoate, propionic acid and sorbic acid but also typical colorants like curcumin, dose-dependently suppressed key features of Th1-type activity, i.e. the IFN-γ-dependent pathways of neopterin production and tryptophan breakdown (Winkler et al. 2006; Wirleitner et al. 2005) (Fig. 17.3). Because of the suppressive effects on Th1-type immunity, these antioxidants may contribute to the upregulation of Th2-type immunity, thereby supporting allergy development (Zaknun et al. 2012).

In addition, Ristow et al. investigated how antioxidant supplements block the benefits of physical exercise comparing trained and untrained participants (Ristow et al. 2009). Exercise enhances ROS production in skeletal muscle (Mrakic-Sposta et al. 2015), which

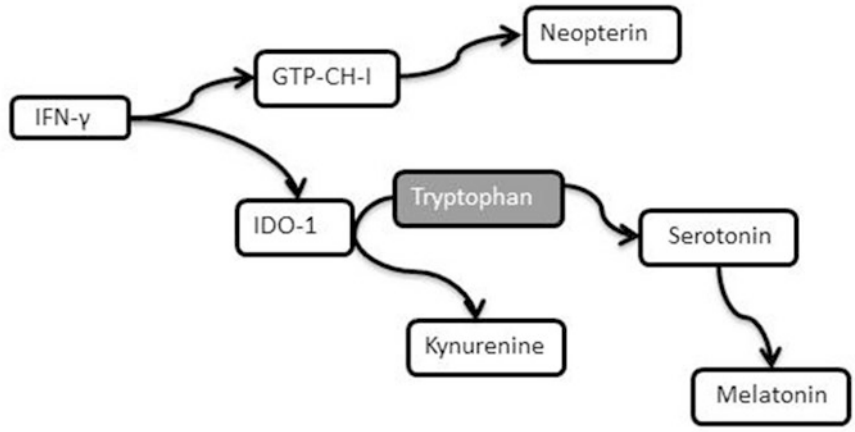

Fig. 17.3 Interferon γ activates formation of the oxidative stress marker neopterin via enzyme GTP-cyclohydrolase (GTP-CH-I) and accelerates tryptophan breakdown along the kynurenine axis due to induction of indoleamine 2,3-dioxygenase (IDO-1) activity. Thus, less tryptophan is available for serotonin and melatonin synthesis

increases insulin sensitivity (Powers and Jackson 2008). Antioxidant supplementation during exercise diminishes ROS levels and thus the induction of ROS detoxifying enzymes like superoxide dismutase and glutathione peroxidase. This positive effect of exercise is significantly attenuated and insulin sensitivity remains unaffected (Peternelj and Coombes 2011; Ristow et al. 2009). As insulin resistance positively influences leptin levels, it can be assumed that an increased leptin production would also not be effectively diminished. Thus, an originally well-intentioned oversupply with supplemented antioxidants may significantly debilitate the endogenous antioxidative protection system over time.

However, this conclusion maybe different in endurance sports vs. recreational sports: The ROS detoxifying enzyme machinery is elicited dependent on the extent of ROS excess. After administration of extra antioxidants, the amounts of ROS will at least partly be detoxified and thereby the oxidizing capacity due to exercise is hampered as is the fat and carbohydrate burning capacity. As a consequence, also the levels of ROS detoxifying enzymes like superoxide dismutase and glutathione peroxidase will be slowed down (Burtscher et al. 2015). To avoid this scenario and if the intention of doing sports is the burning of calories (fat and carbohydrates), the intake of antioxidant supplements like vitamins should be postponed after the periods of exercise, best to training-free days. One may speculate that a different strategy is superior for competitive sports or endurance sports when antioxidant supplements may be able to counteract the insults, which may derive from the inflammation responses from damaged muscle tissue.

Still, the pathways remain unclear by which physical fitness or diet affect the psychological stress/leptin relationship. Evidence suggests that vigorous physical fitness moderates the levels of leptin concentrations, regardless of relevant confounders including total body fat (Jimenez-Pavon et al. 2012). Regular exercise results in higher circulating levels of adiponectin and lower levels of several circulating pro-inflammatory adipokines, including leptin (Ben Ounis et al. 2009). Thus, increased physical activity has the power to reduce systemic low-grade inflammation via a decrease in pro-inflammatory adipokine secretion, which is a direct result of a reduction in abdominal fat mass (Gleeson et al. 2011), and thereby affect the mental stress/leptin relationship.

1.3 IDO-1 Regulates Tryptophan Conversion—A Key Aspect in Caloric Consumption

As a precursor for serotonin (5HT) and melatonin, which are both known satiety and satiation regulators, the essential amino acid tryptophan is a key player in caloric intake regulation (Brandacher et al. 2007; Nduhirabandi et al. 2012). Obese patients far exceed the required minimum daily intake of tryptophan (200 mg), yet the circulating levels of tryptophan are low (Brandacher et al. 2006) and the patients continue to over-consume. Upregulated indoleamine 2,3-dioxygenase (IDO-1) activity by increased Th1-type inflammation decreases the circulating tryptophan levels and ultimately can underlie these effects (Brandacher et al. 2007) (Fig. 17.3).

Serotonin inhibits NPY expression, regulates carbohydrate (Blundell and Lawton 1995; Bray 2001) and fat intake (Halford and Blundell 2000; Hagan et al. 1997), and relieves stress—another caloric intake trigger (Buwalda et al. 2001). In addition, Buwalda et al. found that 5-hydroxytryptamine receptor 1A (5-HT1a) receptors in the central nervous system were desensitized in animals receiving a carbohydrate-based diet (Buwalda et al. 2001). This illustrates one aspect of the "reward-deficiency syndrome": the more carbohydrates a person eats, the more desensitized they will become to serotonin, experiencing delayed satiety, and thus eating more. This syndrome can also be catalysed by a lack of tryptophan for serotonin conversion due to upregulated IDO-1 activity, which converts more tryptophan to kynurenine (Brandacher et al. 2007), and/ or a perceived lack of tryptophan due to defective serotonin receptors/ transporters. Since tryptophan is an essential amino acid that can only be obtained from the diet, a person would crave tryptophan-rich foods like eggs, cheese, flour, etc. It should be noted that the tryptophan levels obtained from these foods wane in comparison to the effect of carbohydrates promoting insulin secretion, which decreases the large neutral amino acid (LNAA) levels, allowing more tryptophan to cross the BBB and to be decarboxylated into serotonin (Wurtman and Wurtman 1995). Moreover, under certain circumstances, IDO-1 can also cleave and degrade other indoleamine derivatives like serotonin as well as melatonin, however with fewer efficacies than tryptophan (Ferry et al. 2005).

Antioxidative compounds like vitamins C and E but also foods and beverages rich in antioxidants like cacao, coffee, wine and beer were observed to suppress Th1-type immune activation *in vitro* (Zaknun et al. 2012). Similar effects were revealed with food supplements like preservatives sodium sulphite and benzoate as well as colorants, e.g., beet root and curcuma, which mainly comprise chemical antioxidants. As a consequence, tryptophan breakdown becomes slowed down and tryptophan availability for serotonin production will be improved. This background may have contributed to a preferred intake of those foods that contain higher levels of antioxidants and thus probably have a mood enhancing effect. Thus, weight gain of the general population during recent decades could at least partly result from the by-passing of hunger regulation by food additives and antioxidant supplements (Fuchs 2012).

Melatonin, one important regulator of sleep, also plays a critical role in the pathology of obesity. This is underlined by the observation that chronic sleep deprivation and altered quality of sleep were found to be associated with the obesity epidemic (Van Cauter and Knutson 2008; Cizza et al. 2011). Sleep duration has been inversely linked to leptin levels and food consumption in chronic, temporal and "rollercoaster" sleep deprivation (Van Cauter and Knutson 2008). Sleep deprivation also upregulates orexin activity, which then activates NPY and induces hunger (Van Cauter and Knutson 2008; Wu et al. 2002; Estabrooke et al. 2001; Zeitzer et al. 2007). In middle-aged rats, melatonin supplementation reduced the body weight, visceral adiposity, and plasma insulin and leptin levels to youthful levels (Rasmussen et al. 1999; Wolden-Hanson et al. 2000). Kitagawa et al. showed that melatonin supplements ameliorated insulin resistance in rats (Kitagawa et al. 2012). This effect is probably due to the fact that melatonin inhibits insulin secretion in rat pancreatic islets during sleep,

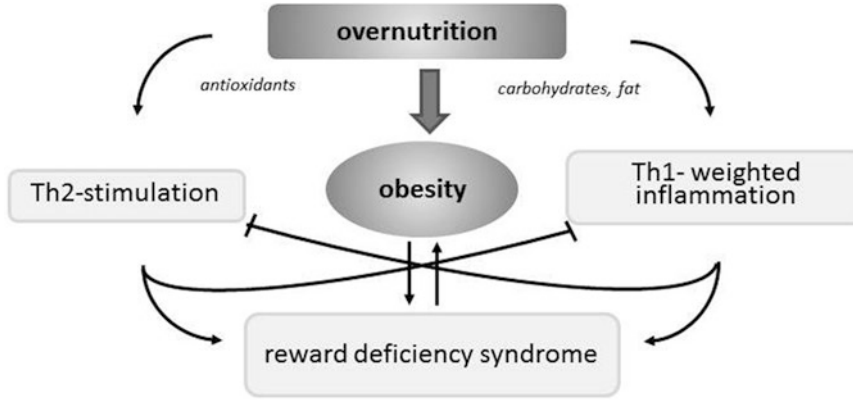

Fig. 17.4 Overnutrition leads to the dysregulation of metabolic and immunological pathways. These changes trigger the reward deficiency syndrome, thereby promoting the intake of more, preferentially antioxidant-enriched food (adapted according Mangge et al. 2013)

allowing them to rest and rejuvenate (Picinato et al. 2002; Cizza et al. 2011; Peschke 2008). Furthermore, melatonin has been found to increase the recruitment and metabolic activity of brown adipocytes in mammals (Tan et al. 2011).

From this, it is critical to regain normal melatonin and serotonin levels by down-regulating IDO-1 activity associated with Th1-type activity and high leptin levels. This could alleviate the "reward-deficiency syndrome" that perpetuates obesity. On the basis that antioxidants have been shown to decrease leptin levels and Th1-type immunity, which both lead to the obesity-propagation cycle, antioxidants have been discussed to be beneficial in weight loss (Bahadoran et al. 2012). However, on the other hand, so far the fact has been neglected that the rise in tryptophan hydroxylation and subsequent decarboxylation to serotonin indirectly caused by antioxidant food supplements could also spark a positive feedback response wherein a person craves too much food highly supplemented with antioxidants to get a heightened/prolonged reward-sensation (Fig. 17.4). Moreover, even better than a diet rich in tryptophan, a diet enriched with antioxidants increases tryptophan availability and serotonin production in the brain. Unlike tryptophan-rich diet, which usually contains also other amino acids at high concentrations including LNAA, antioxidants solely support an increase of available tryptophan and thus the transport across the blood-brain-barrier. In addition, also tetrahydrobiopterin (BH4), the necessary cofactor of tryptophan 5-hydroxylases (TPH) for the production of serotonin, is stabilized in a reductive environment. Thus, antioxidant compounds are able to support serotonin production in many ways. Accordingly, it would be worthwhile to conduct studies, which investigate more in depth such potential negative and positive effects of antioxidant supplements.

1.4 Addiction, Inflammation and Obesity

Drug addiction and obesity share strikingly similar neural activity and properties. They can both be defined as disorders that cause a person to compulsively and impulsively seek a specific type of reward (food or drug), at the expense of all other rewards (health, social acceptance, etc.). The key players discussed so far in this review—insulin, leptin, serotonin and NPY—have also been implicated in the food reward circuitry, in context of addiction behavior and dopamine. Dopamine, one of the central mediators in the brain for the universal metabolic and reactivity system, potentially causes the loss of control seen in both addiction and obesity (Volkow et al. 2012a). Virtually all neurons in the ventral tegmental area (VTA) synthesize dopamine (Opland

et al. 2010) and many of them also express receptors for leptin (Figlewicz et al. 2003; Leshan et al. 2010), insulin (Figlewicz et al. 2008), and orexin (Fadel and Deutch 2002). These hormones/ neuropeptides are centrally involved in the rewarding effects of drug abuse and obesity (Davis et al. 2008; Wellman et al. 2007). Dopamine 2 receptor (D2R) activity has been linked to central (i.e. hypothalamic) leptin signaling (Kim et al. 2010). D2R knock-out mice [D2R(−/−)] displayed an increased leptin sensitivity, and showed reduced food intake and body weight while displaying an increased basal energy expenditure level, compared with their wild type littermates (Kim et al. 2010). Moreover, hypothalamic NPY has been linked to alcoholism, another addiction disorder (Alvaro et al. 1997; Volkow et al. 2012a), and there is also some relevance of an accelerated tryptophan breakdown rate in patients suffering from alcoholism (Gleissenthall et al. 2014). In addition, the nicotine withdrawal behavioral characteristics—mood disturbances, weight gain, and carbohydrate craving—have been associated with impaired serotonin release (Benwell et al. 1988).

The hippocampus is known for its critical role in learning and short-term memory function, and has a high density of insulin receptors (Eichenbaum 2006; Squire 2004; Palovcik et al. 1984). With insulin secreted in proportion to body fat mass, this could be another mechanism leading to the addiction-like behavior in obesity—learning and remembering the desired food that led to euphoria, reinforcing the destructive behavior (Benoit et al. 2004). Thus, compared to lean individuals, obese people obviously have complex alterations of brain reward circuitries, involved in food consumption (Stice et al. 2008; Kenny 2011). A discrepancy between the anticipation and actual reward most likely sustains an over-consumption behavior (Volkow et al. 2012b). However, based on the available studies it is far too simple to conceptualize both obesity and overeating as a food addiction accompanied by corresponding brain changes. There are several fundamental questions still open, which remain to be clarified by future more in depth investigations (Ziauddeen et al. 2012). A recent study from our group used functional magnetic resonance imaging (fMRI) to identify common features between these two conditions. There was a significant positive relationship between plasma insulin levels, waist circumference, and hippocampal activation after stimulation with high-caloric food images. Interestingly, only the waist circumference, and not the BMI, correlated significantly with the hippocampal activation patterns (Wallner-Liebmann et al. 2010). Rocha et al. showed that chronic, Th1-weighted inflammation was preferentially found in abdominal fat tissue (Rocha et al. 2008). Thus, our observation underlines the importance of abdominal fat accumulation for obesity-associated pathologic burden and the potential involvement of Th1-type inflammation in high-calorie food cravings.

1.5 Antioxidative "Stress" in Obesity—Summary of a New Hypothesis

We have summarized that antioxidants, which are abundantly found in the Western diet as preservatives and additives, possess the capacity to influence obesity-related inflammation. More importantly, we have hypothesized a vicious cycle leading to uncontrolled weight gain that is related to so far neglected negative effects of antioxidants (Fig. 17.4). As over-nutrition causes greater Th1-type activity and oxidative stress, the body might attempt to compensate by craving more antioxidants highly supplemented in processed food. Thus, the vicious cycle, creating the disparity between expectation and reward in the reward-deficiency syndrome may be essentially propagated. These cravings may be further heightened by the obesogenic environment—advertisements, constant food availability, and social behavior.

As mentioned, serotonin and thus melatonin production is decreased during Th1 type responses. Both serotonin and melatonin have been inversely correlated with weight gain. Antioxidants can be beneficial in this context by decreasing Th1-type activity and tryptophan breakdown via IDO-1 and by increasing life-span

of BH4. However, potential harmful effects have to be considered. Studies in murine cells have shown antioxidants to reduce leptin levels (Ciardi et al. 2012). Hence, antioxidants may interfere with the body's natural satiety and satiation response. As excessive antioxidant food might increase a person's comfort by increasing the amount of tryptophan available for serotonin synthesis, this person might enter a continual eating cycle to sustain this effect. Additionally, antioxidant supplements diminish the benefits of physical exercise (Ristow et al. 2009; Mangge et al. 2013). This correlates with the theory that antioxidants, which cannot distinguish between toxic and beneficial ROS species, decrease the natural hormesis (Poljsak and Milisav 2012; Nakamura et al. 2010). Finally, antioxidant food supplements, preservatives and colorants seem to play a role in the pathogenesis of allergy and asthma by promoting a Th2-weighted immune response (Zaknun et al. 2012). Thus, the amount of dietary antioxidants could represent the cutting edge for the co-occurrence of adiposity and allergy, which is nowadays well recognized (Flaherman and Rutherford 2006).

Our hypothesis has several limitations. First, it has to be acknowledged that there are considerable differences between humans and mice especially regarding the biochemistry of biogenic amine neurotransmitters as well as nitric oxide radical. In keeping with this, under inflammatory conditions, the biosynthesis of biogenic amines like serotonin or dopamine have been shown to become easily disturbed in humans (Neurauter et al. 2008; Haroon et al. 2012), but less so in animal models. Consequently, any direct conclusion for immune activation and inflammation-associated conditions need to be scrutinized. Moreover, several reported studies on the bioactivites of antioxidants were performed in cell cultures and it still has to be investigated whether these findings can be extrapolated to the *in vivo* situation. Also, the actual immune state of an individual has to be considered when evaluating the effect of controlled antioxidant intake (Gostner et al. 2014). Antioxidant supplements are highly prevalent in the "Western diet", without further consideration of criticism. Interestingly, except in one study investigating patients at risk of cardiovascular events (Violi et al. 2004), nobody has claimed so far that an individual's oxidative stress level must first be evaluated to determine if antioxidant supplements are beneficial. Finally, to our knowledge, no one knows how many antioxidants an average person consumes or actually needs per day.

2 Conclusion

The best recommendation for the prevention of obesity is to maintain a healthy lifestyle through balanced nutrition and physical activity, but this may be impossible to adhere to, once a person enters the circular reward-deficiency syndrome. For this, an escape route from the cycle is of dire importance in an age marred by obesity in industrialized countries. As current drugs fail to sustainably curb human obesity, it is essential to understand underlying key pathomechanisms. Thus, the latest hype for antioxidants, the multitude of modern foods supplemented with them, and their obvious ambivalent role in obesity-related inflammation, highlights the need for further studies (Mangge et al. 2013).

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Adiponectin-Resistance in Obesity

18

Atilla Engin

Abstract

The decrease in adiponectin levels are negatively correlated with chronic subclinical inflammation markers in obesity. The hypertrophic adipocytes cause obesity-linked insulin resistance and metabolic syndrome. Furthermore, macrophage polarization is a key determinant regulating adiponectin receptor (AdipoR1/R2) expression and differential adiponectin-mediated macrophage inflammatory responses in obese individuals. In addition to decrease in adiponectin concentrations, the decline in AdipoR1/R2 mRNA expression leads to a decrement in adiponectin binding to cell membrane, and this turns into attenuation in the adiponectin effects. Within the receptor complex, adaptor protein-containing pleckstrin homology domain, phosphotyrosine-binding domain, and leucine zipper motif 1 (APPL1) is the intracellular binding partner of AdipoR1 and AdipoR2. The expression levels of APPL1 or APPL2 lead to an altered adiponectin activity. Despite normal or high adiponectin levels, an impaired post receptor signaling due to APPL1/APPL2 may alter adiponectin efficiency and activity. However, APPL2 blocks adiponectin signaling through AdipoR1 and AdipoR2 by competitive inhibition of APPL1. APPL1 is also an important mediator of adiponectin dependent insulin sensitization. In this context, adiponectin resistance is associated with insulin resistance and is thought to be partly due to the down-regulation of the AdipoRs in high-fat diet fed subjects. Actually, adiponectin resistance occurs very rapidly after saturated fatty acid feeding, this metabolic disturbance is not due to a decrease in AdipoR1 protein content. Intra-abdominal adipose tissue-AdipoR2 expression is reduced in obesity, whereas AdipoR1 expression is not changed. Adiponectin resistance together with insulin resistance forms a vicious cycle. The elevated adiponectin levels with adiponectin resistance is a compensatory response in the condition of an unusual discordance between insulin resistance and adiponectin unresponsiveness.

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Additionally, different mechanisms are involved in vascular adiponectin resistance at different stages of obesity. Nevertheless, diet-induced hyperlipidemia is the leading cause of vascular adiponectin resistance. Leptin/adiponectin imbalance may also be an important marker of the elevated risk of developing abdominal obesity-associated cardiovascular diseases.

Keywords

Obesity • Adiponectin • High molecular weight (HMW) adiponectin • Globular adiponectin • Insulin resistance • Hyperadiponectinemia • Adiponectin receptor R1/R2 (AdipoR1/R2) • Adaptor protein-containing pleckstrin homology domain, phosphotyrosine-binding domain, and leucine zipper motif 1 (APPL1) • APPL1/APPL2 • Adenosine monophosphate (AMP)-activated protein kinase (AMPK) • Tumour necrosis factor-alpha (TNF-alpha) • Endothelial nitric oxide synthase (eNOS)

1 Introduction

Scherer et al. described a novel 30-kDa adipocyte complement-related protein (Acrp30), that is made exclusively in adipocytes. This molecule is called adiponectin and structurally similar to complement factor C1q. Secretion of adiponectin is enhanced by insulin. Actually its mRNA is induced over 100-fold during adipocyte differentiation (Scherer et al. 1995). Adiponectin is present in the circulation of healthy individuals at very high concentrations accounting for approximately 0.05% of total plasma proteins, with levels ranging from 3 to 30 μg/mL (Arita et al. 1999; Shibata et al. 2009). The median concentrations of plasma adiponectin are 5.5 μg/mL in men and 8.7 μg /mL in women. The subjects are divided into two groups according to their adiponectin concentrations using a cut-off point of 4.0 μg / mL. In men with an adiponectin concentration less than 4.0 μg /mL showed a higher prevalence of abdominal obesity. The prevalence of the metabolic syndrome is higher in subjects with an adiponectin concentration less than 4.0 μg /mL than in those with a concentration more than 4.0 μg / mL. In low concentration group, metabolic syndrome occurs at a rate of 52.3% and 37.5% in men and in women, respectively. Furthermore, the relative risk of adverse cardiovascular events is 1.56-fold higher among patients with the lower adiponectin levels than in those with the higher levels (Ryo et al. 2004). Mean preoperative fasting adiponectin concentration is significantly increased in response to weight loss after bariatric surgery. The plasma adiponectin levels correlate negatively with fat mass. Each kilogram of fat mass lost corresponds to an increase in plasma adiponectin concentration by approximately 6%. The subjects in obese state show no variations in adiponectin plasma concentration fluctuations during the evening and night, whereas in the post-obese state diurnal variation of adiponectin is restored again (Calvani et al. 2004). In obese patients, short-term weight loss does not change total adiponectin levels and insulin resistance, while the distribution pattern of adiponectin oligomers changes due to significant increment of high molecular weight adiponectin and reduction of medium molecular weight adiponectin. Especially, high molecular weight adiponectin is promptly responsive to short-term weight loss prior to changes in insulin resistance (Mai et al. 2014). According to adiponectin hypothesis reduced adiponectin levels can be caused by genetic factors or high-fat diet. In this context, decreased adiponectin signaling or disruption of adiponectin receptors may serve as an upstream pathway of increased oxidative stress, monocyte chemoattractant protein-1 (MCP-1) expression and inflammation in white adipose tissue (Yamauchi et al. 2007). In obese subjects, the

hypertrophic adipocytes cause obesity-linked insulin resistance and metabolic syndrome. The decrease in adiponectin receptors (AdipoR1/R2) mRNA expression leads to a decrement in adiponectin binding, and this turns into attenuation in the adiponectin effects. Consequently, adiponectin resistance together with insulin resistance forms a vicious cycle (Yamauchi and Kadowaki 2008). Recently, new treatment strategies are being developed for the improvement of adiponectin resistance. The up-regulation of the expression and stimulation of adiponectin receptors by using adiponectin receptor agonists would be an effective method to treat obesity-related conditions. However, current drug development based on adiponectin and adiponectin receptors for clinical applications is scarce, and there is a lack of clinical trial data availability (Hossain et al. 2015). Dual activation of peroxisome proliferator-activated receptor-alpha (PPAR-alpha) and peroxisome proliferator-activated receptor-gamma (PPAR-gamma) enhances the action of adiponectin by increasing both adiponectin and AdipoRs, which can result in the amelioration of obesity-induced inflammation and insulin resistance (Tsuchida et al. 2005). Despite the conflicting evidences, leptin administration within the sub-physiological to physiological range increases circulating levels and adipose tissue mRNA expression of adiponectin (Hoffmann et al. 2016). Different insights and existing contradictions about adiponectin resistance could be reconciled by explaining the details of related metabolic pathways. In this respect, this chapter will attempt to clarify the mechanisms and contributing factors of adiponectin resistance in obesity.

2 Regulation of Adiponectin Expression

Adiponectin is found in serum in number of complexes which include trimers and hexamers. These are collectively defined as low-molecular weight (LMW) trimer, medium molecular weight (MMW) hexamer and high-molecular weight (HMW) multimers (Waki et al. 2003; Whitehead et al. 2006).Trimeric and HMW/hexameric adiponectin activate different signal transduction pathways. The hexamer consists of two adjacent trimeric globular domains and a single stalk composed of collagen domains from two trimers. Although they are not necessary for trimer formation or stability, two of the three monomers in an adiponectin trimer are covalently linked by disulfide bonds between cysteine residues (Tsao et al. 2003). The concentrations of total adiponectin and HMW adiponectin are 25% and 45% lower, respectively, in obese children compared to controls. Thereby, HMW to total-adiponectin ratio is lower in the obese children than in the controls, whereas LMW adiponectin/total adiponectin ratio is higher in the obese as compared to the normal-weight children. These ratios are reversed with weight loss in obese patients after weight-reduction programme (Gajewska et al. 2011). HMW complexes circulating in serum represent a precursor pool. Activation of HMW complex is triggered by metabolic stimuli, which may subsequently trigger the induction or activation of a serum reductase. Trimeric adiponectin has a significantly shortened half-life when compared with the endogenous oligomeric complexes. Active adiponectin may be subjected to proteolysis by membrane-bound proteases that are found on the cell surface of target cells. This process leads to the formation of final active ligand that is rapidly cleared (Pajvani et al. 2003).

The adiponectin cDNA encodes a polypeptide of 247 amino acids with a secretory signal sequence at the amino terminus, a collagenous region and a globular domain. The expression of adiponectin is observed exclusively in mature fat cells. By contrast, the stromal-vascular fraction of fat tissue does not contain adiponectin mRNA. Hormone-induced differentiation of pre-adipocytes dramatically increases the level of adiponectin expression, however, the expression of adiponectin mRNA is significantly reduced in the adipose tissues from obese humans (Hu et al. 1996). Acute hyperinsulinaemia results in a significant reduction of total adiponectin. In this case, HMW adiponectin does not change, whereas MMW adiponectin and LMW adiponectin decrease. In the state of obe-

sity, both hyperinsulinaemia and hyperlipidaemia contribute to low adiponectin levels (Bobbert et al. 2009). Regulation of adiponectin expression in non-adipose tissues is different from that in adipose tissue. Thus, adiponectin is up-regulated in human skeletal muscle in response to inflammatory stimuli. The underlying mechanisms may involve the inducible nitric oxide synthase (iNOS)-dependent pathway (Delaigle et al. 2004).

Inhibition of mitogen-activated protein kinase kinase (MAPKK)/extracellular signal-regulated kinase (ERK) 1/2 pathway decreases intracellular and secretory adiponectin levels in adipocytes, whereas adiponectin gene expression is increased. Thereby, MAPKK/ERK1/2 pathway activity plays a critical role in controlling adiponectin protein turnover in adipocytes via the ubiquitin-proteasome pathway. Thus, in adipose tissues of high-fat diet fed mice, ubiquitinated adiponectin protein levels are significantly higher (Gu et al. 2013).

The decrease in adiponectin levels are negatively correlated with chronic subclinical inflammation markers in obesity. Weight loss in morbidly obese patients induces a significant rise in adiponectin, while decreases the chronic inflammatory markers (Kopp et al. 2005). In these cases, profound reducing effects of interleukin-6 (IL-6) plus IL-6 receptor (IL-6R) and tumour necrosis factor-alpha (TNF-alpha) are shown on adiponectin mRNA levels. The inverse relationship between the plasma adiponectin and cytokines *in vivo* and the cytokine-induced reduction in adiponectin mRNA *in vitro* suggest that endogenous cytokines may inhibit adiponectin expression (Bruun et al. 2003). While TNF-alpha, IL-6, and IL-18 downregulate adiponectin production, transcription factors such as PPAR-gamma, CCAAT-enhancer-binding protein (C/EBP) alpha, and forkhead box transcription factor 1 (FOXO1) upregulate the adiponectin expression (Hino and Nagata 2012). In this respect, Vilarrasa et al. showed that massive weight loss induced by gastric bypass in a series of 65 morbidly obese patients had reduced IL-18, TNF-alpha receptors, and C-reactive protein (CRP). However, there was no relationship between either adiponectin and IL-18 or TNF-alpha receptors and CRP. This result suggests that IL-18 and TNF-alpha receptors do not play a remarkable role in the inhibition of the adiponectin production (Vilarrasa et al. 2007).

Leptin secretion rises acutely during feeding or in response to over-nutrition as adipocytes expand in size and number (Unger 2002), whereas adiponectin secretion is increased during under-nutrition and exercise. In the latter case, adipocytes tend to diminish in size because lipolysis is most active in this period (Miyazaki et al. 2010). Actually, leptin and adiponectin have similar lipo-oxidative actions (Unger 2002). In contrast to leptin, adiponectin levels are significantly reduced among obese subjects in comparison with lean controls. Although the majority of adiponectin is secreted from adipose tissue, the mean plasma adiponectin levels are found significantly lower in obese patients (Arita et al. 1999). In this context, leptin and adiponectin are inversely correlated in obesity. However, leptin can not participate directly in adiponectin synthesis. Nontheless, the long-term regulation of adiponectin expression in white adipose tissue appears to be the opposite of that of leptin. Additionally, adiponectin levels are more sensitive to changes in adiposity or insulin sensitivity (Zhang et al. 2002).

3 Adiponectin Receptor Complex and Adiponectin Resistance

Adiponectin exerts its effects by binding to adiponectin receptors, two of which are AdipoR1 and AdipoR2 (Yamauchi et al. 2014). Adiponectin receptors have a suitable structure related to their functions. The seven transmembrane helices of AdipoR1 form a helix bundle, which are arranged circularly in a clockwise manner, from helix-1 to helix-7. The structure of AdipoR2 is quite similar to that of AdipoR1. In both, the AdipoR1 and AdipoR2 structures surround a large internal cavity. This cavity is formed between the four- and three-helix subdomains. Adiponectin may broadly interact with the extracellular face of

receptor, rather than the carboxy-terminal tail of the receptors. Moreover, the adiponectin-binding sites seem to be shared by AdipoR1 and AdipoR2 (Tanabe et al. 2015). In addition to AdipoR1 and AdipoR2, T-cadherin has been identified as a receptor for the HMW form of adiponectin, but not for trimeric or globular adiponectin (gAD). Although lacking in the known cellular functions, T-cadherin is expressed in endothelial and smooth muscle cells, where it is positioned to interact with adiponectin (Hug et al. 2004). Circulating levels of adiponectin, particularly HMW form of adiponectin are elevated in T-cadherin-deficient mice (Denzel et al. 2010).

Actually, adiponectin receptors bind to globular and full-length adiponectin and mediate increased adenosine monophosphate (AMP)-activated protein kinase and PPAR-alpha ligand activities. Thereupon, adiponectin also enhances glucose uptake and fatty-acid oxidation (Kadowaki and Yamauchi 2005). Basically, the adiponectin receptors are transmembrane receptors that undergo conformational changes and couple the intracellular domain with other signaling molecules upon extracellular adiponectin binding. Adaptor protein [adaptor protein containing pleckstrin homology (PH) domain, phosphotyrosine binding (PTB) domain and leucine zipper motif (APPL)] directly binds to the intracellular domains of AdipoR1 and AdipoR2 via its C-terminal PTB and CC domains (Ruan and Dong 2016). The first identified adaptor proteins that interact directly with adiponectin receptors are APPL1 and APPL2 (Mao et al. 2006). Within the receptor complex, APPL1 is the intracellular binding partner of AdipoR1 and AdipoR2 (Mao et al. 2006). Furthermore, the general function of PTB domain is to act as an adaptor or scaffold for the binding of proteins, particularly those in signaling pathways. The PTB domain of APPL1 is located near the -COOH terminus, away from BIN-Amphiphysin-Rvs (BAR)-PH domain. Therefore, PTB is an easily accessible structure for its binding partners (Deepa and Dong 2009). APPL1 mediates the actions of adiponectin in the regulation of energy metabolism and insulin sensitivity (Ruan and Dong 2016). Thus, insulin-stimulated activation of protein kinase B (Akt) and suppression of gluconeogenesis in hepatocytes are enhanced by APPL1 overexpression, but are attenuated by APPL1 deficiency. In this context, APPL1 is an important mediator of adiponectin dependent insulin sensitization in skeletal muscle. Decreased APPL1 expression reduces insulin-dependent Akt activation. Moreover, while adiponectin treatment itself does not affect Akt phosphorylation, co-treatment of myotubes with both adiponectin and insulin leads to a synergistic increase in Akt phosphorylation (Hosch et al. 2006). APPL1 also plays an important role in insulin-stimulated insulin-responsive glucose transporter-4 (GLUT4) translocation in muscle and adipose tissues and its N-terminal portion may be critical for APPL1 function. Thereby, deficiency of APPL1 attenuates insulin-mediated Akt phosphorylation, glucose uptake and GLUT4 translocation in adipocytes. APPL1 is localized mainly in the cytosol, and it shows a small degree of re-localization to the microsomes and nucleus in response to insulin (Saito et al. 2007b) (Fig. 18.1).

Eventually, the expression levels of APPL1 or APPL2 lead to an altered adiponectin activity. Decreases in AMP-activated protein kinase (AMPK) activation, hepatic glucose uptake and free fatty acid oxidation increase the de novo lipogenesis and gluconeogenesis in liver. Final stage of this process involves intrahepatic lipid accumulation and fatty liver disease. Genetically, C-APPL1/A-APPL2 allele combination is associated with an increase in nonalcoholic fatty liver disease (NAFLD) occurrence, with a more severe degree of hepatic steatosis and with reduced cytoprotective effects of adiponectin on hepatocytes. NAFLD risk alleles have been found to be associated with hepatocellular injury and a more severe hepatic steatosis. Actually, these effects may be independent of the plasma adiponectin levels. Despite normal or high adiponectin levels, an impaired post receptor signalling due to APPL1/APPL2 single nucleotide polimorphisms may alter adiponectin efficiency and activity (Barbieri et al. 2013). Thus, APPL2 blocks adiponectin signaling through AdipoR1

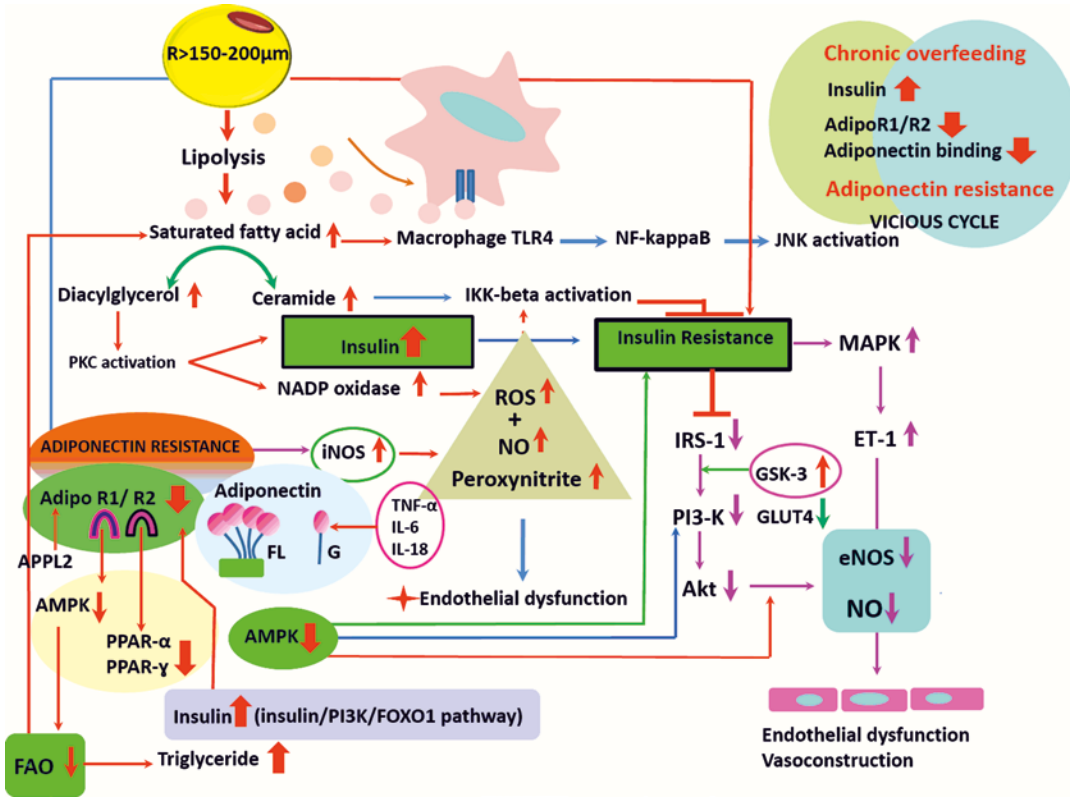

Fig. 18.1 Adiponectin resistance together with insulin resistance forms a vicious cycle. Insulin-adiponectin resistance cross talk causes intracellular signal transduction disturbances. Adiponectin resistance is a compensatory response in the condition of an unusual discordance between insulin resistance and adiponectin unresponsiveness (*PKC* protein kinase C, *AMPK* 5′ adenosine monophosphate-activated protein kinase, *GLUT4* insulin-responsive glucose transporter-4, *NADP* nicotinamide adenine dinucleotide phosphate, *iNOS* inducible nitric oxide synthase, *NO* nitric oxide, *ROS* reactive oxygen radicals, *AdipoR1/R2* adiponectin receptors, *TLR4* Toll-like receptor-4, *NF-kappaB* nuclear factor-kappa B, *JNK* c-Jun N-terminal kinase, *IKK* inhibitor kappa B kinase, *IRS-1* insulin receptor substrate-1, *PI3-K* Phosphatidylinositol 3-kinase, *Akt* protein kinase B, *eNOS* endothelial nitric oxide synthase, *MAPK* mitogen-activated protein-kinase, *ET-1* endothelin-1, *TNF-alpha* Tumor necrosis factor-alpha, *GSK-3* glycogen synthase kinase-3, *FL* full-length human adiponectin, *G* globular adiponectin, *IL-6* interleukin-6, *IL-8* interleukin-8, *PPAR-ɣ* peroxisome proliferator-activated receptor-gamma, *PPAR-α* peroxisome proliferator-activated receptor-alpha, *FAO* fatty acid oxidation, *FOXO1* forkhead box transcription factor 1, *APPL2* adaptor protein-containing pleckstrin homology domain, phosphotyrosine-binding domain, and leucine zipper motif 2)

and AdipoR2 by competitive inhibition of APPL1 (Ruan and Dong 2016).

On the other hand, DNA methylation in the obesity decreases adiponectin expression. The AdipoR2 is highly methylated in adipocytes from high fat diet-fed obese mice as compared with lean mice. Moreover, AdipoR2 methylation levels are inversely correlated with the amounts of adiponectin mRNA. By contrast, the AdipoR1 methylation is unaltered regardless of obesity. In human adipocytes, the AdipoR2 methylation is not only positively correlated with body mass index but also negatively associated with adiponectin transcripts. DNA methylation involves the particular region of adiponectin promoter. Thus, the AdipoR2 methylation is mediated by DNA methyltransferase, and induces the subsequent formation of heterochromatin structure to suppress adiponectin gene expression in obesity (Kim et al. 2015). While the AdipoR1 activates the AMPK pathways, AdipoR2 induces the PPAR-alpha pathways such as increased expres-

sion of uncoupling protein 2 (UCP2). In this respect, disruption of AdipoR1 results in the abrogation of adiponectin-induced AMPK activation, whereas that of AdipoR2 results in decreased activity of PPAR-alpha signaling pathway. Furthermore, simultaneous disruption of both AdipoR1 and R2 abolishes adiponectin binding and actions. Collective elimination of the adiponectin effects results in increased tissue triglyceride content, inflammation and oxidative stress, and thus leads to insulin resistance (Yamauchi et al. 2007). Conversely, UCP2 could exert anti-steatotic and anti-inflammatory activities through promoting mitochondrial respiration, attenuating non-esterified mitochondrial fatty acid accumulation and reactive oxygen species (ROS) production, and activating AMPK (Baffy 2005; Zhou et al. 2010). Acute adiponectin loading fails to lower glycemia, which is consistent with systemic adiponectin resistance. The mechanism for this failure appears to correlate with a blunted response of AMPK activation but not with changes in AdipoR1 and AdipoR2 mRNA expression. Actually, adiponectin resistance correlates with impaired hepatic AMPK response to adiponectin. The reduced AMPK response is associated with increased basal AMPK phosphorylation and AMPK resistance (Lin et al. 2007). Tsuchida et al. have previously shown that the expression of AdipoR1/R2 is inversely correlated with plasma insulin levels *in vivo*. Moreover, the expressions of AdipoR1/R2 in obese mice are significantly decreased in skeletal muscle and adipose tissue, which is correlated with decreased adiponectin binding to membrane fractions of skeletal muscle and decreased AMPK activation by adiponectin (Tsuchida et al. 2004). AdipoR1 mRNA expression in skeletal muscle myotubes derived from lean healthy individuals is stimulated 1.8- and 2.5-fold with gAD and leptin, respectively. No increase in AdipoR1 gene expression is measured in myotubes derived from obese subjects. AdipoR2 mRNA expression is unaltered after gAD and leptin exposure in myotubes (McAinch et al. 2006). Evidences of adiponectin resistance have been shown in peripheral tissues of obese humans. In this manner, decrease in the expressions of AdipoR1/R2 and adiponectin concentration reduces the adiponectin binding to cell membrane (Tsuchida et al. 2004). Furthermore, AdipoR1 expression in human adipose tissue is also reduced in obese subjects. Thus, it suggests that adiponectin might have reduced biological effects in adipose tissue due to low levels of adiponectin receptors in omental adipocytes of obese individuals (Rasmussen et al. 2006). Receptor endocytosis is a key event in regulation of signaling transduction. Enhanced adiponectin-stimulated AMPK phosphorylation involves the downregulation of adiponectin signaling through the endocytosis of AdipoR1. Indeed, AdipoR1 is internalized through a clathrin- and Rab5-dependent pathway and that endocytosis may play a role in the regulation of adiponectin signaling. Thus, blocking clathrin-mediated endocytosis abolishes adiponectin internalization (Ding et al. 2009).

4 Adiponectin and Insulin Resistance

Overexpression of glycogen synthase kinase (GSK)-3 in skeletal muscle of obese type 2 diabetic humans are associated with an impaired ability of insulin to activate glucose disposal and glycogen synthase. Inhibition of GSK-3 causes improvements in insulin-stimulated glucose transport activity by enhancing post-insulin receptor insulin signaling and GLUT4 glucose transporter translocation (Henriksen and Dokken 2006). Chronic treatment of insulin-resistant, prediabetic obese rats with a highly selective GSK-3 inhibitor improves insulin receptor substrate-1 (IRS-1)-dependent insulin signaling in skeletal muscle and enhances whole body insulin sensitivity (Dokken and Henriksen 2006). Thus, GSK-3 overactivity in obesity is associated with enhanced IRS-1 serine phosphorylation and defective IRS-1-dependent signaling, ultimately resulting in reduced GLUT4 translocation and glucose transport activity in skeletal muscle (Henriksen 2010). Actually, insulin-induced signaling involves in activation of phosphatidylinositol 3-kinase (PI3K) and Akt. Akt inactivates

GSK-3. PI3K-dependent GSK3 inactivation causes the inhibition of adiponectin formation in diet-induced obesity. The expression of PI3K-resistant-GSK3 stimulates the production of adiponectin and protects from diet-induced obesity (Chen et al. 2016). In obesity-linked insulin resistance, both adiponectin and adiponectin receptors are downregulated. Up-regulation of adiponectin/adiponectin receptors or enhancing adiponectin receptor function has protective effect against obesity-linked insulin resistance (Caselli 2014). AdipoR1 overexpression increases glucose uptake and glycogen accumulation in the high-fat diet-fed rats, and it locally ameliorates muscle insulin resistance. These effects are associated with increased phosphorylation of IRS-1, Akt, and GSK-3. A direct role for adiponectin action via AdipoR1 in the enhancement of insulin sensitivity involves activation of the PI3K and AMPK signaling pathways (Patel et al. 2012). APPL1 has been shown to interact directly with Akt (Saito et al. 2007b). However, IRS-1 phosphorylation is also increased by either APPL1 or AdipoR1 overexpression (Patel et al. 2012). APPL1 promotes glucose disposal into skeletal muscle in vivo through activation of the PI3K pathway (Cleasby et al. 2011). Plasma concentrations of adiponectin in obese subjects are significantly lower than those in non-obese subjects (Arita et al. 1999). Adiponectin resistance is initially described in obesity. This condition is associated with insulin resistance and is thought to be partly due to the down-regulation of the AdipoR1 due to high-fat diet (Mullen et al. 2009). Additionally, the severe degree of insulin resistance occurs in patients with genetic defect of the insulin receptors. These cases have the combination of high plasma adiponectin with low leptin (Semple et al. 2006). This discordance between high plasma adiponectin and extreme insulin resistance may be accounted for their either direct effects in adipocytes leading to the loss of insulin receptor function, or their effects on the developed adipose tissue that has severely impaired insulin receptor function (Semple et al. 2006). The hyperadiponectinaemia in states of insulin receptor dysfunction is explained by a superiority of HMW multimers to the other counterparts. HMW adiponectin shows a significant correlation with the dissociation between plasma adiponectin and insulin sensitivity when compared to total plasma adiponectin (Semple et al. 2007). Furthermore, deficiency of both IRS-1 and 2 in adipocytes reduces adiponectin secretion. Lack of insulin receptor or IRSs in adipocytes increases adiponectin mRNA expression, but reduces adiponectin secretion. Adiponectin mRNA expression shows no significant response to insulin receptor deficiency, but is modestly increased by IRS-1 and 2 deficiency. Loss of insulin receptor function may affect adiponectin levels indirectly through alteration of adipocyte turnover (Groeneveld et al. 2016). The expressions of both AdipoR1 and AdipoR2 are significantly decreased in most of the insulin-sensitive tissues of genetically obese mice as compared with the controls. FOXO1 increases the AdipoR1/R2 expressions. In these animals, insulin can reduce the AdipoR1/R2 expressions via down-regulation of FOXO1 activity. Actually, the expression levels of AdipoR1/R2 might regulate adiponectin binding and AMPK activation by adiponectin. This process is referred as adiponectin sensitivity. Chronic overfeeding and attending elevation of insulin results in the decreased expression of AdipoR1/R2. The decrease in AdipoR1/R2 mRNA leads to a decrease in adiponectin binding, and this turns into a decrease in the adiponectin effects. This is defined as adiponectin resistance. Adiponectin resistance together with insulin resistance forms a vicious cycle (Tsuchida et al. 2004). The changes in AdipoR2 levels may reflect decreased FOXO1-dependent transcription. In contrast, AdipoR1 mRNA expression is not changed in liver and tend to be reduced in skeletal muscle of both normoglycemic and hyperglycemic conditions. This reflects the differential regulation of AdipoR1 and AdipoR2 under insulin-resistant state. The reduced AdipoR1 expression in muscle also contributes to adiponectin resistance in addition to insulin resistance. In any way possible, insulin resistance can result in hyperadiponectinemia and adiponectin resistance. In contrast, Lin et al. showed that stepwise decreases in adiponectin and AdipoR1/AdipoR2 expressions are associated with the pro-

gression from insulin resistance to overt diabetes (Lin et al. 2007). During the prolonged hyperglycemia-associated oxidative stress, acetylated FOXO1 is retained in the nucleus, where it engages sirtuin 1 (SIRT1). Deacetylation of FOXO1 by SIRT1 promotes FOXO1-dependent transcription and accelerates FOXO1 degradation (Kitamura et al. 2005). During prolonged incubation with insulin, insulin-stimulated glucose uptake is significantly reduced. Insulin resistance and adiponectin mRNA expression develops in these adipocytes. In insulin-resistant adipocyte, adiponectin deficiency does not change insulin-stimulated glucose uptake, whereas in insulin-sensitive adipocytes, adiponectin deficiency suppresses insulin signaling, expression of IRS-1 and GLUT4, and GLUT4 translocation to the membrane (Chang et al. 2015) (Fig. 18.1).

5 Discordance Between Adiponectin Response and Insulin Resistance

The variation in fat cell size that occurs in obesity may have an important impact on adipose tissue function. Adipose tissue morphology correlates with insulin levels and is linked to the total adipocyte number independent of sex and body fat level (Arner et al. 2010). However, in visceral fat cells, the lipolytic activity is higher than in subcutaneous fat cells owing in part to less marked insulin action and lower alpha 2-adrenergic receptor mediated antilipolytic effect of catecholamines (Engfeldt and Arner 1988). Adipose tissue displays a variable balance between increases in fat cell size and increases in fat cell number in obesity. Thus, the total adipocyte number is greatest in pronounced hyperplasia and smallest in pronounced hypertrophy (Arner et al. 2010). Adipose tissue expands via the combination of hypertrophy and hyperplasia in diet-induced obesity. Subcutaneous adipose tissue has a primary role in supporting circulating adiponectin levels in lean subjects. Fat cell diameter and percentage of hypertrophic fat cells are greatest in subcutaneous adipose tissue and correlate negatively with both serum and secreted adiponectin levels in obese subjects, because increases in fat cell size have been shown to impair fat cell function. In contrast, hyperplastic expansion appears predominant in visceral adipose tissue, which contained a significantly greater percentage of small fat cells and a significantly smaller mean fat cell diameter. Visceral adipose tissue correlated positively with both serum HMW and secreted total adiponectin. Eventually, reductions in the percentage of small fat cells in subcutaneous adipose tissue are associated with the reductions in both secreted and circulating levels of total and HMW adiponectin (Meyer et al. 2013). Actually, AMPK activity is lower in visceral than in subcutaneous abdominal adipose tissue of insulin sensitive obese or insulin-resistant obese patients. Furthermore, AMPK activity is lower in all adipose tissues of obese patients who are insulin resistant than in body mass index-matched insulin sensitive subjects. These evidences indicate the close links between reduced AMPK activity, increased inflammation in white adipose tissue and whole-body insulin resistance in obese humans (Gauthier et al. 2011). In fact, AMPK is a heterotrimeric enzyme complex consisting of a catalytic subunit alpha and two regulatory subunits beta and gamma. AMPK is activated by the rising of AMP and decreasing of ATP. AMPK system is a regulator of energy balance that once activated by low energy status, switches on ATP-producing catabolic pathways such as fatty acid oxidation and glycolysis, and switches off ATP-consuming anabolic pathways such as lipogenesis. The AMPK system also regulates food intake and energy expenditure by mediating the insulin sensitizing effect of adiponectin (Viollet et al. 2007). Elevated adiponectin protein expression in adipocytes and elevated adiponectin serum concentrations could help to compensate the adipocyte-specific insulin resistance in fat-specific insulin receptor deficient mice. Thus, hyperadiponectinemia results from a lack of insulin signalling in adipose tissue (Blüher et al. 2002). Furthermore, Kim et al. showed that the elevated adiponectin levels with adiponectin resistance is a compensatory response in the con-

dition of an unusual discordance between insulin resistance and adiponectin responsiveness. However, this type of adiponectin resistance could not be explained by decreased adiponectin receptor gene expression or AMPK phosphorylation in skeletal muscle and liver. These mice are resistant or unresponsive to metabolic actions of both insulin and adiponectin (Kim et al. 2006). Serum adiponectin is significantly associated with AMPK phosphorylation in skeletal muscles of men but not in women. Serum adiponectin is significantly and negatively associated with skeletal muscle ceramide content in men. Furthermore, ceramide content is negatively associated with AdipoR1 expression in skeletal muscles of men. These associations suggest that the insulin-sensitizing effect of adiponectin on human male skeletal muscles may be mediated via AdipoR1 leading to lowering of ceramide content (Høeg et al. 2013). For women only, there is a negative correlation between C16-ceramide and plasma adiponectin and a positive correlation between total ceramide content and insulin resistance (Blachnio-Zabielska et al. 2012). Evidence of adiponectin resistance has been shown in peripheral tissues of obese humans. Thus, decrease in the expressions of AdipoR1/R2 or adiponectin concentration in obese mice reduces the adiponectin binding to membrane fractions of skeletal muscle (Tsuchida et al. 2004).

Actually, adiponectin stimulates fatty acid oxidation and improves insulin sensitivity in humans, due in part to the activation of AMPK and subsequent deactivation of acetyl coenzyme A carboxylase (ACC). Although both high fat diets result in the loss of ability to stimulate fatty acid oxidation of adiponectin, high saturated fat diet shows reduced rates of maximal insulin-stimulated glucose transport compared with high unsaturated fat diet. The lack of stimulation of fatty acid oxidation in response to adiponectin is supported by the lack of ACC phosphorylation in high fat diets. The development of adiponectin resistance does not necessarily coincide with the development of impaired glucose transport (Mullen et al. 2007). gAD increases glucose uptake in skeletal muscle cells via GLUT4 translocation and subsequently reduces the rate of glycogen synthesis and shifts glucose metabolism toward lactate production. These effects are consistent with the increased phosphorylation of AMPK and ACC and oxidation of fatty acids, which is induced by gAD (Ceddia et al. 2005). Thus, gAD increases AMPK activity and ACC phosphorylation and decreased malonyl CoA concentration in muscle. In fact, adiponectin is known to increase fatty acid oxidation in skeletal muscle by the inactivation of ACC, and reducing inhibition of carnitine palmitoyl transferase 1 (CPT1) by malonyl-CoA, leading to increased fatty acid uptake in the mitochondria (Tomas et al. 2002). In contrast, the stimulatory effect of gAD on fatty acid oxidation in skeletal muscle is blunted in obese humans. Combined exposure of insulin and globular head group of adiponectin exhibits an additive effect on glucose uptake in lean and obese individuals but this effect is reduced by 50% in obese muscle. In accordance with glucose, fatty acid oxidation is significantly increased with adiponectin in both lean and obese subjects. The absolute change in fatty acid oxidation in response to gAD is 58% lower in obese subjects. While the ratio of palmitate esterification to oxidation is significantly elevated in obese muscle, the stimulation of AMPK-alpha2 by gAD is impaired. In this case, resulting metabolic changes are attributed to reduced AMPK activity. These evidences indicate that adiponectin resistance develops during the progression of obesity (Bruce et al. 2005). Interestingly, reduced activation of AMPK signaling and fatty acid oxidation in obese and obese diabetic myotubes is not associated with reduced protein expression of AMPK-alpha and ACCbeta or the expression and activity of the upstream AMPK kinase. Moreover, obese subjects tend to have higher AdipoR1 expression. Thus, reduced activation of AMPK by gAD in obese subjects is not caused by reduced adiponectin receptor expression (Chen et al. 2005). Practically, only the animals fed with the saturated diet become insulin resistant, whereas polyunsaturated-fed animals are insulin responsive. By high fat feeding, increased fatty acid translocase (FAT/CD36) at the plasma membrane of skeletal muscle accompanies by

increased total fatty acid uptake in accumulation of reactive diacylglycerol (DAG) and ceramide lipid species and impaired insulin response. Virtually, a 60% saturated-fat diet can induce skeletal muscle adiponectin resistance, as evidenced by a failure of gAD to increase fatty acid oxidation or phosphorylate ACC above basal levels. However, it is unclear whether adiponectin resistance precedes intramuscular lipid accumulation and the development of insulin resistance. Nevertheless, adiponectin resistance occurs very rapidly after saturated fatty acid feeding, and this is not due to a decrease in AdipoR1 protein content (Mullen et al. 2009).

On the other hand, leptin stimulates AMPK activity and increases AMPK Thr172 and ACC-beta Ser222 phosphorylation and fatty acid oxidation in lean myotubes but that in obese subject leptin-dependent AMPK signaling and fatty acid oxidation are suppressed. Reduced activation of AMPK is associated with elevated expression of IL-6 and suppressor of cytokine signaling 3 (SOCS3) mRNA in myotubes of obese subjects. SOCS3 has been shown to inhibit leptin activation of AMPK in human myotubes and contributes to leptin resistance observed in obese subject (Steinberg et al. 2006). Furthermore, leptin and adiponectin stimulates fatty acid oxidation through similar mechanisms, it is possible that SOCS3 may interfere with intracellular gAD signal transduction. Adiponectin resistance worsens insulin resistance in obese mice. The expression of AdipoR1/R2 is regulated by insulin via the insulin/ PI3K/FOXO1 pathway and is correlated with adiponectin sensitivity (Tsuchida et al. 2004). According to Cui et al. Foxo1 silencing inhibits AdipoR1 expression and the activation of AMPK. Insulin induces a decrease in skeletal muscle AdipoR1 expression in a PI3K/Akt/FOXO1-dependent fashion (Cui et al. 2012). It is well-known that Akt regulates gene transcriptions through the inactivation of FOXO1. As such, the PI3K/Akt/FOXO1 axis has a central role in energy metabolism and signal transduction of insulin, governing insulin sensitivity (Mullen et al. 2009). Obesity decreases the expression of adiponectin receptors, thereby reducing adiponectin sensitivity finally leads to insulin resistance (Kadowaki and Yamauchi 2005). These observations confirm the important connections between insulin and AdipoR1. Eventually, elevated insulin levels may result in a decreased expression of AdipoR1, leading to diminished binding of adiponectin and a reduction in PPARgamma coactivator 1alpha (PGC-1alpha). These events ultimately trigger adiponectin resistance by simultaneously increasing the sphingolipid-ceramide levels (Sente et al. 2016). PPAR-gamma2 is a key regulator of adipogenesis, and PPAR-gamma2 transcription is activated by C/EBP-delta through the direct binding of C/EBP-delta to the PPAR-gamma2 promoter. By contrast, glucocorticoid-induced leucine-zipper protein (GILZ) binds to the PPAR-gamma2 promoter element and inhibits C/EBP-delta-mediated transcription. GILZ inhibits the transcription of the PPAR-gamma2 gene and blocks adipocyte differentiation (Shi et al. 2003). GILZ overexpression decreases leptin mRNA and protein secretion. Although GILZ silencing decreases adiponectin mRNA levels, it does not affect the amount of adiponectin secreted. While GILZ silencing increases basal ERK1/2 and c-Jun N-terminal kinase (JNK) phosphorylation, it decreases MAPK phosphatase-1 (MKP1) protein levels. Thus, adipose tissue GILZ mRNA levels are reduced in proportion to the degree of obesity (Lee et al. 2016). Surprisingly, adiponectin overexpression reduces MKP1 protein levels. High adiponectin levels enhance MAPK/PGC-1alpha signaling and mitochondrial biogenesis in skeletal muscle by suppressing MKP1 protein expression (Qiao et al. 2012). Despite the low level of adiponectin in obesity, the reason for this discrepancy is unclear.

Furthermore, a high-fat maternal diet decreases AdipoR1 expression in offspring, which could contribute to reduced sensitivity to adiponectin (Hou et al. 2015). Thus, AdipoR1 deficiency leads to diet-induced metabolic dysfunction. These mice have greater body weight and fat mass, hepatic steatosis, impaired glucose disposal rate, and elevations in serum insulin and leptin, whereas, AdipoR2-deficient mice are protected from diet-induced weight gain and metabolic perturbations (Parker-Duffen et al. 2014).

In contrast, the AdipoR1 overexpressing mice resist diet-induced obesity while decreasing lipid accumulation, oxidative stress and autophagic damage (Chou et al. 2014a). On the other hand, the adiponectin promoter drives expression of lipoprotein lipase (LPL) in adipocytes and increases the amount of lipase in adipose tissue lipid storages. Thereby, adipose tissue protects against the accumulation of ectopic lipids in the peripheral tissues by storing fat as neutral lipid (triglyceride). In addition to lipid diversion, another predicted effect of increased adipose LPL is the stimulation of PPAR transcription factors by the free fatty acids, which are generated by lipoprotein hydrolysis. Consequently, increased LPL in adipocytes improves adipocyte function and protects against glucose and insulin intolerance in diet-induced obesity (Walton et al. 2015). Simultaneous disruption of both AdipoR1- and R2-adiponectin binding and actions result in increased tissue triglyceride content, inflammation and oxidative stress, and thus lead to insulin resistance and marked glucose intolerance. In this regard, AdipoR1 and R2 play important roles in the regulation of glucose and lipid metabolism (Yamauchi et al. 2007). Actually, adiponectin exerts insulin-sensitizing effects through binding to own receptors. This signaling primarily leads to activation of AMPK and PPAR-alpha. As mentioned above, in obesity-linked insulin resistance, both adiponectin and adiponectin receptors are downregulated. From this perspective, up-regulation of adiponectin or enhancing adiponectin receptor functions may be a therapeutic strategy for obesity-linked insulin resistance (Caselli 2014) (Fig. 18.1).

Fifty-four obese patients with NAFLD showed significantly lower serum adiponectin levels when compared with the normal participants; and also its level is lower in nonalcoholic steatohepatitis (NASH) patients in comparison to patients with simple steatosis. Furthermore, adiponectin receptor gene expression in liver biopsy of NASH patients is lower in comparison to non-NASH patients. Adipo R2 receptor depletion in these patients suggests that not only adiponectin deficiency has a role in progression of severity of NAFLD, but also adiponectin resistance is important in the pathogenesis of NAFLD (Salman et al. 2015). Adiponectin potently stimulates ceramidase activity through AdipoR1 and AdipoR2. Ceramide catabolism is enhanced and its anti-apoptotic metabolite, sphingosine-1-phosphate (S1P) is formed independent from AMPK activity. Ceramidase activity is impaired in cells lacking both adiponectin receptor isoforms, leading to elevated ceramide levels and enhanced susceptibility to palmitate-induced cell death (Holland et al. 2011). Actually, AdipoR1-deficiency markedly reduces gAD-induced neutral ceramidase activation, whereas AdipoR2-deficiency only slightly inhibits ceramidase. More importantly, small interfering RNA-mediated neutral ceramidase-deficiency markedly blocks the effect of adiponectin on TNF-alpha-induced intercellular adhesion molecule-1 (ICAM-1) expression. Eventually, adiponectin inhibits TNF-alpha-induced inflammatory response via ceramidase recruitment and activation in an AdipoR1-dependent fashion (Wang et al. 2014). Actually, ceramide impairs insulin sensitivity in peripheral tissues by blocking the plasma membrane translocation and promoting dephosphorylation of Akt (Holland and Summers 2008). Upon binding adiponectin, the adiponectin receptors convert ceramides into sphingosines by stimulating ceramidase activity. Hence, adiponectin-related activities depend primarily on the lowering of cellular ceramides or the concomitant increase in sphingosines (Ye and Scherer 2013). AdipoRon, synthetic small-molecule agonist of the AdipoR1, exhibits very similar effects to adiponectin in muscle and liver, such as activation of AMPK and PPAR-alpha pathways by binding to both AdipoR1 and AdipoR2. Moreover, AdipoRon ameliorates insulin resistance and glucose intolerance in mice fed a high-fat diet or genetically obese mice (Okada-Iwabu et al. 2013). Consequently, treatment with AdipoRon increases AdipoR1/AdipoR2, AMPK, and PPAR-alpha mRNA expressions as well as the mRNA levels of genes involved in fatty acid beta-oxidation. Moreover, AdipoRon contributes to increased mitochondrial biogenesis through the up-regulation of PGC-1alpha expression (Sente et al. 2016).

As a dietary saturated fatty acid, palmitate not only rapidly inhibits transcription of the adiponectin gene and the release of adiponectin from adipocytes but also stimulates lysosomal degradation of newly synthesized adiponectin (Karki et al. 2011). Palmitate induces a 45% decrease in insulin-stimulated glucose uptake in adipocytes. In accordance with this, the mRNA and protein expression of adiponectin are reduced by 43% and 36%, respectively, by palmitate treatment. These changes are accompanied by a 54% increase in intracellular ROS levels (Xi et al. 2007). Palmitate also increases the expression levels of iNOS and endoplasmic reticulum (ER) stress, ER stress response markers, and decreased mitochondrial protein contents. Palmitate-induced mitochondrial dysfunction is the primary event that leads to iNOS induction, ER stress, and decreased adiponectin synthesis in adipocytes (Jeon et al. 2012). Nitric oxide exerts its action on specific amino acid residues within target proteins by increasing generation of the nitric oxide-derivide peroxynitrite. iNOS represses adipose PPAR-gamma expression. In contrast, iNOS disruption sensitizes adipose tissue to PPAR-gamma agonism, thus raising plasma high-molecular weight adiponectin levels. This leads to increased AMPK activation in the liver and improvement of hepatic insulin sensitivity and glucose tolerance in obese mice (Dallaire et al. 2008) (Fig. 18.1). Lipid peroxidation-induced protein carbonylation is a potential mechanism underlying mitochondrial dysfunction (Frohnert and Bernlohr 2013). Protein carbonylation plays a major stimulating role in cytokine-dependent mitochondrial dysfunction and may be linked to the development of insulin resistance in the adipocyte (Curtis et al. 2012). Actually, mitochondrial function is closely linked to adiponectin synthesis in adipocytes, and mitochondrial dysfunction in adipose tissue decreases plasma adiponectin levels in obesity (Koh et al. 2007). Impaired mitochondrial function increases ER stress, and reduces adiponectin transcription via activation of JNK and consequent induction of activating transcription factor (ATF)-3 (Koh et al. 2007). ATF-3 negatively regulates human AdipoR1 expression via binding to an ATF-3-responsive region in the promoter, which plays an important role in attenuation of adiponectin signaling and induction of insulin resistance (Park et al. 2010). Further, palmitate-induced ER stress induces adiponectin resistance, via AMPK phosphorylation and reducing APPL1 expression (Park et al. 2015). The induction of ER stress is also accompanied by a decrease in adiponectin multimerization in adipocytes (Mondal et al. 2012). APPL1 interacts with adiponectin receptors in mammalian cells and this interaction is stimulated by adiponectin. Overexpression of APPL1 increases adiponectin signalling and adiponectin-mediated downstream events; such as lipid oxidation, glucose uptake and the membrane translocation of GLUT4, whereas suppression of APPL1 reduces all these processes (Mao et al. 2006). Adaptor protein APPL1 as a critical molecule promotes IRS 1/2-insulin receptor interaction. APPL1 forms a complex with IRS1/2 under basal conditions, and this complex is then recruited to the insulin receptor in response to insulin or adiponectin stimulation. The interaction between APPL1 and insulin receptor depends on insulin- or adiponectin-stimulated APPL1 phosphorylation, which is greatly reduced in insulin target tissues in obese mice (Ryu et al. 2014).

AdipoR2 is induced by both PPAR-alpha and PPAR-gamma in primary and THP-1 macrophages. Actually, AdipoR1 is more abundant than AdipoR2 in monocytes and its expression decreases upon differentiation into macrophages, whereas AdipoR2 remains constant (Chinetti et al. 2004). Decreased HMW adiponectin plays a crucial and causal role in obesity-linked insulin resistance and metabolic syndrome. Decreased adiponectin action and increased MCP-1 form a vicious adipokine network causing obesity-linked insulin resistance and metabolic syndrome. While PPAR-gamma upregulates HMW adiponectin, PPAR-alpha upregulates AdipoRs (Yamauchi and Kadowaki 2008). The abundance of MCP-1 mRNA in adipose tissue and the plasma concentration of MCP-1 are increased in white adipose tissue of both genetically obese diabetic mice and mice with obesity induced by a high-fat diet. MCP-1 expression in adipose tissue contributes to the macrophage infiltration into

this tissue (Kanda et al. 2006). Initially, the C-C motif chemokine receptor-2 (CCR2) regulates monocyte and macrophage recruitment in adipose tissue. Thereby CCR2 is necessary for macrophage-dependent inflammatory responses. Adipose tissue-derived MCP1 (also known as CCL2) is a major chemoattractant, which is responsible for macrophage infiltration and activation. This cytokine is a high-affinity ligand for CCR2 and is significantly elevated in obesity. Consequently, the high amount of CCR2 influences the development of obesity-associated adipose tissue inflammation and systemic insulin resistance (Weisberg et al. 2006). Free fatty acid-stimulated expression of monocyte chemotactic factors is dose and time dependent in obesity. Inhibition of the JNK pathway partially reduces free fatty acid-induced upregulation of MCP-1 and MCP-3. JNK is not only important for mediating free fatty acid-induced insulin resistance but also involves in free fatty acid-induced expression of monocyte chemotactic factors in adipocytes. Adipocytes-derived chemokines contribute to obesity-related white adipose tissue macrophage infiltration through the stimulation of free fatty acids (Jiao et al. 2009). High mobility group box 1 (HMGB1) is a pro-inflammatory adipocytokine involved in white adipose tissue inflammation and insulin resistance in patients with obesity. HMGB1 is secreted from TNF-alpha-induced adipocytes through JNK signaling. Adiponectin protects against HMGB1-induced adipose tissue inflammation (Shimizu et al. 2016). Additionally, adiponectin also promotes macrophage polarization toward the anti-inflammatory M2 phenotype. In this respect, adiponectin stimulates the expression of M2 markers and attenuates the expression of M1 markers in human monocyte-derived macrophages and stromal vascular fraction cells isolated from human adipose tissue (Ohashi et al. 2010). Macrophage polarization controls AdipoR1 and AdipoR2 expression. Activation of classical M1 macrophages suppress AdipoRs expression, whereas alternatively activated (M2) macrophages preserve AdipoRs. Recombinant full-length human adiponectin treatment largely restores AdipoR levels of M1-polarized macrophages compared with those in nonpolarized macrophages, even in the presence of M1 cytokines. In contrast to M1 macrophages, M2 macrophages maintain higher AdipoR levels compared with nonpolarized macrophages and exhibit no AdipoR up-regulation in response to adiponectin. Adiponectin exerts a strong proinflammatory response by inducing IL-12, IL-6, and TNF-alpha in M1 macrophages. Notably, this proinflammatory adiponectin response is absent in nonpolarized and M2 macrophages. In contrast, in M2 macrophages, adiponectin induces the anti-inflammatory cytokine IL-10 without altering AdipoR expression (van Stijn et al. 2015). It is clear that macrophage polarization is a key determinant regulating AdipoR expression and differential adiponectin-mediated macrophage inflammatory responses (van Stijn et al. 2015).

Preadipocytes have a heightened inflammatory cytokine response following acute saturated fatty acid and monounsaturated fatty acid exposure, when compared to mature adipocytes. This effect is pronounced for MCP-1. Preadipocytes also demonstrate activation of the NF-kappaB pathway following fatty acid exposure (Dordevic et al. 2014). Adiponectin pre-treatment significantly reduces the increase in MCP-1 mRNA in stimulated adipocytes. Thus, adiponectin exerts anti-inflammatory activity by suppressing IL-6 and MCP-1 production from inflamed adipocytes. This anti-inflammatory action may be mediated through inhibition of NF-kappaB activity as well as through increased PPAR-gamma expression. Furthermore, adiponectin significantly attenuates nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha (IkappaB-alpha) and IkappaB kinase (IKK) gene expression (Zoico et al. 2009). Irrespective of abdominal visceral fat, low adiponectin and high free fatty acid levels are associated with insulin resistance, and that the effect of low adiponectin is stronger than that of high free fatty acid levels in circulation (Medina-Urrutia et al. 2015). An excess flux of fatty acids causes an altered adipokine production and an increase in oxidative stress in mature adipocytes. Free fatty acids exposed adipocytes demonstrate decreased FOXO1 protein levels in

a dose-dependent manner. The FOXO1 down-regulation correlates with an increase in the production of ROS and a proinflammatory adipokine pattern, which is characterized with a decrease in adiponectin and an increase in IL-6, plasminogen activator inhibitor-1 (PAI-1), and MCP-1 mRNA expression (Subauste and Burant 2007). In hepatic cells, adiponectin stimulation produces a transient burst of ROS through activation of the small guanosine triphosphatase (GTPase) Rac1 and 5-lipoxygenase. Furthermore, adiponectin-induced oxidants cause the oxidation and inhibition of protein-tyrosine phosphatase 1B (PTP1B), one of the major phosphotyrosine phosphatases that involves in the control of insulin receptor phosphorylation. Adiponectin causes increased association of PTP1B to insulin receptor. ROS is a critical regulator of the cross-talk between adiponectin and insulin pathways and provides a redox-based molecular mechanism for the insulin-sensitizing function of adiponectin (Fiaschi et al. 2007).

The transcript level of ACC and fatty acid synthase is upregulated by saturated fatty acid treatment. In this case, AdipoR1 reverses the effect induced by palmitate and enhances fatty acid metabolism. Actually, AdipoR1 also increases the gene expression of cytochrome C oxidase, PPAR-alpha, and decreases the gene expression of PGC1-alpha, AMPK-alpha in palmitate-treated human hepatocytes (Chou et al. 2014b). Adiponectin alleviates the endothelial dysfunction caused by elevated free fatty acids concentration through the cross talk between cyclic AMP (cAMP) and NF-kappaB signaling pathway (Wang et al. 2012). The homeostatic model assessment (HOMA)-insulin resistance is associated positively with body mass index, serum non-esterified fatty acid, leptin, IL-6, and TNF-alpha levels, but negatively with adiponectin. Of these, only serum level of leptin, and in a lesser degree IL-6 and adiponectin are independent determinants of the severity of insulin resistance (Peti et al. 2011). The long-chain saturated fatty acids are positively associated and delta 9–18 desaturase activity is significantly but inversely associated with adiponectin concentrations after dietary fat intake (Gallo et al. 2010).

Plasma adiponectin level correlates positively with peripheral and hepatic insulin sensitivity and negatively with fasting proinsulin and the proinsulin-to-insulin ratio. Indeed, adiponectin, but not adiposity, is the significantly independent determinant of the proinsulin-to-insulin ratio (Bacha et al. 2004). Nevertheless, compared with subcutaneous-derived adipocytes, adiponectin secretion from omental adipocytes is increased by insulin. Thereby, reduced secretion of adiponectin from the omental adipose depot may account for the decline in plasma adiponectin in obesity (Motoshima et al. 2002). Thus, Drolet et al. showed that omental adipocyte adiponectin release is reduced to a greater extent in visceral obese women. Hence, omental adiponectin release contributes to hypo-adiponectinemia on a large scale (Drolet et al. 2009). C/EBP and Nuclear Factor-Y (NF-Y) bind on the −117/−73 region of the adiponectin promoter. This region is critical for the activity of the adiponectin. The C/EBP binding increases in both re-fed animal and high glucose-treated adipocytes (Park et al. 2004). The expression of adiponectin mRNA is significantly reduced in the adipose tissues from obese humans (Hu et al. 1996), whereas mRNA levels of key adiponectin-regulatory transcription factors, including PPAR-gamma2 or C/EBP-alpha, are not significantly changed (Kim et al. 2015). The higher storage capacity of adipocytes prevents the formation of lipotoxic intermediates in adipocytes from the excessive fatty acids. Thereby, excessive lipids efficiently form triglyceride-enriched lipid droplets. If lipid influx into a cell exceeds the oxidative or storage capacity of the cell, then lipotoxic lipid intermediates are likely to accumulate (Unger et al. 2013). Both adiponectin and leptin increase fatty acid oxidation and protect the lipid-intolerant cells from the lipotoxic consequences of fatty acid spillover and aberrant ceramide accumulation (Unger et al. 2013). In particular, adiponectin increases IkappaB-alpha and PPAR-gamma levels to prevent high-fat diet-induced impairment of insulin signalling in adipose tissue. Furthermore, gAD administration is able to improve pathways of insulin signaling and lipid storage in adipose tissue of high-fat diet-fed rats (Matafome et al.

2014). In addition, chronic adipose tissue hypoxia induces dysfunction of adipocytes by triggering oxidative stress in human adipocytes and reduce the production of adiponectin in obesity (Netzer et al. 2015). In this case, antioxidants improve insulin resistance and restore adiponectin production. Recent studies have demonstrated that adiponectin protects against oxidative stress-induced damage in the vascular endothelium (Matsuda and Shimomura 2014). Intra-abdominal adipose tissue-AdipoR2 expression is reduced in obesity, whereas AdipoR1 expression is not altered by obesity. While AdipoR1 expression is directly associated with plasma free fatty acids concentration, AdipoR2 is inversely correlated with plasma levels of triglycerides (Morínigo et al. 2006).

Considering the beneficial metabolic effects, adiponectin-induced AMPK activation is associated with increased lipid oxidation. In this case, adiponectin initially enhances the association of AdipoR1 with APPL1, subsequent binding of APPL1 with AMPKalpha2, leads to phosphorylation and inhibition of ACC and increases fatty acid oxidation (Fang et al. 2010). Additionally, adiponectin also stimulates the ceramidase activity through AdipoR1 and AdipoR2, and enhances ceramide catabolism and formation of its anti-apoptotic metabolite, S1P, independent of AMPK. Overproduction of adiponectin decreases caspase-8-mediated death, whereas genetic ablation of adiponectin increases apoptosis *in vivo* through a sphingolipid-mediated pathway (Holland et al. 2011). Collectively, adiponectin may directly oppose lipotoxicity by targeting the degradation of ceramide, which is most commonly implicated as a mediator of lipotoxic cell death. It has been shown that adiponectin-mediated enhancements in adipose tissue expansion are highly effective at limiting lipid spillover to other tissues and diminishing formation of lipotoxic metabolites in nonadipose tissues (Unger et al. 2013). Saturated fatty acid-induced ER stress also induces adiponectin resistance, assessed via AMPK phosphorylation, via reducing APPL1 expression (Park et al. 2015). On the other hand, toxic free fatty acids can activate the intrinsic lipoapoptosis pathway in hepatocytes via JNK. Reduced adiponectin levels increase vulnerability to lipotoxicity, which promotes progression from simple steatosis to nonalcoholic steatohepatitis and even advanced hepatic fibrosis (Wree et al. 2011).

6 Adiponectin Resistance and Endothelial Dysfunction in Obesity

Adiponectin is protective against endothelial dysfunction through its pleiotropic actions in obesity. Data from human investigations demonstrate that adiponectin is an important component of the adipo-vascular axis that mediates the cross-talk between adipose tissue and vasculature (Li et al. 2011). High fat diet abolishes microvascular responses to either gAD or insulin and decreases insulin-stimulated glucose disposal by approximately 60%. In this respect, high fat diet induces vascular adiponectin and insulin resistance but gAd administration can restore vascular insulin responses and improve the metabolic action of insulin via an AMPK- and nitric oxide-dependent mechanisms (Zhao et al. 2015). Actually, adiponectin exerts anti-inflammatory and anti-atherogenic properties via its ability to stimulate vascular endothelial nitric oxide production (Chen et al. 2003). Indeed, both gAd and full-length adiponectin induce a relevant dose-dependent vasodilation in Zucker lean rats, but not in hypoadiponectinemic Zucker fatty diabetic rats. This vasodilator effect is totally nitric oxide-dependent. AdipoR1 is much more highly expressed than AdipoR 2 in both animal groups, but APPL1 is significantly decreased in obese rats. The endothelial nitric oxide synthase (eNOS) expression is not significantly different between Zucker lean and obese Zucker fatty diabetic rats. Adiponectin exerts a nitric oxide-dependent vasodilation in resistant arteries of normoglycemic Zucker lean rats, but not Zucker fatty diabetic rats. It is reasonable to claim that alterations in the expression of APPL1 may be involved in the resistance to adiponectin in obese rats (Schmid et al. 2011). Furthermore, the recombinant globular domain of human adipo-

nectin reduces the generation of endothelial ROS, which are induced by oxidized low density lipoprotein (Motoshima et al. 2004). Additionally, adiponectin suppresses high-glucose-induced peroxide production in endothelial cells. Suppression of excess ROS production via adiponectin is mediated by a cAMP/protein kinase A (PKA)-dependent pathway, because globular domain of adiponectin increases cellular cAMP content in hyperglycemic conditions. Inhibition of PKA blocks the effect of both globular domain and full-length adiponecting to suppress ROS generation (Ouedraogo et al. 2006). On the other hand, both superoxide and peroxynitrite productions are increased in adiponectin deficient vessels. Furthermore, although the eNOS expression is normal, nitric oxide production and eNOS phosphorylation is significantly reduced in adiponectin-deficient vessels. Thus, the globular domain of adiponectin reduces aortic superoxide production, increases eNOS phosphorylation, and improves vasodilatory response to acetyl choline. Increased nitric oxide inactivation combined with decreased basal nitric oxide production contributes to endothelial dysfunction in adiponectin deficient subjects. The replacement of plasma adiponectin may improve endothelial function, and reduces cardiovascular complications (Cao et al. 2009).

As mentioned above, in hyperlipidemic state endothelial dysfunction is mainly dependent on decreased synthesis of bioavailable nitric oxide. By contrast, stimulation of nitric oxide production and inhibition of superoxide radical production would provide protection against endothelial dysfunction. However, increase in the production of nitric oxide simultaneously with superoxide radical production leads to formation of peroxynitrite and aggravates vascular injury (Li et al. 2007). In this context, membrane-associated reduced nicotinamide adenine dinucleotide phosphate (NAD(P)H)-dependent oxidases, which can be activated by PKC in the hyperlipidemic condition, is the primary source of superoxide anion. Peroxynitrite formation is a result of the swift reaction between nitric oxide and superoxide anion (Mohazzab et al. 1994). In this respect, treatment with the globular domain of adiponectin significantly enhances eNOS but reduces iNOS activity in hyperlipidemic vessels. Indeed, globular domain of adiponectin reduces superoxide production and peroxynitrite formation in hyperlipidemic vascular segments by approximately 78%. Collectively, these results indicate that adiponectin protects the endothelium against hyperlipidemic injury in obesity by multiple mechanisms, including promoting eNOS activity, inhibiting iNOS activity, preserving bioactive nitric oxide, and attenuating oxidative and nitrative stress (Li et al. 2007). Insulin-resistance decreases nitric oxide bioavailability. In accordance with the insulin resistance, adiponectin-resistance specific pathways decrease nitric oxide bioavailability by enhancing oxidative/nitrative stress. Consequently, insulin-adiponectin resistance cross talk causes intracellular signal transduction disturbances (Li et al. 2010a). In this context, the major intracellular pathway activated by adiponectin, AMPK is responsible for vascular protective and anti-ischemic properties of adiponectin. Conversely, adiponectin resistance is a serious risk factor for cardiovascular injury including atherosclerosis and endothelial dysfunction (Lau et al. 2011). Adiponectin promotes the phosphorylation of AMPK, Akt and eNOS in endothelial cells. Both AMPK and Akt signals are required for adiponectin-induced endothelial migration and differentiation. PI3K functions upstream from the Akt-eNOS regulatory axis in adiponectin-stimulated endothelial cells (Ouchi et al. 2004). Additionally, AMPK inhibition does not abrogate the reduction of high-glucose–induced ROS production by gAD in endothelial cells. This suggests that the involvement of the PKA pathway in this process is not dependent on AMPK. Furthermore, increasing cAMP levels or blocking PKA activity in human endothelial cells do not effect the ability of gAD to activate AMPK (Ouedraogo et al. 2006).

Endothelial cell is activated by various inflammatory stimuli, including TNF-alpha, and this results in the synthesis of adhesion molecules and increases the adherence of monocytes. This monocyte adhesion to the arterial endothelium is considered to be crucial for the development of vascular diseases (Ross 1993). Physiological

concentrations of adiponectin dose-dependently inhibit TNF-alpha-induced THP-1 adhesion and expression of vascular cell adhesion molecule-1 (VCAM-1), E-selectin, and ICAM-1 on human aortic endothelial cells (Ouchi et al. 1999). Overexpression of AdipoR1 and 2 in endothelial cells significantly enhances the suppressive effect of a sub-effective dose of adiponectin on TNF-alpha-induced ICAM-1 expression and NF-kappaB activation. Upregulation of AdipoRs in endothelial cells potentiates the anti-inflammatory effects of adiponectin (Zhang et al. 2009) (Fig. 18.1).

Adiponectin over-expression in diet-induced obese mice lead to the inhibition of macrophage infiltration and the elimination of crown-like structures. In this context, adiponectin transgenic mice display a remarkable sensitivity to insulin, and thus the hepatic steatosis diminish. Increased circulating adiponectin levels are associated with increased adipose tissue vascularization and perfusion under conditions of long term diet-induced obesity, subsequently, metabolic functions improve despite the obese environment (Aprahamian 2013). Leptin activates cellular signaling pathways, and increases adiponectin mRNA in the adipose tissue from normal-weight individuals, but the same increase in adiponectin mRNA can not be achieved in the adipose tissue from obese participants. First of all, weight gain increases adiponectin expression in healthy humans. In this case leptin up-regulates adiponectin expression during weight gain. Actually, obese subjects have increased caveolin-1 expression, which attenuates leptin-signal. This leptin signal impairment may prevent concordant increases in adiponectin levels in obese subjects despite their high levels of leptin. In other words, increases in leptin to adiponectin ratio may suggest decreased leptin sensitivity, and altered insulin sensitivity (Singh et al. 2016). Actually, the leptin to adiponectin ratio is proposed as a good biomarker for the prevalence of metabolic syndrome in comparison to the adiponectin and leptin levels alone. However, this ratio is closely dependent on visceral fat accumulation and cardiorespiratory performance (Kumagai et al. 2005). In addition, a high serum leptin to adiponectin ratio and high levels of serum triglycerides may be indicators of "at-risk" obesity, independent of other obesity markers, especially in young severely obese individuals (Labruna et al. 2011).

Despite unchanged or elevated plasma adiponectin levels, vascular AMPK and eNOS phosphorylation levels are significantly reduced in high fat-diet animals. The disassociation between plasma adiponectin levels and vascular AMPK/eNOS phosphorylation in obese/hyperlipidemic rats suggests that high fat-fed animals have reduced vascular response to adiponectin. Although different mechanisms are involved in vascular adiponectin resistance in high fat-fed rats at different stages of obesity, diet-induced obesity/hyperlipidemia causes significant vascular adiponectin resistance. In advanced stages of high fat-diet, adiponectin inactivation, adiponectin receptor downregulation, and circulating adiponectin reduction collectively occur (Li et al. 2010b). In obesity-related cardiovascular diseases, the beneficial effects of perivascular adipose tissue on vascular functions are impaired. The contribution of perivascular adipose tissue dysfunction to obesity-related cardiovascular diseases is associated with decreased levels of adiponectin and increased levels of TNF-alpha. These changes lead to increased quantity of adipose tissue, inflammation, cell proliferation and endothelial dysfunction (Ozen et al. 2015). Furthermore, TNF-alpha is upregulated by leptin. In contrast, adiponectin downregulates the expression and release of a number of proinflammatory immune mediators. Therefore, leptin/adiponectin imbalance may be an important mediator of the elevated risk of developing abdominal obesity associated cardiovascular diseases (López-Jaramillo et al. 2014). Adiponectin and its receptor system have an important protective role against oxidative stress- or TNF-alpha-mediated myocardial insulin resistance and dysfunction after ischemic heart disease (Saito et al. 2007a). It is well-known that in the advanced stages of heart failure, there is a downregulation in fatty acid oxidation, increased glycolysis and glucose oxidation, reduced respiratory chain activity, and an impaired reserve for mitochondrial oxidative capacity (Stanley et al. 2005). Thus, the abroga-

tion of TNF-alpha-induced suppression of AdipoR1 expression might account for the improvement of glucose uptake. Furthermore, adiponectin and its receptor system may have an important protective role against oxidative stress- or TNF-alpha-mediated myocardial insulin resistance and dysfunction after myocardial injury (Saito et al. 2007a). In the myocardium, adiponectin-mediated protection from ischemia-reperfusion injury is linked to cyclooxygenase-2 (COX-2)-mediated suppression of TNF signaling, inhibition of apoptosis by AMPK, inhibition of iNOS and NADPH-oxidase protein expression and resultant excessive peroxynitrite-induced oxidative and nitrosative stress (Goldstein et al. 2009; Tao et al. 2007).

Clinical studies have demonstrated the impaired production of eNOS in the vasculature in subjects with decreased adiponectin levels. Consequently, endothelium-dependent vasorelaxation decreases due to lack of nitric oxide availability (Adya et al. 2015). Obesity and type 2 diabetes are associated with low plasma adiponectin concentrations in different ethnic groups and indicate that the degree of hypoadiponectinemia is more closely related to the degree of insulin resistance and hyperinsulinemia than to the degree of adiposity and glucose intolerance (Weyer et al. 2001). gAd acts as a critical physiological factor which protects against fluctuating high glucose-induced endothelial damage. It may act via attenuating apoptosis and increasing synthesis of nitric oxide through both the PI3K/Akt and AMPK signaling pathway to reduce oxidative stress and cell apoptosis (Xiao et al. 2011). Globular adiponectin reverses high glucose-impaired endothelial progenitor cells functions through nitric oxide- and p38 MAPK-related mechanisms (Huang et al. 2011). Both full-length adiponectin and gAD increase endothelial COX-2 expression, with gAD -mediated upregulation of COX-2 that is dependent on AdipoR1 and NFkappaB activation. With respect to full-length adiponectin, gAD more efficiently increases activation of NFkappaB signaling pathways, resulting in COX-2 overexpression and COX-2-dependent prostacyclin 2 release. In contrast to the full-length adiponectin, gAD also increases p38 MAPK phosphorylation and VCAM-1 expression, ultimately enhancing adhesion of monocytes to endothelial cells (Addabbo et al. 2011).

7 Conclusion

In obesity, the interactions between insulin and adiponectin are extremely complex. While insulin negatively regulates the expression of AdipoRs and adiponectin sensitivity, insulin resistance may result in hyperadiponectinaemia and adiponectin resistance. Adaptor protein of AdipoRs, APPL1 also promotes the insulin receptor substrat-insulin receptor interaction. On the other hand, saturated fatty acid-induced ER stress enhances adiponectin resistance, via reducing APPL1 expression. This condition is also associated with insulin resistance and is thought to be partly due to the down-regulation of the adiponectin and AdipoR1 in high-fat diet.

Even, new treatment strategies are being developed for the improvement of adiponectin resistance, lack of clinical evidences complicates the solution of the problems associated with obesity. Further understanding of the molecular mechanisms of the AdipoRs expression or stimulation, adiponectin-AdipoRs interaction and adiponectin/insulin resistance would facilitate the treatment of obesity-related conditions and adipose tissue dysfunction.

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Non-Alcoholic Fatty Liver Disease

19

Atilla Engin

Abstract

Non-alcoholic fatty liver disease (NAFLD) is in parallel with the obesity epidemic and it is the most common cause of liver diseases. The development of hepatic steatosis in majority of patients is linked to dietary fat ingestion. NAFLD is characterized by excess accumulation of triglyceride in the hepatocyte due to both increased inflow of free fatty acids and de novo hepatic lipogenesis. Insulin resistance with the deficiency of insulin receptor substrate-2 (IRS-2)-associated phosphatidylinositol 3-kinase (PI3K) activity causes an increase in intracellular fatty acid-derived metabolites such as diacylglycerol, fatty acyl CoA or ceramides. Lipotoxicity-related mechanism of NAFLD could be explained still best by the "double-hit" hypothesis. Insulin resistance is the major mechanism in the development and progression of NAFLD/Non-alcoholic steatohepatitis (NASH). Metabolic oxidative stress, autophagy, and inflammation induce NASH progression. In the "first hit" the hepatic concentrations of diacylglycerol increase with rising saturated liver fat content in human NAFLD. Activities of mitochondrial respiratory chain complexes are decreased in liver tissue of patients with NASH. Furthermore, hepatocyte lipoapoptosis is a critical feature of NASH. In "second hit" reduced glutathione levels due to oxidative stress lead to overactivation of c-Jun N-terminal kinase (JNK)/c-Jun signaling that induces cell death in the steatotic liver. Accumulation of toxic levels of reactive oxygen species (ROS) is caused by the ineffectual cycling of the endoplasmic reticulum (ER) oxidoreductin (Ero1)-protein disulfide isomerase oxidation cycle through the downstream of the inner membrane mitochondrial oxidative metabolism and Kelch like-ECH-associated protein 1 (Keap1)- Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) pathway.

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Keywords

Acetyl-CoA carboxylase (ACC) 1 • Atypical protein kinase C (aPKC) • Autophagy • Ceramide • Cirrhosis • Diacylglycerol (DAG) • Diacylglycerol acyltransferase (DGAT) • Fatty acid synthase (FAS) • Hepatic steatosis • Mammalian target of rapamycin complex 1 (mTORC1) • Non-alcoholic fatty liver disease (NAFLD) • Non-alcoholic steatohepatitis (NASH) • Obesity • Protein kinase C (PKC) • Serum alanine aminotransferase (ALT) • Sterol regulatory element-binding protein-1c (SREBP-1c) • Triglyceride • Tumor necrosis factor-alpha (TNF-alpha) • Unfolded protein response (UPR)

1 Introduction

Hypertrophic obesity leads to a dysregulated and dysfunctional subcutaneous adipose tissue and the accumulation of ectopic fat in many depots (Gustafson and Smith 2015). Thus, the release of fatty acids from dysfunctional and insulin-resistant adipocytes results in lipotoxicity, which is caused by the accumulation of triglyceride-derived toxic metabolites in ectopic tissues such as liver, muscle, pancreatic beta cells. Subsequent activation of inflammatory pathways, cellular dysfunction and lipoapoptosis are inevitable consequences of ectopic fat accumulation (Cusi 2012).

Due to its association with obesity and insulin resistance, the impact of nonalcoholic fatty liver disease (NAFLD) is growing worldwide (García-Ruiz et al. 2013). The prevalence of NAFLD in the general adult United States population is 30–40%. However, in the other parts of the world the range of NAFLD prevalence varies between 6 and 35% (Vernon et al. 2011). Approximately 18 million people in the United States have coexisting type-2 diabetes and NAFLD. The prevalence of non-alcoholic steatohepatitis (NASH) and advanced fibrosis is 69.2% and 41.0%, respectively in patients with diabetes and NAFLD (Bazick et al. 2015). Although NAFLD is not initially associated with higher mortality, there is a progressive increase in mortality with advancing fibrosis scores (Kim et al. 2013). NAFLD comprises a disease spectrum which includes variable degrees of simple steatosis with non-alcoholic fatty liver, NASH and cirrhosis (Vanni et al. 2010). The annual NAFLD fibrosis score changes in patients who died are significantly higher than those in patients who survived. The NAFLD fibrosis score is used to separate NAFLD patients with and without advanced liver fibrosis. In this respect, patients are classified into three subgroups according to the progression pattern of liver fibrosis by comparing the initial phase of NAFLD fibrosis score to the NAFLD fibrosis score at the end of the follow-up period. While the majority of patients are either in stable-fibrosis (60%) or in progressive-fibrosis (37%) phase, only 3% are in the regressive fibrosis (Treeprasertsuk et al. 2013). Patients with fibrosis stage 3–4, irrespective of the NAFLD activity score, have increased mortality. Indeed fibrosis stage predicts both overall and disease-specific mortality (Ekstedt et al. 2015). Free fatty acids are also elevated in NASH patients. The amount of free fatty acid in plasma positively correlates with the disease severity (Diraison et al. 2003; Nehra et al. 2001). Therefore, NASH is characterized by hepatic fat accumulation coincidental with inflammation, reduced liver function and subsequent advanced fibrosis (Neuschwander-Tetri and Caldwell 2003). Progression to NASH occurs in approximately 10–25% of NAFLD patients (Vanni et al. 2010). In average, twenty percent of NASH patients are reported to develop cirrhosis, and 30–40% of patients with NASH cirrhosis experience liver-related death (Cusi 2012).

Recently, worldwide increase in NAFLD prevalence is in parallel with the obesity epi-

demic. In many developed countries, NAFLD is the most common cause of liver diseases. NASH-related cirrhosis is the third most common indication for liver transplantation (Charlton et al. 2011; Zezos and Renner 2014). Between 2004 and 2013 in the United States, NASH became the second-leading disease among liver transplant waitlist registrants increased by 170% (Wong et al. 2015). Indeed, patients with NAFLD with advanced fibrosis are at greatest risk of developing complications of end-stage liver disease. Therefore, non-invasive methods require further validation to select those patients with NAFLD who needs liver biopsy (Castera et al. 2013). Overall, 37.5% of normal serum alanine aminotransferase (ALT) group has NASH or advanced fibrosis, whereas 53% of elevated ALT has no NASH or advanced fibrosis. In this respect, higher ALT values correlate with higher specificity, but lower sensitivity for both NASH and advanced fibrosis (Verma et al. 2013). Evaluation of the ALT/serum aspartate aminotransferase (AST) ratio, fibrosis-4 (FIB-4) score [(age × AST (IU/L)/platelet count × 10^9/L) x radical ALT (IU/L)] together with the NAFLD fibrosis scores is reliable to exclude advanced fibrosis in a high proportion of patients with NAFLD (McPherson et al. 2010). Nevertheless, the FIB-4 score yields the best diagnostic accuracy for advanced fibrosis in patients with normal or elevated ALT and reduces the need for liver biopsy (McPherson et al. 2013). NAFLD recurs in at least one-third of patients transplanted for NASH cirrhosis (Zezos and Renner 2014). Development of de novo liver steatosis in 31.1% and NASH in 3.8% of the recipients is open to debate (Dumortier et al. 2010; Vallin et al. 2014).

2 The Mechanism of Non-alcoholic Fatty Liver Disease

Reasonably enlargement of visceral adipose tissue coincides with the secretion of free fatty acid into the portal circulation. In addition to the high amount of free fatty acid supply, the compensatory hyperinsulinemia stimulates fatty acid synthesis and inhibits fatty acid catabolism in the hepatocytes. When fatty acid input exceeds the capacity of beta-oxidation, accumulating acyl-CoA is drained by triglyceride synthesis, which leads to steatosis in the liver. Actually, accumulation of fatty acids or fatty acyl-CoAs is more harmful to hepatocytes than the deposition of triglycerides (Zámbó et al. 2013). The circulating non-esterified fatty acids accounts for approximately 60% of the hepatic triglyceride content in NAFLD patients. Remaining arises from the de novo lipogenesis or from the diet (Donnelly et al. 2005). Thus, de novo lipogenesis plays a substantial role in the pathogenesis of NAFLD, accounting for 26% hepatic triglycerides in human subjects (Donnelly et al. 2005). For de novo lipogenesis, glucose is converted to acetyl CoA through glycolysis and the oxidation of pyruvate. Acetyl-CoA is then converted to malonyl-CoA by acetylCoA carboxylase (ACC). Fatty acid synthase (FAS) catalyzes the formation of palmitic acid from malonyl-CoA and acetyl-CoA. Glucose and insulin promote lipogenesis by activation of carbohydrate-response element binding protein (ChREBP) and sterol regulatory element binding protein-1c (SREBP-1c), respectively (Kawano and Cohen 2013).

The regulation of food intake by the cannabinoid system coordinates energy homeostasis via central orexigenic as well as peripheral lipogenic mechanisms. The cannabinoid type 1 (CB1) receptor-specific activation enhances lipogenesis in primary adipocyte (Cota et al. 2003), which results in increased free fatty acids release into the liver. On the other hand, activation of hepatic CB1 receptors contributes to the ectopic accumulation of fat in the liver, but not to the increase in whole body adiposity (Osei-Hyiaman et al. 2008). The visceral adiposity index (VAI) is a scoring system based on body mass, triglycerides, high density lipoprotein (HDL) cholesterol and waist circumference. The VAI is used to be a marker of visceral fat distribution and dysfunction. It is claimed that VAI, insulin resistance, metabolic syndrome are related to steatosis, liver inflammation and NASH in morbidly obese patients (Díez-Rodríguez et al. 2014). Recently in NAFLD patients, VAI has been suggested as an indicator of both qualitative and quantitative

adipose tissue dysfunction (Petta et al. 2012). More recently, according to others, VAI is not related to the severity of hepatic inflammation or fibrosis in nondiabetic patients with NAFLD (Ercin et al. 2015; Vongsuvanh et al. 2012).

Overall the liver lipid content is dependent on high-fat feeding. Thus, the development of hepatic steatosis in a majority of patients is linked to dietary fat ingestion rather than body mass index (Gauthier et al. 2006). The eventual development of insulin resistance leads to continuous lipolysis from insulin-resistant intraabdominal visceral fat depots. Subsequently free fatty acids, especially saturated free fatty acids are released into the portal circulation, where they are translocated to the liver and may be lipotoxic (Verna and Berk 2008). Hence, NAFLD is characterized by excess accumulation of triglyceride in the hepatocyte both due to increased inflow of free fatty acids and de novo hepatic lipogenesis (Rolo et al. 2012). Virtually, the initial stage of NAFLD involves accumulation of triglycerides in the liver. Later on, enhanced non-esterified fatty acid release from adipose tissue (lipolysis), increases de novo lipogenesis and decreases beta-oxidation. These are consistent with the potential sources of hepatic lipids (Postic and Girard 2008).

Fatty acid-binding proteins (FABPs) are members of the superfamily of lipid-binding proteins. So far, nine different FABPs, with tissue-specific distribution, have been identified including liver. Fatty acids translocation mainly occurs via three more fatty acid transporters in addition to FABP; fatty acid transport proteins (FATP), fatty acid translocase (FAT/CD36) and caveolin-1. Eventually, the accumulation of fat in the form of lipid droplets within the hepatocytes results in hepatic steatosis (Canbay et al. 2007; Chmurzyńska 2006). FAT/CD36 is predominantly located at the plasma membrane of hepatocytes in patients with NAFLD (Miquilena-Colina et al. 2011). Intracellular lipid droplets or "adiposomes" contain the molecular machinery to synthesize, store, utilize, and degrade various lipids derivatives from the enzymatic activity of hydroxymethylglutaryl-coenzyme A reductase and ACC (Liu et al. 2004). Thereby, non-esterified fatty acids are stored in lipid droplets in the form of triglyceride, which could reduce the lipotoxicity of cytosolic fatty acids. Meanwhile, lipid droplet-binding protein, protein perilipin 5 (PLIN5) expression is increased in steatotic livers, whereas PLIN5 deficiency reduces hepatic lipid content and the size of lipid droplets (Wang et al. 2015). PLIN coats lipid droplets and protects triglycerides from the lipolytic action of hormone-sensitive lipase (Londos et al. 1999). Furthermore, overexpression of lipid storage droplet protein 5 enhances lipid accumulation in the hepatic cells. Lipid storage droplet protein 5 (LSDP5) contributes to triglyceride accumulation by negatively regulating lipolysis and fatty acid oxidation in hepatocytes (Li et al. 2012). Some other lipid droplet proteins also play a role in the pathophysiology in the fatty liver disease. Thus, high fat diet-induced peroxisome proliferator-activated receptor-gamma (PPAR-gamma) increases the expression of PLIN2 and/or Fat-specific protein 27 (FSP27) and develops fatty liver (Okumura 2011). In an alternative pathway, lipid droplet formation is enhanced due to increased diacylglycerol O-acyltransferase 1 expression without increased PPAR-gamma expression and decreased hormone-sensitive lipase expression (Kohjima et al. 2007). In humans, hepatic lipase activity positively correlates with intra-abdominal fat content (Carr et al. 1999). In fact, hyperinsulinemia is associated with the downregulation of insulin receptor substrate-2 (IRS-2) in the liver. This pathway includes a cascade of consecutive events; firstly, insulin-mediated stimulation of tyrosine phosphorylation of IRS-2 is diminished. Subsequent to decrease in IRS-2 associated phosphatidylinositol 3-kinase (PI3K) activity, phosphorylation of Akt is severely depressed. Despite the reduction in IRS-2, insulin continues to increase SREBP-1c. Eventually, the combination of glucose overproduction with the enhanced fatty acid synthesis leads to further increase in insulin secretion and resistance by creating a vicious cycle (Shimomura et al. 2000).

Triglyceride synthesis is a critical metabolic pathway contributing to the lipid content in cells (Li et al. 2012). Primarily, liver-lipoprotein lipase achieves a twofold increase in liver triglyceride

content. Insulin resistance with the deficiency of IRS-2-associated PI3K activity causes an increase in intracellular fatty acid-derived metabolites such as diacylglycerol (DAG), fatty acyl CoA or ceramides (Kim et al. 2001). Hepatic insulin responsiveness may be improved by decreasing the transcription factor, cyclic adenosine monophosphate (cAMP)-responsive element-binding protein (CREB) expression. Thus, decreased hepatic CREB expression leads to reduced accumulation of intrahepatic DAG content and protein kinase C (PKC)-epsilon activation. An eventual protection against fat-induced hepatic steatosis and hepatic insulin resistance may be ensured (Erion et al. 2009) (Fig. 19.1). It has been recently demonstrated that the transcription factor hepatocyte specific CREB (CREB-H) mRNA promoter activity is induced by fatty acids and co-expression of PPAR-alpha (Danno et al. 2010). Additionally, hepatic CREB-H is transcriptionally activated by fasting. Active CREB-H induces apolipoproteins (Apo); Apoa4, Apoa5, and Apoc2, which exhibit stimulatory effects on lipoprotein lipase. These apolipoproteins facilitate triglyceride clearance from plasma, whereas CREB-H-deficiency causes hypertriglyceridemia (Lee 2012). Non-esterified fatty acids enter into hepatocytes mainly through CD36, FATP2, FATP4, and FATP5. Overexpression of human ApoC3 can inhibit lipoprotein lipase activity. Thereby, restriction of peripheral fat uptake promotes postprandial hyperlipidemia (Samuel and Shulman 2012). Actually, carriers of the ApoC3 variant alleles increase fasting plasma ApoC3 concentrations and fasting hypertriglyceridemia, which enhances hepatic steatosis. The prevalence of NAFLD has been reported as 38% among variant-allele carriers who have marked insulin resistance (Petersen et al. 2010). The common variant I148M of the enzyme patatin-like phospholipase domain containing 3 (adiponutrin or PNPLA3) has emerged as a major genetic determinant of hepatic steatosis and non-alcoholic steatohepatitis in 40–50% of Europeans. PNPLA3 encodes a lipid droplet-associated, carbohydrate-regulated lipogenic and/or lipolytic enzyme (Krawczyk et al. 2013). PNPLA3-I148M is associated with triglyceride accumulation by limiting triglyceride hydrolysis. Therefore, the I148M variant interferes with hepatic triglyceride hydrolysis, affecting the association of PNPLA3 with the lipid droplets (He et al. 2010). Eventually, homozygosity for the I148M allele is associated with a 3.3-fold increased risk of both NASH and liver fibrosis (Valenti et al. 2010). Greco et al. showed that a total of 1060 genes were significantly associated with liver fat content in a series of 30 NAFLD patients. Of these genes, 419 were positively and 641 negatively correlated with liver fat. While the expression of PLIN increases, hormone-sensitive lipase expression simultaneously decreases with the elevation of hepatic fat content. In these patients, the chemokines, chemokine (C-C motif) ligand 2 (CCL2)/ monocyte chemoattractant protein-1 (MCP-1) and CCL4 along with fatty acid transporters, FABP4 and CD36 are positively correlated with the liver fat content (Greco et al. 2008). As noted above, expression of several genes induces the synthesis of inflammatory mediators in hepatic steatosis without histologically detectable inflammation. In particular, serum levels of C-reactive protein (CRP), tumor necrosis factor-alpha (TNF-alpha), interleukin-6 (IL-6), CCL2/MCP-1, and CCL19 increase in NAFLD. Moreover, the initially elevated TNF-alpha and CCL2/MCP-1 levels remain high in NASH (Haukeland et al. 2006). Among these, IL-6 levels as well as MCP-1/CCL2 and CCL4 concentrations positively correlate with the intrahepatic triglyceride content (Greco et al. 2008; Hwang et al. 2007).

Although it is proposed that the "double-hit" hypothesis is recently obsolete, as it is inadequate to explain the several molecular and metabolic changes that take place in NAFLD, lipotoxicity-related mechanism of NAFLD could be explained still best by this hypothesis. Actually, the "double-hit" hypothesis comprises the alterations of metabolic pathways, which are included in "multiple hit" hypothesis (Buzzetti et al. 2016). According to "double-hit" hypothesis, triglyceride accumulation is the "first hit" that predisposes to liver damage in the progression from NAFLD to NASH (Tacke et al. 2009). The ratio between triglyceride and total phospholipids in the cells

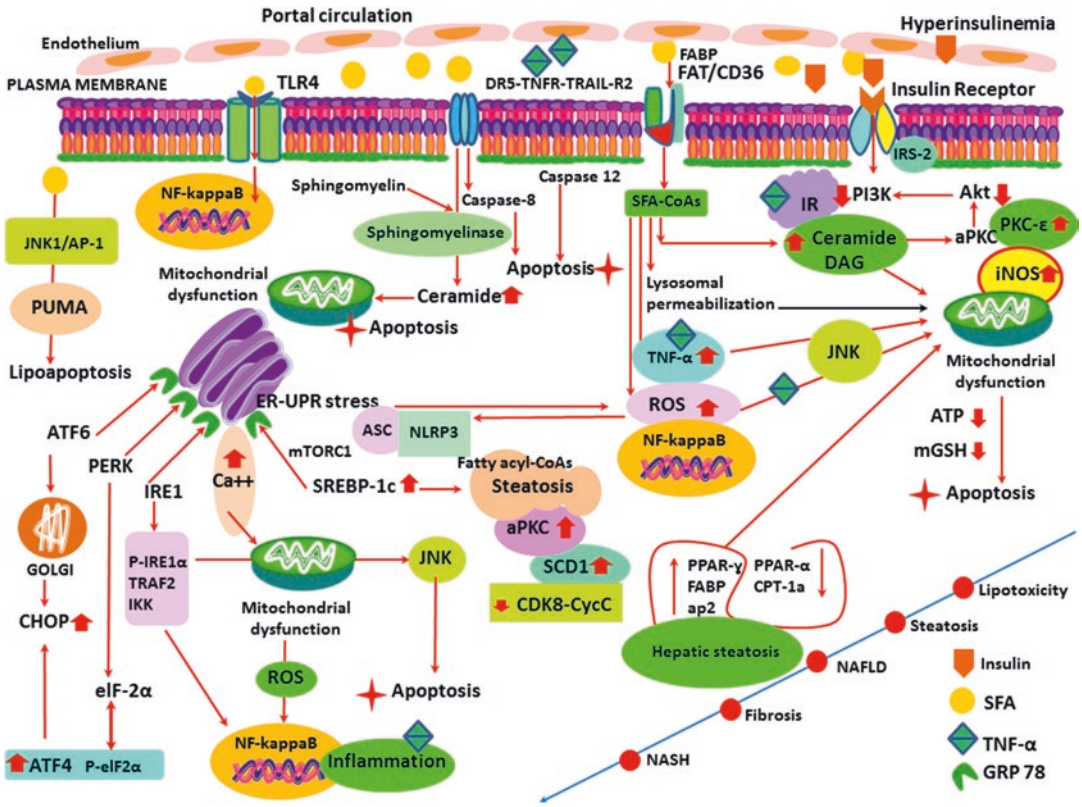

Fig. 19.1 NAFLD is characterized by excess accumulation of triglyceride in the hepatocyte both due to increased inflow of free fatty acids and de novo hepatic lipogenesis. Insulin resistance enhances triglyceride lipolysis, release of pro-inflammatory cytokines and inhibits esterification of free fatty acids within adipose tissue. Ceramides promote hepatic insulin resistance and attenuate Akt signaling. Dissociation of ATF6, IRE1, PERK and SREBP from GRP78 leads to activation of a series of signaling pathways which result in oxidative stress, inflammation, apoptosis and hepatic steatosis (*JNK* c-Jun N-terminal kinase, *AP-1* Activator protein-1, *PUMA* Pro-apoptotic protein p53 up-regulated modulator of apoptosis, *ATF6* Activating transcription factor 6, *CHOP* CCAAT/enhancer-binding protein homologous protein, *ATF4* Activating transcription factor 4, *eIF2α* alpha-subunit of Eukaryotic Initiation Factor 2, *p eIF2α* Phosphorylated alpha-subunit of Eukaryotic Initiation Factor 2, *IKK* inhibitor kappa B kinase, *TRAF2* TNF receptor-associated factor 2, *pIRE1* Phosphorylated inositol requiring enzyme 1, *IRE1* Inositol requiring enzyme 1, *PERK* PRKR-like endoplasmic reticulum kinase, *NF-kappaB* Nuclear factor-kappa B, *ROS* Reactive oxygen species, *TLR4* Toll-like receptor-4, *ER* Endoplasmic reticulum, *UPR* Unfolded protein response, *mTORC1* Mammalian target of rapamycin complex1, *SREBP-1c* Sterol regulatory element-binding protein-1c, *ASC* Apoptosis-associated speck-like protein containing a CARD adaptor protein, *NLRP3* NOD-like receptor (NLR) subfamily of PRRs and NALP3, *TNFR1* TNF receptor-1, *TRAIL-R2* TNF-related apoptosis-inducing ligand, *DR5* Death receptor 5, *aPKC* Atypical protein kinase C, *CDK8* Cyclin-dependent kinase 8, *CycC* Cyclin C, *aP2* Activator protein-2, proximal promoter region of the adipocyte P2 gene mRNA, *FABP* Fatty acid-binding protein, *PPAR-γ* Peroxisome proliferator-activated receptor-gamma, *CPT-1a* Carnitine palmitoyl transferase-1a, *NASH* Non-alcoholic steatohepatitis, *NAFLD* Non-alcoholic fatty liver disease, *TNF-α* Tumor necrosis factor-alpha, *SFA-CoA* Saturated fatty acyl-CoA, *FAT/CD36* Fatty acid translocase, *FABP* Fatty acid-binding protein, *IRS-2* Insulin receptor substrate-2, *IR* Insulin resistance, *PI3K* Phosphatidylinositol 3-kinase, *Akt* Protein kinase-B, *PKC-ε* Protein kinase C-epsilon, *mGSH* Mitochondrial glutathione, *iNOS* Inducible nitric oxide synthase, *GRP78* 78-kD glucose-regulated/binding immunoglobulin protein

increases twofold with oleic acid versus palmitic acid. Moreover, when compared to palmitic acid, triglyceride secretion is also twofold higher with the oleic acid (Cohen et al. 2015). Thus, free fatty acids lead to steatosis or lipoapoptosis according to the abundance of saturated free fatty acids to unsaturated ones. Saturated fatty acid-induced hepatocyte death is inhibited by diverting satu-

rated fatty acid to triglyceride and decreasing lysophosphatidylcholine content. Thereby, cytosolic triglyceride accumulation is considered to be hepatoprotective (Han et al. 2008). In brief, increased de novo synthesis and uptake of fatty acids lead to further fatty acid accumulation in hepatocytes. In this regard increased expressions of ACC1, FAS, SREBP-1c, and adipose differentiation-related protein (ADRP) are consistent with the first hit in NAFLD (Kohjima et al. 2007).

3 Insulin Resistance and Non-alcoholic Fatty Liver Disease

Insulin resistance is the major mechanism in the development and progression of NAFLD, therefore, the potential therapeutic effect of insulin sensitizers on NAFLD/NASH has a great importance (Takahashi et al. 2015). Actually, mean adipocyte size positively correlates with intrahepatic fat. Although the enlargement of subcutaneous abdominal adipocytes is not directly related to insulin action, it predicts an increased liver fat content in obese individuals. Nevertheless, liver fat content is the only independent predictor of reduced peripheral and hepatic insulin action (Koska et al. 2008). Recently an inverse relationship has been found between hepatic lipid content and insulin sensibility (Hage Hassan et al. 2014). In fact, insulin resistance is secondary to progressive mitochondrial dysfunction. This may be the acceptable primary event that triggers obesity-associated NAFLD development (Rector et al. 2010). Insulin resistance in NAFLD is characterized by reduced whole-body, hepatic, and adipose tissue insulin sensitivity. Insulin resistance is often associated with chronic low-grade inflammation, and numerous mediators that are released from immune cells and adipocytes and may contribute to the liver damage and liver disease progression (Ercin et al. 2015). Very strong inverse correlation between peripheral insulin sensitivity and intrahepatic triglyceride and visceral fat contents suggest that hepatic insulin resistance may precede peripheral insulin resistance (Hwang et al. 2007; Perseghin 2009). However, there are no convincing data indicating that the development of NAFLD precedes the onset of whole body insulin resistance. But some animal studies indicated that the development of peripheral insulin resistance is secondary to hepatic fat infiltration and hepatocytic insulin resistance (Perseghin 2009).

In the scope of "double-hit" hypothesis, the second hit includes metabolic oxidative stress, autophagy, and inflammation induced NASH progression (Berlanga et al. 2014). Of all, obesity triggers a chronic inflammatory state and cytokine release from either adipocytes or from macrophages that are infiltrating adipose tissue. All of these antagonize insulin action. According to other perspective, lipid accumulation in non-adipose tissues leads to the build up of bioactive sphingolipids. Thereby ceramides link both excess saturated fatty acids and TNF-alpha to the induction of insulin resistance (Samad et al. 2011; Summers 2006). The mean DAG and triacylglycerol levels increase significantly in NAFLD, but the free fatty acids remain unaltered. A stepwise increase in the mean triacylglycerol/DAG ratio is observed in the normal livers progressing to NAFLD and finally ending up at NASH. However, the total phosphatidylcholine decreases in both NAFLD and NASH, whereas the free cholesterol to phosphatidylcholine ratio increases progressively (Puri et al. 2007). While unsaturated fatty acids lead to triglyceride synthesis and storage, saturated fatty acids may be used for synthesis of DAG and ceramide (Hage Hassan et al. 2014). Ceramides accumulate in the liver during the elevated hepatic influx of free fatty acids and take a part among the lipid bilayer components of cell membranes (Hannun and Obeid 2008). Furthermore, these two lipid second messengers; DAG and ceramides have deleterious actions on insulin signaling (Hage Hassan et al. 2014). Nevertheless, obese mice with hepatic steatosis have elevated triacylglycerol levels, in addition to putative activated ceramide-synthesis pathways (Yetukuri et al. 2007). DAG has been shown to accumulate in insulin resistant liver. The hepatic concentrations of DAG increase with rising saturated liver fat content in human NAFL. The human fatty liver is also characterized by depletion of long polyunsat-

urated fatty acids in the liver and increases in hepatic stearoyl-CoA desaturase-1 (SCD1) and lipogenic activities (Kotronen et al. 2009). Indeed SCD1 plays a direct role in the development of fatty liver diseases (Narce et al. 2012) (Fig. 19.1).

Since NAFLD is associated with obesity and peripheral insulin resistance, insulin resistance increases lipolysis and promotes free fatty acid delivery to the liver. Final step of triglyceride synthesis in liver is catalyzed by acyl-coenzyme A: DAG acyltransferase (DGAT) (Choi and Diehl 2008). Thus, a twofold increase in DGAT2 mRNA levels results in a fivefold increase in liver triglyceride content. Overexpression of hepatic DGAT2 develops hepatic steatosis. However, DGAT-mediated hepatic steatosis does not lead to insulin resistance (Monetti et al. 2007). On the other hand, suppression of DGAT-2 protects against fat-induced hepatic insulin resistance by lowering hepatic DAG concentrations and PKC activation through decreased SREBP1c-mediated lipogenesis and increased hepatic fatty acid oxidation (Choi et al. 2007).

A negative correlation has been found between insulin-mediated suppression of hepatic glucose production and intrahepatic DAG, but not with intrahepatic ceramide or acylcarnitine in obese adults (Magkos et al. 2012). According to data obtained from obese patients, nondiabetic individuals, hepatic DAG content in lipid droplets is the best predictor of insulin resistance in humans. Therefore, increase in hepatic DAG content indicates the NAFLD-associated hepatic insulin resistance (Kumashiro et al. 2011). In this respect, hepatic insulin resistance could be attributed to an almost 12-fold increase in hepatic DAG content along with a 3.6-fold increase in PKC-epsilon activation. While subsequent insulin-stimulated IRS-2 tyrosine phosphorylation decreases by 52%, phospho serine/threonine-specific protein kinase (phosphor-protein kinase B, pAkt) to Akt ratio is reduced by 64% in comparison with basal conditions (Jornayvaz et al. 2011).

As mentioned above, PKC family members also play a role in the development of hepatic insulin resistance (Raddatz et al. 2011). Increase in hepatic DAG content leads to activation of PKC-epsilon and subsequently results in decreased insulin signaling. Actually, the DAG-PKC-epsilon hypothesis can explain the occurrence of hepatic insulin resistance observed in most cases of NAFLD associated with obesity (Birkenfeld and Shulman 2014). During obesity, ceramide is mainly generated either from long saturated fatty acids de novo with the condensation of palmitate and serine to form 3-keto-dihydrosphingosine or from the sphingomyelin hydrolysis through the sphingomyelinase pathway. Furthermore, ceramide is recovered through the breakdown of glycosylsphingolipids in salvage pathway (Hage Hassan et al. 2014). While a positive correlation is found between human adipose tissue ceramide content and plasma adiponectin concentration, a negative correlation is evident between total ceramide content and homeostasis model of insulin resistance (HOMA-IR) index (Błachnio-Zabielska et al. 2012). The role of ceramides in the onset of hepatic insulin resistance is more debated. However, accumulation of ceramides contributes to the progression of steatohepatitis by modulating several cell functions (Marí and Fernández-Checa 2007). Inhibition of ceramide de novo synthesis reduces hepatic lipid accumulation in rats with NAFLD, this may lead to amelioration of hepatic steatosis (Kurek et al. 2014). In high-fat-fed mice, hepatic insulin resistance involves ceramide-induced activation of atypical protein kinase C (aPKC), which selectively impairs Akt-dependent forkhead box O1 protein (FOXO1) phosphorylation on scaffolding protein WD40/ProF (Sajan et al. 2015). aPKC activation in the liver is dependent on IRS-2/PI3K ratio. The activation of aPKC remains in high-fat feeding and obesity. Elevated activation of hepatic aPKC in hyperinsulinemic states may enhance the expression of SREBP-1c, which controls genes that increase the hepatic lipid synthesis (Farese et al. 2005). In this cycle, first of all, insulin enhances the synthesis of fatty acids and triglycerides through increasing the active nuclear fragment of SREBP-1c by more than 25-fold. Subsequently, the rate of gluconeogenesis is reduced via inhibition of phosphoenolpyruvate carboxykinase (PEPCK) mRNA by more than 95%. Meanwhile activation of mammalian target of rapamycin

complex 1 (mTORC1) leads to increased production of SREBP-1c. Simultaneously intrahepatic triglyceride storage is enhanced. In this process, insulin-mediated increase in SREBP-1c mRNA and the decrease in PEPCK mRNA are both blocked by inhibitors of PI3K and Akt. mTORC1 mediates the insulin induction by activating two transcription factors, liver X receptors (LXR) and SREBP (Li et al. 2010). On the other hand, active form of SREBP-1c seems to control the transcription of genes involved in fatty acid biosynthesis (Repa et al. 2000). Although some studies indicate that mTORC1 regulates SREBP-1 activation at multiple levels, only the mTORC1 stimulation is not sufficient to trigger SREBP-1 activation and lipid biogenesis *in vivo* (Bakan and Laplante 2012). Insulin induces SREBP-1c activation through Akt-mediated suppression of a liver specific isoform of insulin-induced gene product (Insig) and stimulation of mTORC1 signaling. However, Akt-Insig2a is a major mTORC1-independent pathway, which downregulates Akt in the liver by regulating SREBP-1c activation (Yecies et al. 2011). According to Ferré and Foufelle, the activation of SREBP-1c and subsequent insulin-induced de novo lipogenesis in hepatocytes is the response to endoplasmic reticulum (ER) stress in hepatic steatosis (Ferré and Foufelle 2010). Recently, markedly lower levels of cyclin-dependent kinase 8 (CDK8) and Cyclin C (CycC) proteins have been observed in obesity. In these cases, higher levels of nuclear SREBP-1c (nSREBP-1c) proteins and usually lower levels of CDK8 protein are found in human NAFLD biopsy samples as compared to normal livers. An inverse correlation arises between CDK8 and nSREBP-1c proteins in human NAFLD. Thereupon, the ratio of nSREBP-1c to CDK8 is more than fivefold higher in NAFLD human livers. Diminution of the CDK8-CycC complex by mTORC1 is an important contributing factor of hepatic de novo lipogenesis in NAFLD and insulin resistant states (Feng et al. 2015). SREBP-1c is not only regulated by itself but also by liver X receptors (LXRs). Increase in SREBP-1c mRNA by LXR is accompanied by a simultaneous increase in the SREBP-1c protein and subsequent stimulation of fatty acid synthesis (Repa et al. 2000). Actually, the elevated hepatic lipogenesis is the result of the elevated insulin levels. Insulin enhances transcription of lipogenic genes by increasing the ability of LXR-alpha or LXR-beta to activate the SREBP-1c promoter in liver (Chen et al. 2004). LXR specifically activates SREBP-1 gene transcription through an LXR response element (LXRE). Subsequent activation of lipogenic genes such as FAS, ACC, and SCD-1 occur secondary to SREBP-1 gene activation (Schultz et al. 2000).

4 Mitochondrial Dysfunction and Non-alcoholic Fatty Liver Disease

Activities of mitochondrial respiratory chain complexes are decreased in liver tissue of patients with non-alcoholic steatohepatitis. This dysfunction displays a negative correlation with serum TNF-alpha, insulin resistance, as well as higher body mass index values (Pérez-Carreras et al. 2003).

It is well known that the steady state balance of hepatic triglycerides is controlled by the hepatic uptake and the consumption of fatty acids. Actually the amount of hepatic triglycerides is not fixed but can readily be changed due to triglycerides/free fatty acid partitioning and triglycerides-free fatty acid metabolism by mitochondrial beta-oxidation (den Boer et al. 2004). The main controller for fatty acid entry into the matrix and of the hepatic mitochondrial beta-oxidation flux is malonyl-CoA sensitive carnitine palmitoyl transferase-I (CPT-I). It localizes in the inner zone of the mitochondrial outer membrane (Eaton 2002). Actually, malonyl-CoA participates in two opposing pathways; a substrate for fatty acid synthesis and a regulator of fatty acid oxidation (Mao et al. 2006). Malonyl-CoA is generated by two isoforms of ACC, ACC1 and ACC2 (Ha et al. 1996). Malonyl-CoA produced by the cytosolic ACC1, which catalyzes the rate-limiting step in the biosynthesis of long-chain fatty acids. In this respect, ACC1 is an important regulator of de novo fatty acid synthesis (Mao et al. 2006). The impairment in mitochondrial

fatty acid oxidation due to increased expression of mRNA for hepatic PPAR-gamma, adipose fatty acid binding protein, activator protein-2 (ap2) and suppressed expression of mRNA for hepatic PPAR-alpha and CPT-1a are crucial in the pathogenesis of hepatic steatosis (Cong et al. 2008). CPT-1 regulates the transfer of long-chain acyl-CoAs from the cytosol into the mitochondria, where they are oxidized. Decrease in both ACC1 and ACC2 expressions increase hepatic fat oxidation in fed state, whereas, suppression of ACC1 alone inhibits lipogenesis (Savage et al. 2006). Overall hepatic lipid turnover is regulated by transcription factors, such as ChREBP, SREBP-1c, CCAAT-enhancer-binding protein alpha (C/EBP alpha), and PPARs (Giby and Ajith 2014). ChREBP is a main regulator of lipogenesis in response to glucose. Glucose activates ChREBP by regulating its entry from the cytosol to the nucleus, thereby promotes its binding to carbohydrate responsive element in the promoter regions of glycolytic and lipogenic genes including liver-pyruvate kinase and ACC in addition to FAS, respectively (Poupeau and Postic 2011). In cases of hepatic steatosis and obesity, downregulation of PPAR-alpha is related to insulin resistance and favors lipogenesis over fatty acid oxidation. However, PPAR-gamma upregulation promotes lipogenesis (Tyagi et al. 2011). New fatty acids are the endogenous activators of physiologically distinct pools of PPAR-alpha (Chakravarthy et al. 2005). In obesity conditions, hyperinsulinemia with insulin resistance might contribute to the liver fat accumulation by inducing FAT/CD36 of hepatocytes during the development of fatty liver (Buqué et al. 2012). Actually, hyperinsulinemia have a direct role in stimulating hepatic CD36 expression and thus, the development of hepatosteatosis, hepatic insulin resistance, and dysglycemia (Steneberg et al. 2015). As described above, insulin facilitates de novo lipogenesis through upregulation and activation of SREBP1c and induction of ACC1. Malonyl-CoA produced by the cytosolic ACC1 is a regulator of de novo fatty acid synthesis. Decrease in malonyl-CoA responds by up-regulating the fatty acid synthesis pathway and down-regulating the fatty acid oxidation pathway (Mao et al. 2006). Firstly, excessive hepatic lipid accumulation in obesity may play a central, pathogenic role in insulin resistance. The other possibility is that hepatic insulin resistance itself contributes to alterations in mitochondrial oxidative capacity (Patti and Corvera 2010). As another option, lower hepatic ATP production results in insulin resistance, however the lack of ATP with the higher circulating plasma triglycerides or free fatty acids suggest that increased lipid availability cannot explain the abnormality of hepatic ATP production (Schmid et al. 2011). It is thought that, primary defects in mitochondrial fatty acid oxidation capacity can lead to fourfold increase in DAG accumulation, PKC-epsilon activation, and hepatic insulin resistance (Zhang et al. 2007). Others have reported evidences for increased rates of fatty acid oxidation due to elevated lipid burden, which results in excessive formation of reactive oxygen species (ROS) (Satapati et al. 2012). The latter findings suggest that increased mitochondrial activity could promote oxidative stress within the liver. Decreased antioxidant capacity of liver may play a critical role in the progression from benign NAFLD to inflammatory NASH and cirrhosis (Pessayre and Fromenty 2005). In this setting, the increased mitochondrial fatty acid beta-oxidation rate and the delivery of electrons to the respiratory chain cause the imbalance between a high electron input and a restricted outflow. Ultimate accumulation of electrons within the mitochondrial respiratory chain complexes increase the ROS formation. ROS-induced release of TNF-alpha and FAS triggers mitochondrial membrane permeabilization and apoptosis (Pessayre et al. 2004). Mitochondrial respiratory chain dysfunction and liver lesions reflect the tyrosine nitration of mitochondrial proteins by peroxynitrite or peroxynitrite-derivative radicals. Increased hepatic TNF and inducible nitric oxide synthase (iNOS) expression enhance peroxynitrite formation and subsequent inhibition of mitochondrial respiration (García-Ruiz et al. 2006). In addition to reduced anti-oxidant defense capacity and increased inflammatory response, NASH patients have higher mitochondrial mass, but lower maximal respiration, which is associated with greater

hepatic insulin resistance, mitochondrial uncoupling, and leaking activity (Koliaki et al. 2015).

The generation of ceramides in the liver may also be mediated by TNF-alpha and various associated death ligands. TNF-alpha can initiate ceramide synthesis by binding to TNF receptor-1 (TNFR1) and activating acid sphingomyelinase (Marí and Fernández-Checa 2007). The acid sphingomyelinase is required for the activation of key pathways that regulate steatosis, fibrosis and lipotoxicity. These processes comprise ER stress, autophagy and lysosomal membrane permeabilization (Garcia-Ruiz et al. 2015). Protein translocation through membranes consists of a carrier-type mechanism, exocytosis, membrane damage, and channel formation. The ceramide-induced protein release from mitochondria indicates the formation of single ceramide channels in individual mitochondria (Colombini 2010). Actually, these ceramide channels are formed in the mitochondrial outer membrane rather than a simple release mechanism (Siskind et al. 2002). Both the short-chain model compound, N-acetyl-D-erythro-sphingosine (C2-ceramide), and a typical naturally occurring long-chain ceramide, N-hexanoyl-D-erythro-sphingosine (C16-ceramide) are found to be equally potent to permeabilize the mitochondrial outer membrane and to release the proteins. The impermeability of the mitochondrial outer membrane to proteins could be restored by removing ceramide (Elrick et al. 2006). Furthermore, it is suggested that bcl-2-like protein 4 (Bax) oligomerization and mitochondrial outer membrane permeabilization are two critical steps in cell death. Mari et al. found that mitochondrial outer membrane-localized oligomeric Bax is not sufficient for TNF-induced mitochondrial outer membrane permeabilization and cell death. Thereby mitochondrial glutathione (mGSH) depletion and acidic sphingomyelinase activity are necessary for liver injury (Marí et al. 2008). The disassembly of ceramide channels is initiated by ceramidase. Eventually, sphingosine markedly reduces the ability of ceramide to induce the release of intermembrane space proteins from mitochondria by destabilizing ceramide channels (Elrick et al. 2006) (Fig. 19.1).

5 Hepatocyte Lipoapoptosis in Non-alcoholic Fatty Liver Disease

Hepatocyte lipoapoptosis is a critical feature of non-alcoholic steatohepatitis. Despite equal cellular steatosis, exposure to saturated free fatty acid provokes more apoptosis than unsaturated ones (Malhi et al. 2006). Saturated free fatty acids stimulate protein phosphatase 2A activity by threefold via FOXO3a activation. Direct binding of FOXO3a to the intracellular death mediator, Bim promoter enhances its expression and lipoapoptosis (Barreyro et al. 2007). Anti-apoptotic proteins destabilize the channels, whereas pro-apoptotic proteins act synergistically with ceramide to increase membrane permeability. Bcl-2 family proteins control these channels (Colombini 2013). The Bcl-2 protein family consists of approximately 20 members. Collectively, these proteins trace incoming stress signals and help to determine cell fate by altering the balance between pro-and anti-apoptotic family members. Bcl-2 proteins may play a direct role in saturated fatty acid-induced hepatocyte cell death and the progression of NAFLD (Gentile et al. 2011). The stage of liver steatosis increases the expression of proapoptotic proteins. Antagonistic protein Bcl-2 is diminished together with the progression of liver steatosis. However, as a proapoptotic protein, Bax expression has a minor role in the process of steatosis (Panasiuk et al. 2006). Saturated free fatty acids induce c-Jun N-terminal kinase (JNK)-dependent hepatocyte lipoapoptosis by activating the proapoptotic Bcl-2 proteins, Bcl-2-interacting mediator of cell death (Bim) and Bax. Of these proteins, Bax trigger the mitochondrial apoptotic pathway (Malhi et al. 2006).

A potent pro-apoptotic protein p53 up-regulated modulator of apoptosis (PUMA) contributes to free fatty acids-induced lipoapoptosis in liver cells by a JNK1/activator protein-1 (AP-1) complex signaling cascade (Cazanave et al. 2009). A direct interaction between PUMA and the first alpha helix of Bax, which promotes Bax translocation to the mitochondria, triggers pro-apoptotic activity (Cartron et al. 2004).

Mitochondria are the key controller of fatty acids removal and modify hepatocytes to counteract the excessive fat storage. Thereby, mitochondrial dysfunction participates at different levels in the pathogenesis of non-alcoholic steatohepatitis. Consequently, functional disorder of the mitochondria impairs fatty liver homeostasis and induces overproduction of ROS that in turn trigger cell death (Serviddio et al. 2008). In this context, Mcl-1 is an anti-apoptotic member of the Bcl-2 family. Saturated free fatty acid induces Mcl-1 degradation by the ubiquitin-dependent proteasome degradation pathway. The novel PKC isoform, PKCtheta promotes Mcl-1 degradation by cytotoxic free fatty acid in hepatocytes. Inhibition of Mcl-1 degradation attenuates saturated free fatty acid-induced apoptosis (Masuoka et al. 2009). Bcl-xL is another member of the anti-apoptotic Bcl-2 family. Overexpression of Bcl-xL blocks Bax-induced lysosomal permeabilization and attenuates saturated free fatty acid-induced apoptosis *in vitro*. Consistent with these evidences, Bax has a regulatory role on free fatty acid-mediated lysosomal permeabilization and subsequent cell death (Feldstein et al. 2006).

Although both NAFL and NASH are associated with eukaryotic translation initiation factor-2alpha (eIF 2alpha) phosphorylation, there is a failure to activate downstream recovery pathways; activating transcription factor 4 (ATF4)-C/EBP homologous protein (CHOP)-DNA damage-34(GADD34) (Puri et al. 2008). ER stress signaling has an important role in the attenuation of lipogenesis and cell protection through the overexpression of transcription factors ATF6 and ATF4, respectively (Ito et al. 2004). The JNKs, which are encoded by three different JNK loci, are now known to be regulated by many other stimuli, from pro-inflammatory cytokines to obesity. JNKs play important roles in numerous cellular processes, including programmed cell death, negative regulation of insulin signaling and control of fat deposition (Karin and Gallagher 2005). JNK is activated by oxidants and cytokines and regulates hepatocellular injury and insulin resistance (Schattenberg et al. 2006). Cell death is caspase-dependent and associated with mitochondrial membrane depolarization in addition to cytochrome c release. All these indicate activation of the apoptotic mitochondrial pathway. Thus JNK-dependent lipoapoptosis is associated with activation of Bax, a known mediator of mitochondrial dysfunction (Malhi et al. 2006). Interestingly, while JNK1 promotes steatosis and hepatitis, JNK2 inhibits hepatocyte death by blocking the mitochondrial death pathway. Actually, JNK1 mediates insulin resistance and both the development and progression of steatohepatitis (Singh et al. 2009c).

PUMA contributes to free fatty acid-induced lipoapoptosis in liver cells. The saturated free fatty acid induces PUMA expression by a JNK1/AP-1 signaling cascade. PUMA up-regulation with subsequent Bax activation is also demonstrated in human liver samples from patients with NASH (Cazanave et al. 2009) (Fig. 19.1).

Excessive triglycerides and cholesterol are retained in lipid droplets because of the decreased rate of lipolysis and the resultant reduction in fatty acid beta-oxidation in cells (Amir and Czaja 2011). Potentially toxic cell constituents are directed to the lysosome for degradation through autophagic pathways. Indeed, autophagy provides protection against cell death. Three types of autophagy are known: macroautophagy, chaperone-mediated autophagy and microautophagy. Macroautophagy is a formation of autophagosome that engulfs and damages macromolecules and organelles (Mehrpour et al. 2010). Macrolipophagy is required for the breakdown of lipid droplets components by lysosomal enzymes, whereas autophagic defects are associated with an increased accumulation of triglycerides and promote disease states (Dong and Czaja 2011; Singh et al. 2009a). Eventually, inhibition of macroautophagy provides a mechanism for the progression of simple steatosis to NASH. On the other hand, both cellular lipid accumulation and hyperinsulinemia may impair autophagic function of hepatocyte. The progression of NAFLD to NASH may also be promoted by these metabolic abnormalities (Amir and Czaja 2011). Since NASH is characterized by the accumulation of megamitochondria with the linear crystalline inclusions (Caldwell et al. 1999), autophagic turnover or mitophagy eliminates dysfunctional

or damaged mitochondria to prevent unnecessary cell loss (Green et al. 2011). Furthermore, these mechanisms also control the intracellular lipidome (Singh et al. 2009a).

ROS-mediated mitochondrial permeability transition and mitochondrial DNA (mtDNA) mutations may favor the release of mtDNA, in turn compromising oxidative phosphorylation and initiating a vicious cycle of mitochondrial collapse. In this case, triggering the nucleotide-binding domain, leucine-rich repeat/pyrin domain-containing-3 (NALP3) inflammasome and activating pro-inflammatory signaling via retinoic acid-inducible gene 1 (RIG-I) and a member of RIG-I-like receptor (RLR) family, melanoma differentiation-associated protein 5 (MDA5) may integrate autophagy, cell death, and inflammation (Ganz and Szabo 2013; Green et al. 2011). Indeed, ROS can activate NOD-like receptor family pyrin domain-containing 3 (NLRP3) inflammasome and its adaptor protein ASC at the interface between mitochondria and the ER. Thus, NLRP3 inflammasome senses mitochondrial dysfunction (Jin and Flavell 2010; Zhou et al. 2011). Consequently, release of mtDNA into the cytosol depends on the NALP3 inflammasome and mitochondrial ROS. Autophagic proteins regulate NALP3-dependent inflammation by preserving mitochondrial integrity (Nakahira et al. 2011). During the autophagy, cytoplasmic cargo including portions of the cytosol, mitochondria, and other organelles can be sequestered and digested (Green et al. 2011).Caspase inhibition is generally not sufficient for survival following mitochondrial outer-membrane permeabilization; instead cells undergo a "caspase-independent cell death" (CICD). Protection from CICD reflects an increase in and a dependence upon autophagy, associated with a transient decrease in mitochondrial mass (Colell et al. 2007). Macroautophagy is critical to remove damaged mitochondria in hepatocyte. Loss of macroautophagy leads to overactivation of the JNK/c-Jun signaling pathway that induces cell death. Cell-death occurs as a consequence of activation of the mitochondrial death pathway with cellular adenosine triphosphate (ATP) depletion, mitochondrial cytochrome c release, and caspase activation. Chaperone-mediated autophagy provides a resistance to death from oxidative stress (Wang et al. 2010), whereas defective autophagy may promote overactivation of the JNK/c-Jun signaling pathway in response to the oxidative stress that occurs in NASH. Increased JNK signaling from oxidative stress and decreased autophagy may promote steatohepatitis (Singh et al. 2009c). In fact, JNK/c-Jun/AP-1 activation promotes apoptosis (Singh and Czaja 2007). JNK/c-Jun signaling has been implicated in the regulation of oxidative stress-mediated liver injury. The result of oxidant-induced JNK activation on cell death reflects the net effect of JNK-mediated resistance and the promotion of cell death by c-Jun (Amir et al. 2012). In this case, reduced glutathione (GSH) depletion leads to over-activation of JNK/c-Jun signaling at the level of mitogen-activated protein kinase kinase 4 (MAPKK4) that induces cell death in the steatotic liver (Singh et al. 2009b) (Fig. 19.1).

Alterations in Bcl-2 proteins occur downstream of CHOP and JNK, and inhibition of the Bcl-2 homology 3 (BH-3) domain proteins Bim or PUMA prevents saturated fatty acid-mediated hepatocyte cell death (Malhi et al. 2006). JNK1/c-Jun-induced PUMA transcriptional up-regulation with subsequent Bax activation is an integral step promoting saturated free fatty acid-mediated apoptosis. This process is also observed in NASH patients and suggests a possible contribution of PUMA to the development of chronic hepatic lipotoxicity (Cazanave et al. 2009). The mono-unsaturated free fatty acid palmitoleate, is an adipose tissue-derived lipid hormone that strongly stimulates muscle insulin action and suppresses hepatosteatosis (Cao et al. 2008). Palmitoleate inhibits saturated free fatty acid-induced apoptosis and ER stress, in particular, CHOP induction in hepatocytes. Furthermore, it inhibits free fatty acid-induced dysregulation of pro-apoptotic Bcl-2 proteins (Akazawa et al. 2010). The toxic saturated FFAs directly up-regulates death receptor 5 (DR5) by apoptotic transcription factor CHOP-dependent mechanism, whereas genetic deletion of DR5 expression attenuates lipoapoptosis. Saturated free fatty acid directly induces plasma mem-

brane DR5 association with lipid rafts and receptor clustering independent of its cognate ligand TNF-related apoptosis-inducing ligand (TRAIL) resulting in caspase-8 activation and cellular demise (Cazanave et al. 2011). DR5 mRNA expression is significantly elevated in patients with non-alcoholic steatohepatitis. Free fatty acid induced hepatocyte steatosis sensitizes to TRAIL by a DR5 mediated JNK dependent mechanism (Malhi et al. 2007). DR-mediated apoptosis is regulated at the cell surface by the density of DRs. In addition, saturated fatty acids promote increased cell surface expression of DR5 (Cazanave et al. 2011). Cellular levels of DR5 are transcriptionally upregulated by CHOP-dependent mechanisms (Yamaguchi and Wang 2004).

6 Oxidative Stress in Non-alcoholic Fatty Liver Disease

As mentioned above, the "first hit" involves the accumulation of fat in the liver, the "second hit" includes oxidative stress-mediated inflammation, stellate cell activation and fibrogenesis (Chitturi and Farrell 2001).

Activities of mitochondrial respiratory chain complexes are decreased in liver tissue of patients with NASH. This dysfunction displays close correlation with serum TNF-alpha, insulin resistance, and body mass index values. Eventually oxidative phosphorylation is decreased in individuals with NASH (Pérez-Carreras et al. 2003). Overall mitochondrial respiratory chain dysfunction and liver lesions reflect the tyrosine nitration of mitochondrial proteins by peroxynitrite or peroxynitrite-derivative radicals (García-Ruiz et al. 2006). Actually, nitro-oxidative stress plays a major role in the pathogenesis of mitochondrial dysfunction provoked by high fed diet. Inhibition of oxidative phosphorylation is due to decreased synthesis and increased degradation of its subunits by the nitro-oxidative stress (García-Ruiz et al. 2014). Incubation of mitochondrial proteins with peroxynitrite induces the degradation of the oxidative phosphorylation subunits (García-Ruiz et al. 2010). Indeed, elevated oxidative stress has been well documented in NAFLD patients. Oxidative stress destroys lipid, protein, and DNA molecules by triggering the inflammatory signaling pathways, which promotes the progression from steatosis to NASH. Meanwhile, overproduced ROS may directly deplete antioxidant capacity by consuming GSH and inhibiting the activities of antioxidant enzymes (Liu et al. 2015). In this case, liver antioxidant pathways enhance to neutralize ROS (Kohjima et al. 2007). Virtually, peripheral insulin resistance, increased fatty acid oxidation, and hepatic oxidative stress are evident in both fatty liver and NASH. Additionally, NASH is associated with mitochondrial structural defects (Sanyal et al. 2001). Chronic oxidative stress is significantly higher in NAFLD livers and strongly influences steatosis progression (Perlemuter et al. 2005). Oxidative stress is generated by free fatty acids, pro-inflammatory cytokines and TNF alpha. The disturbances of lipid peroxidation in hepatocytes can lead to their extensive accumulation (Panasiuk et al. 2006). Mitochondrial free cholesterol loading is a result of the hepatocellular sensitivity to TNF and FAS-induced steatohepatitis due to mGSH depletion (Marí et al. 2006). Oxidative stress is exacerbated further in patients with steatohepatitis, which is associated with the total content of cytochrome P450 (CYP450) induction. Thereby, in patients with steatohepatitis the total content of CYP450 are significantly increased compared with controls (Videla et al. 2004). Mitochondrial dysfunction affects the accumulation of lipids in hepatocytes and promotes lipid peroxidation, the production of ROS, release of cytokines causing inflammation and cell death (Camps and Joven 2015). Defective mitochondrial oxidative phosphorylation hallmarks prominently in NAFLD (Pérez-Carreras et al. 2003). Regarding hepatic lipid homeostasis, the ratio of nicotinamide adenine dinucleotide (NAD^+) to NADH is dramatically increases in liver. The activators and inhibitors of the cellular respiration respectively increases and decreases the [NAD^+]/[NADH] ratio. Increasing the concentration of NAD^+ stimulates complete oxidation of fatty acids. Moreover, NAD^+ recovers impaired fatty acid oxidation in hepatocytes, which are deficient for either oxidative phosphor-

ylation or NAD-dependent deacetylase sirtuin-3 (SIRT3) (Akie et al. 2015). These changes are accompanied by both PKC-epsilon activation via increased hepatic DAG and an increase in mitochondrial ROS. Actually, hepatic DAG content in lipid droplets is the best predictor of insulin resistance in humans. Thus, NAFLD-associated hepatic insulin resistance is caused by an increase in hepatic DAG content (Kumashiro et al. 2011; Seki et al. 2002). Metabolic pathways related to high-fat diet-induced obesity have been implicated in hepatic insulin resistance, and thus, proposed as important mediators of hepatic insulin resistance in NAFLD. Additionally, a high-fat diet increases lipid metabolites but decreases lipid metabolism intermediates and the NAD to NADH ratio. High-fat diet results in fat accumulation via decreased beta-oxidation (Kim et al. 2011) (Fig. 19.1). Livers of mice fed on the high fat diet have reduced SIRT3 activity, a threefold decrease in hepatic NAD^+ levels and increased mitochondrial protein oxidation. SIRT3 depletion results in hyperacetylation of critical mitochondrial proteins that protect against hepatic lipotoxicity under conditions of nutrient excess (Kendrick et al. 2011).

The serum levels of oxidized low density lipoprotein (oxLDL) are significantly higher in NASH patients compared to the controls. Furthermore, insulin resistance is independently associated with the circulating levels of lipid peroxidation products (Chalasani et al. 2004). Metabolic syndrome patients exhibit a strong association between increased abdominal fat storage, liver steatosis, and systemic oxidative alterations (Palmieri et al. 2006). Oxidative stress develops in the liver of NAFLD patients with steatosis and exacerbates further in patients with steatohepatitis. Furthermore, in these patients the co-existence of a diminished antioxidant capacity and protein oxidation in the liver is associated with steatohepatitis (Videla et al. 2004). Hepatocyte oxidant injury depends on the interactions between the MAPK, extracellular signal-regulated kinase 1/2 (ERK1/2), JNK, and the nuclear factor kappaB (NF-kappaB) pathways. ERK1/2 typically induces resistance to oxidant stress, whereas JNK promotes cell death (Czaja 2007). Patients with NASH have significantly higher systemic levels of lipid peroxidation products (Chalasani et al. 2004). There is a strong association between increased abdominal fat storage, liver steatosis, and systemic oxidative alterations in metabolic syndrome patients (Palmieri et al. 2006). Increased ROS secretion into peripheral blood from accumulated fat depot in obesity is also involved in induction of insulin resistance. In only white adipose tissue but not the other tissues of obese mice, the mRNA expression levels of NADPH oxidase subunits increase, and mRNA expression levels and activities of antioxidant enzymes decrease. In accumulated fat, elevated levels of fatty acids activate NADPH oxidase and induce ROS production (Furukawa et al. 2004).

The ER must have an oxidation machinery capable of rapidly disposing of the excess electrons. ER-resident protein ER oxidoreductin (Ero1p) is an essential component of this oxidation machinery. Ero1 is a glycosylated flavoenzyme that is tightly associated with the lumenal face of the ER membrane. The membrane associated flavoprotein Ero1 is a significant source of oxidizing equivalents for the ER lumen and thus, is responsible for setting the ER oxidation state (Pollard et al. 1998). Non-catalytic cysteine pairs in Ero1p sense the level of potential substrates in the ER and correspondingly modulate Ero1p activity as part of a homeostatic regulatory system governing the thiol-disulfide balance in the ER (Sevier et al. 2007). Flavin adenine dinucleotide (FAD)-bound Ero1p oxidizes protein disulfide isomerase (PDI), which then subsequently oxidizes folding proteins directly. FAD-bound Ero1p passes electrons to molecular oxygen and produces ROS. Use of molecular oxygen as the terminal electron acceptor can lead to oxidative stress through the production of ROS and oxidized glutathione (Tu and Weissman 2004). Eventually, glutathione competes with protein thiols for the oxidizing machinery and increased Ero1 activity in the ER enhances glutathione oxidation (oxidized glutathione (GSSG) formation) (Cuozzo and Kaiser 1999). A higher GSH concentration is needed to balance oxidative folding in cells overexpressing Ero1 alpha.

Cytosolic GSH and lumenal Ero1 alpha play antagonistic roles in controlling the ER redox. Moreover, the overexpression of Ero1 alpha significantly increases the GSH content (Molteni et al. 2004). Actually, balancing Ero1 and GSSG oxidation of PDI could regulate the [GSH]/[GSSG] ratio. Any perturbation of this balance might cause an excess of ROS to accumulate and ultimately lead to cell death (Chakravarthi et al. 2006). RNA-dependent protein kinase-like ER eukaryotic initiation factor-2 alpha kinase kinase or PRKR-like endoplasmic reticulum kinase (PERK) is thought to defend against Ero1-mediated oxidative stress through ATF4-mediated activation of antioxidant responses and cysteine sufficiency. Actually, ROS levels are reduced with the exogenous addition of cysteine (Harding et al. 2003). In fact, ROS are generated from the unfolded protein response (UPR)-regulated oxidative folding machinery in the ER and mitochondria. Accumulation of toxic levels of ROS is caused by the ineffectual cycling of the Ero1-PDI oxidation cycle along with the depletion of the ROS scavenger glutathione (Haynes et al. 2004). Actually, the UPR is an adaptive signaling pathway utilized to sense and alleviate the stress of protein folding in the ER. The UPR is mediated through three proximal sensors PERK/Pancreatic eukaryotic initiation factor-2alpha kinase (PEK), inositol-requiring enzyme 1 (IRE1), and ATF6. IRE1/X-box binding protein 1 (XBP1), PERK/eIF2alpha, and ATF6 are activated in ER stress (Shen et al. 2005). UPR-mediated ROS generation is at least partly due to activation of CHOP, also known as GADD153 (Oyadomari and Mori 2004). The PERK and IRE1 arms of the UPR activate transcription factors that upregulate expression of the proapoptotic factor CHOP/GADD153 (Harding et al. 2000). As mentioned above, CHOP protein expression is regulated by the ATF4 and PERK by direct binding to the site of CHOP promoter (Ma et al. 2002). On the other hand, PERK, IRE1alpha, and ATF6alpha sense protein-misfolding stress in the ER. ATF6-alpha is required in cells to regulate protein folding, secretion, and degradation during ER stress and thus to facilitate recovery from stress (Wu et al. 2007). The Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) transcription factor as a novel PERK substrate and Nrf2 is a critical effector of PERK-mediated cell survival. In this case, PERK-mediated activation of Nrf2 maintains redox homeostasis and prevents cell death following ER stress. Nrf2- antioxidant responsive element (ARE) transcriptional pathway plays a critical role in the detoxication and elimination of ROS. ARE is the promoter of genes encoding the two major detoxication enzymes, glutathione S-transferase A2 (GSTA2) and NADPH: quinone oxidoreductase 1 (NQO1) (Cullinan et al. 2003; Nguyen et al. 2009). Interestingly, Nrf2 deficiency is associated with the decreased levels of GSH, detoxifying enzymes, catalase, and superoxide dismutase activity. Its deletion results in rapid onset and progression of steatohepatitis induced by a methionine-choline deficient diet (Sugimoto et al. 2010). The accumulation of ROS occurs either as a consequence of downstream of the inner membrane mitochondrial oxidative metabolism or free fatty acid-mediated oxidative stress, which induces toxicity through the Kelch-like ECH-associated protein (Keap1)-Nrf2 pathway (Mota et al. 2016) (Fig. 19.1).

7 Conclusion

Accumulation of triglycerides is increased in obesity. Conversely, increased metabolism of cellular triglyceride content in obesity facilitates NAFLD development. The harmful effects of high concentrations of lipids and lipid derivatives is associated with NAFL and NASH. ATF6alpha, PERK/ATF6alpha, PERK/eIF2alpha, IRE1alpha/XBP1 pathways can regulate the development of steatosis and lipoapoptosis in the liver. The JNK pathway is stimulated by both oxidative stress and ER stress, as one of the key mediators of insulin resistance and fatty acid-induced hepatotoxicity. Furthermore, stimulation of TRAIL receptor-2 and DR5 expression resulting in caspase-8 activation and cellular demise are implicated as DR signaling pathways in lipotoxicity. Additionally, elevated oxidative stress in NAFLD patients promotes the progression from steatosis to NASH.

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Lipotoxicity-Related Hematological Disorders in Obesity

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Abstract

Lipotoxicity can mediate endothelial dysfunction in obesity. Altered endothelial cell phenotype during the pathobiological course of the lipotoxicity may lead to the hemostatic abnormalities, which is a hallmark of several hematological disorders. Impaired hemostasis could also be directly related to the numerous metabolic diseases such as hypertension, diabetes and atherosclerosis. On the other hand, local hematopoietic bone marrow (BM) renin-angiotensin system (RAS) contributes to the development of atherosclerosis via acting on the lipotoxicity processes. Local BM RAS, principally an autocrine/ paracrine/ intracrinehematological system, is located at the crossroads of cellular regulation, molecular interactions and the lipotoxicity-mediated vascular endothelial dysfunction. The positive regulatory role of plasma LDL on AT1 receptor-mediated hematopoietic stem cell (HSC) differentiation and the production of pro-atherogenic monocytes had been described. LDL-regulated HSC function may explain in part hypercholesterolemia-induced inflammation as well as the anti-inflammatory and anti-atherosclerotic effects of AT1 receptor blockers. The role of local adipose tissue RAS is directly related to the pathogenesis of metabolic derangements in obesity. There may be a crosstalk between local BM RAS and local adipose tissue RAS at the genomics and transcriptomics levels. The aim of this chapter is to review hematological alterations propagating the pathological influences of lipotoxicity on the vascular endothelium.

Keywords

Hematological disorders • Lipotoxicity • Obesity

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1 Introduction

Apart from the circulating renin-angiotensin system (RAS), there is a local RAS in many different organs. It is well known that the local RAS has different paracrine, autocrine, and intracrine effects. Obesity is defined as abnormal body weight with a body mass index (BMI, kg body weight/height $(m)^2$) of greater than 30 and is related with increases in all-cause mortality, including death from cardiovascular disease and heart failure. Obesity is considered as a part of the metabolic syndrome and at present, obesity is an epidemic health disaster that is widely seen in the world population. It was shown that lipotoxicity can mediate endothelial dysfunction in obesity (Kim et al. 2012). The obesity pandemic currently affects several hundred million people worldwide, comprising increasing numbers of patients in developing countries such as India and China. In the US, the incidence of childhood obesity and obesity-related hypertension has reached to an alarmingly high level, and preventive measures have been taken to fight this disease. In the nineteenth century Rudolf Virchow defined inflammation as well as injury of endothelial cells in atherosclerotic vascular tissues. Also it was demonstrated that augmented proliferation of vascular smooth muscle cells after mechanical endothelial injury proposing that intact endothelial cells protect against atherosclerosis. Endothelial cells form the inner lining of arterial and venous blood vessels and were afterward found to secrete vasoactive and trophic substances such as prostacyclin endothelium-derived relaxing factor/nitric oxide (NO), angiotensin II as well as endothelin-1. At the present time it is well known that these endothelial substances control vascular growth, vasomotion, platelet function, immunologic and inflammatory responses as well as plasmatic coagulation. Blood pressure, blood flow, fluid volume, hemodynamic status, and electrolyte balance are managed by circulating RAS and therefore there is relationship between the RAS and development of atherosclerosis (Ferrario and Strawn 2006). In experimental studies it was proven that AT1a receptor (AT1R) is associated with atherosclerosis development (Daugherty et al. 2010). Endothelial and regenerating vascular cells are shown to be effected by RAS (Becher et al. 2011). In a study which was conducted with mice, Ang II–AT1R pathway in the BM is shown to increase atherosclerosis development (Kato et al. 2005). RAS is associated with atherosclerosis because RAS increases endothelial dysfunction, cellular proliferation and programmed inflammation. Hematopoietic cells that contain AT1Rs augment the migration of BM-derived inflammatory cell into the vessel walls (Kato et al. 2005; Sata and Fukuda 2010). Also, chronic hypertension is developed due to the AT1R receptors limited mononuclear cell accumulation in the renal tissue by the modulation of vasoactive cytokines (Crowley et al. 2010). Therefore altered endothelial cell phenotype during the pathobiological course of the lipotoxicity may lead to the hemostatic abnormalities, which is a hallmark of several hematological disorders. The aim of this chapter is to review hematological alterations propagating the pathological influences of lipotoxicity on the vascular endothelium.

2 The Relationship Between Ras, Endothelium and Lipotoxicity

The components of the metabolic syndrome can be affected by the stability of hemostasis. In many studies it was shown that impaired hemostasis could also be directly related to the numerous metabolic diseases such as hypertension, diabetes and atherosclerosis (Aryan et al. 2009; Atalar et al. 2005; Erdem et al. 1999; Guven et al. 2006; Yildiz and Haznedaroglu 2006). On the other hand, local hematopoietic bone marrow (BM) renin-angiotensin system (RAS) (Haznedaroglu and Beyazit 2013) contributes to the development of atherosclerosis via acting on the lipotoxicity processes (Beyazit et al. 2010). Local BM RAS, principally an autocrine/ paracrine/ intracrine hematological system, is located at the crossroads of cellular regulation, molecular interactions and the lipotoxicity-mediated vascular endothelial dysfunction (Beyazit et al. 2010; Haznedaroglu and Beyazit 2010). The major active peptide in RAS is Angiotensin II (Ang II) which is trans-

formed from Angiotensin I (Ang I) by angiotensin converting enzyme (ACE). Ang I is produced from angiotensinogen by Renin (Paul et al. 2006). However Ang II may also be generated by chymase which is an enzyme in the form of serine protease. Although there are other enzymes such as tonin and cathepsins, chymase is the main alternative enzyme for Ang II generation since it has the highest specifity to the substrate. This pathway is not inhibited by ACE inhibition (Urata et al. 1996). Chymase is blamed for RAS related arteriosclerosis since it is widely present in vascular walls (Arakawa and Urata 2000). AT1R and AT2R are receptors that facilitate the effects of Ang II (De Gasparo et al. 2000). AT1R actions results in vasoconstriction and aldosterone secretion. In vessel walls, kidney, heart, and brain RAS is locally stimulated (Ruiz-Ortega et al. 2001). Stimulation of AT1R leads to intracellular free radical production that contributes to tissue injury by increasing mitochondrial dysfunction. Inhibition of Ang II provides protection for neurodegenerative processes and stimulates longevity in experimental models. The development of atherosclerosis is related with RAS activity. Ang II stimulates the endothelial cell apoptosis thus it compromises the structural integrality of the endothelial barrier. Oxidative lipoprotein alteration, smooth muscle cell relocation from the media into the intima, proliferation, and conversion from a contractile to a synthetic phenotype are stimulated by oxidative stress and hyperthrombotic state by inflammatory response in the vessel intimal layer comprising T lymphocytes and macrophages with RAS stimulation. This process eventually leads to shrinkage of the vessel lumen. Although AT1aR expression on vascular cells is the main reason for these effects, AT1aR expression on BM-derived cells contributes to this process by quickening of infiltration of BM-derived inflammatory cells to the vessel wall.

Both angiotensin II and endothelin-1, the major effector peptide of the endothelin family were known as vasoconstrictor peptides and are secreted by endothelial and other vascular cells. At the present time it is also known that these peptides are not only take part in the management of vascular tone, but also have strong growth-stimulating and pro-inflammatory effects in different cell types. Besides RAS stimulation, also endothelin might also contribute to experimental and human hypertension and that inhibition of action or secretion of endothelin preserves functional and structural alterations in kidney and vasculature. Stimulation of G-protein coupled receptors—in response to either angiotensin II or endothelin-1—and the intracellular events downstream of receptor stimulation seems to be vital to maintain these disease progressions. Furthermore, both proteins potentiate each other's actions since angiotensin II induces expression of endothelin-1 in vitro and in vivo and effects of the enzymes involved in endothelin-1 formation.

Endothelium-dependent vasomotion is imperfect in obese humans and animals and it is not much known regarding the mechanisms of this vascular "dysfunction". It is known that obesity result in stimulation of thromboxane receptor gene expression that is one of the main goals for vasoconstrictor prostanoids. Obesity was related with boosted prostanoid-controlled vasoreactivity that in vascular beds is preserved by endogenous endothelin. These alterations were not related with systemic blood pressure, so this makes us think that obesity per se stimulates vasoconstriction. The role of perivascular adipose tissue for vasomotion is important that reduces vasoconstriction. An adipose-tissue related relaxing factor has been also detected. Local stimulation of vasoactive systems in fat cells raises actions of the sympathetic nervous system in obese patients. The RAS and the ET system stimulate sympathetic system since blockade of ACE, AT1 receptors or ETA receptors affects this stimulation. Thus, obesity-related vasoconstriction could promote hypertension, atherosclerosis, and thrombosis.

3 Metabolic Effects of the Local Bone Marrow RAS

The local BM RAS was first described in 1996 (Haznedaroglu et al. 1996). Since then many studies have been conducted in order to further clarify the role of local BM RAS. Intracellularly produced Ang II in BM stem and progenitor cells

contribute to the hematopoietic niche Ang II relations for autocrine/paracrine effects and to intracellular levels for intracrine effects. The BM microenvironment and blood cell types of the health and disease, the intracrine pathway could be the main mechanism of the effects of angiotensin peptides. Ang II and gene expression of the RAS were found in human mast cells which are considered as the "mobile RAS" controlled by cytokine effects (Hara et al. 2004). Also T and NK cells are also contain RAS elements and they could secrete and transfer AngII to inflammation sites. AngII stimulates the cellular chemotaxis that causes a potential inflammatory amplification system in the vasculature (Jurewicz et al. 2007). It was shown that regulatory T cells can improve Ang II-related cardiac damage (Kvakan et al. 2009).

Human primitive lympho-hematopoietic cells, embryonic, fetal and adult hematopoietic tissues also have ACE activity (Zambidis et al. 2008). Human umbilical cord blood cells are shown to exert Renin, AGT, and ACE mRNAs (Goker et al. 2005; Acar et al. 2007). ACE and other angiotensin elements activity in human hematopoietic stem cells (HSCs) during hematopoietic ontogeny and adulthood (Zambidis et al. 2008), thus local RAS may also have a role in HSC plasticity, and various hematological neoplastic diseases (Haznedaroglu and Beyazit 2010). The extensive pathobiological features of local BM RAS are related with the presence of ACE on leukemic blast cells within leukemic BM (Aksu et al. 2006; Beyazit et al. 2007), on erythroleukemic cells, ACE-expressing macrophages in lymph nodes of Hodgkin lymphoma (Koca et al. 2007), renin action in leukemic blasts (Wulf et al. 1996), Ang II as an autocrine growth factor for AML (Pinto et al. 2004), augmented renin gene action throughout NUP98-HOXA9 boosted blast development (Takeda et al. 2006), increased levels of BB9/ACE (+) AML isoforms, and changed JAK-STAT pathway as a link between RAS and leukemia (Haznedaroglu and Beyazit 2010; Haznedaroglu et al. 2000). JAK-STAT is a critical pathway between the upstream local BM RAS and neoplastic hematopoiesis (Haznedaroglu and Beyazit 2010; Haznedaroglu et al. 2000). In myeloproliferative diseases imatinib mesylate decreases the abnormally enhanced expressions of the main RAS components (Sayitoglu et al. 2009). The choice between to produce either blood or endothelial cells of the hemangioblast is affected by RAS (Slukvin 2009).

4 Local Hematopoietic Bone Marrow Renin-Angiotensin System and Atherosclerosis

The cardiac RAS and the hematopoietic BM RAS are closely associated with each other (Haznedaroglu et al. 1996; Haznedaroglu and Ozturk 2003). Myocardial tissue repair by hematopoietic stem cell plasticity could represent an association between local cardiac RAS and hematopoietic RAS (Ozturk et al. 2004). Blood cells predominantly macrophages/monocytes, neutrophils and T-lymphocytes exist in all phases of atherosclerosis. In a previously published study it was proposed that there is a lipid-angiotensin system connection within the BM that is responsible for the predisposition of immune cells to home to coronary arteries and lead to development of atherosclerosis (Strawn et al. 2003; Strawn and Ferrario 2008). This novel hypothesis takes the former lipid hypotheses together and certificates for an immunological stimulation model that arises as early as alterations in the BM that lead to the development of stimulated circulating monocytic phenotypes which leads to atherogenesis. The effects of modified LDL on hematopoietic management within the BM control the homing features of monocytes which is critical for the initiation of atherogenesis. The "bone marrow response-to-lipid" theory combines the idea that pro-atherogenic fetaures of hematopoietic and non-hematopoietic progenitors are managed by the local effects of modified LDL on the expression of local RAS genes (Strawn et al. 2003). LDL-regulated HSC function could clarify in part hypercholesterolemia-related inflammation and also the anti-inflammatory or anti-atherosclerotic properties of angiotensin receptor blockers (Strawn and Ferrario 2008). Relations between modified LDL

and the hematopoietic BM RAS could stimulate some monocytic phenotypes which lead to atherosclerosis (Strawn et al. 2003; Strawn and Ferrario 2008). BM recipient AT1a receptors are shown to be necessary to stimulate Ang II related atherosclerosis in hypercholesteromic mice in which BM transplantation trials were performed (Cassis et al. 2007). AT1a receptors which are expressed on infiltrating cells manage the Ang II-related atherosclerosis. Moreover, AT1a receptors on resident tissue are vital for the Ang II-related atherosclerosis and on aneurysms abdominal aorta. The pro-atherogenic activity of Ang II controlled differentiation/proliferation of monocyte-lineage cells was shown in a study (Kato et al. 2008). In this study investigator produced BM chimeric apoE negative mice repopulated with AT1-deficient (Agtr1−/−) or wild-type (Agtr1+/+) BM cells. The atherosclerosis process was considerably decreased in apoE−/−/BM-Agtr1−/− mice compared with apoE−/−/BM-Agtr1+/+ mice, along with a reduced numbers of BM granulocyte/macrophage progenitors and peripheral blood monocytes. As a result it can be concluded that Ang II manages the expression of c-Fms in HSCs and monocyte-lineage cells over BM stromal cell originated TNF-alpha to augment M-CSF–stimulated differentiation/proliferation of monocyte-lineage cells and may play role in atherosclerotic activity (Kato et al. 2008). The immunity and isolated lymphocytes are stimulated by Ang II managed autocrine loop. The immuno-stimulator activity of Ang II, particularly shown in T subset, could be harmful when local RAS are mismanaged as in cardiovascular atherosclerotic disease (Coppo et al. 2008). AT1aR in BM cells take role in atherosclerosis development by detecting numerous BM chimeric mice whose BM cells were positive or negative for AT1aR (Sata and Fukuda 2010). It was shown that the number of smooth muscle progenitor cells is increased after Ang II infusion. These smooth muscle progenitor cells were originally peripheral blood cells that transform to α-smooth muscle actin-positive cells after culture in the presence of PDGF-BB. Destabilization of atherosclerotic plaques is stimulated by these smooth muscle-like cells which exert abundant matrix metalloproteinase-9 (MMP-9). As a result it can be concluded that in order to prevent atherosclerosis, inhibition of AT1R should be performed in vascular cells as well as in BM (Kato et al. 2005; Sata and Fukuda 2010). To summarize, the hematopoietic BM RAS with local vasculature RAS have effects in the development and advancement of atherosclerosis, thus leading the development of cardiovascular diseases. Pathophysiology of atherosclerotic diseases gained a new perspective by RAS stimulation of cellular proliferation and programmed inflammation and in future this new approach may lead to a new therapeutic strategy. In a study it was reported that there is no change in atherosclerotic process in LDL receptor knock-out mice by transplantation with BM from AT1a receptor knock-out mice (Cassis et al. 2007), different from the studies indicating that (Kato et al. 2005; Yamada et al. 2007) AT1 receptor inhibition in BM cells may prevent atherosclerosis. In study it was also demonstrated that the beneficial effects of angiotensin receptor blocker (ARB) in end-organ injuries are because of the inhibition of AT1 receptor expressed in the end organs, but not in BM derived cells (Kato et al. 2008). The investigators proposed that distinct results detected in the kidney injury and atherosclerosis is possibly from the alterations in the pathogenesis of mouse models. Furthermore they have wondered that depending upon the tissues and model systems inspected, AT1 receptor activity in BM originated cells may have differential action points. Numerous physiological and pathophysiological stimuli or drugs control endothelial progenitor cell (EPC) mobilization. Furthermore, it was shown in a study levels of circulating EPCs is related with cardiovascular risk and left ventricular remodeling after myocardial infarction (Leone et al. 2009). Moreover, EPC pool in the myocardial microenvironment is associated to the bone marrow cellular proliferation. Vessel development and endothelial regeneration is affected by bone marrow-derived endothelial progenitor cells, monocytic cells, and mature endothelial cells (Steinmetz et al. 2010). Our current knowledge indicates that there are both cells that integrate into the vasculature, true EPC, and cells

with hematopoietic indicators that stimulate neovascularization. The interrogation of RAS activity on vasculogenesis-associated progenitor cells is also significant in the optimization of RAS intervention or regenerative treatment (Roks et al. 2011). To summarize the hematopoietic BM RAS and local vasculature RAS, manages the beginning and advancement of atherosclerosis, therefore effect the development of cardiovascular diseases.

5 The Role of RAS in Atherosclerotic Lesions

Cardiovascular risk factors are related with stimulation of the tissue renin–angiotensin system. It is widely accepted that the inhibition of the RAS can effectively delay the development of vascular diseases and related clinical events, including myocardial infarction. Cardiovascular protection has been predominantly confirmed with angiotensin converting enzyme inhibitors however also angiotensin receptor antagonists seem to be effective. It is notable that not only for vascular diseases but also for renal diseases these agents are very important therapeutic options. This makes us to think that stimulation of the RAS per se is one of the key factors defining initiation and development of abnormal cell growth and actions in the cardiovascular system, resulting in vascular and cardiac hypertrophy, mesangial cell growth and development of atherosclerosis. Atherosclerosis is characterized with chronic inflammation of the vascular wall including gathering of lipids, lipoproteins and mononuclear cells such as monocytes and T cells in the subendothelial space. This process results in a series of events in blood vessels leading to a remodeling of the arterial wall and a decrease in lumen size. Recent advances in biotechnology and molecular techniques have enabled us to discover the molecular pathways which initiate and stimulate the inflammatory activity in the development of atherosclerotic lesions. Even though RAS have a part in the control of cardiovascular and renal function, over-stimulation of this system contributes atherosclerotic process by triggering a series of cellular and molecular reactions detecting in the atherosclerotic lesions (Pastore et al. 1999).

Previously, Ang II was thought to effect atherosclerosis through its hemodynamic features however recently it has been proven that, the structural alterations in the vessel wall seen in atherosclerosis was a direct cellular effect of Ang II (Sata and Fukuda 2010). All of the elements of the RAS are expressed in the vessel wall and the activity of Ang II are generally controlled by the G-protein coupled receptors AT1 and AT2 (Iwai and Inagami 1992). Both AT1R and AT2R have been detected in the vessel wall, AT1R is thought to be accountable from most of the atherogenic activity of Ang II (Sayeski and Bernstein 2001). In a study, it has been showed that in hypercholesterolemic atherosclerosis in rabbits, the density of AT1 receptors in the media of diseased blood vessels is enlarged five-fold compared to healthy animals (Yang et al. 1998). In this study it was also showed that there is a significant AT1R binding capacity in the neointima of the diseased arteries. The most high receptor density in the vessel wall was detected on vascular smooth muscle cells (VSMCs); however cell culture investigations also established a important AT1R facilitated reactions in endothelial cells and macrophages and AT2Rs include only about 10% of total angiotensin receptors in healthy blood vessels (Yang et al. 1998). These data proposed that not only systemic but also local Ang II–AT1R pathway may contribute to beginning and advancement of atherosclerosis in blood vessels. It has been detected that serum ACE levels were considerably higher in cases with hepatoportal sclerosis (HPS) in comparison to the healthy controls (Beyazit et al. 2011). This data was obtained by measuring circulating ACE concentrations. Therefore it seems that ACE may be related with the pathological thrombotic events in the microenvironment of the portal circulation in HPS. Furthermore, it may be suggested that intracellular processes which controls the secretion/expression of vasoactive substances such as angiotensin peptides may stimulate the vasculopathy in portal hypertension (Beyazit et al. 2011).

6 RAS Effect on Vascular Endothelial Cells and Obesity

Ang II is directly related with endothelial function. Ang II is secreted by endothelium and it has significant effects on the endothelium. It is shown that Ang II has a direct role on endothelial function (Ruiz-Ortega et al. 2001). Vascular endothelium is considered as a metabolically active secretory tissue. It functions as a thromboresistant surface to blood and makes a macromolecular block between blood and the vessel. Structural functional defects of endothelial cells are the reason of not only vascular diseases containing atherosclerosis but also definite visceral diseases (Robinson et al. 1995). Vessel tonus, coagulation state, cell development and apoptosis, and leukocyte trafficking are controlled by the factor that are secreted from endothelial cells., VSMCs which are managed by endothelium and other factors, are also able to secrete cytokines and growth factors which may affect vascular cellular phenotype and development (Ruiz-Ortega et al. 2001). The cytokines control inflammation and immunity which may apply both pro-and anti-atherogenic effects on vascular wall cells. The actions of vascular wall cells controlled by cytokines can affect the beginning, development, or complication of the atherosclerotic lesions. Therefore, cytokines may influence multiple stages of atherogenesis and offer novel and interesting targets for treatment approaches. Endothelial cells control vascular tonus in a firm balance between nitric oxide (NO) and Ang II. This equilibrium is significant for to sustain a healthy endothelium. The inequality to Ang II by itself may result in several vital alterations in the endothelium which set the development of atherosclerosis (Neutel 2004). In several studies it has been proved that Ang-II stimulates the adhesion molecules, growth factors, cytokines and chemokines and applies a proinflammatory action on leucocytes, endothelial cells and VSMCs (Sata and Fukuda 2010; Han et al. 1999). Furthermore it has been shown that Ang II augments the expression of vascular endothelial growth factor (VEGF) that considerably stimulates the adventitial angiogenesis (Williams et al. 1995). Ang II starts an inflammatory cascade of decreased nicotinamide-adenine dinucleotide phosphate oxidase, reactive oxygen species (ROS) and nuclear factor-kappa B via type 1 receptor, that controls the transcription and gene expression and stimulates chemokines and adhesion molecules (Dandona et al. 2007). Augmented ROS and reduced MMP-9 effects in bone marrow decreases EPC mobilization in the initial post-infarction stage. ACE blockade or statin therapy stimulates EPC levels with distinct drug-specific actions on bone marrow molecular changes (Thum et al. 2006). The Ang II type 1a (AT1a) receptor is present on multiple cell types in atherosclerosis, containing BM-originated cells and vascular wall cells, and controls inflammation and development. Certainly, Ang II stimulates atherogenesis in hyperlipidemic mice via producing monocytes and by triggering vascular wall cells. In advanced atherosclerotic lesions, Ang II augments matrix metalloproteinases (MMPs) and plasminogen activator inhibitor-1, thus leading to destabilization of atherosclerotic plaque and changes of fibrinolytic equilibrium (Galis and Khatri 2002). Ang II has roles in vascular remodeling separately from stimulating oxidative stress, endothelial damage, thrombosis and inflammation. It acts as a bifunctional growth factor which triggers secretion of growth factors and vasoactive peptides in VSMCs (Itoh et al. 1993). Ang II can stimulate vascular remodeling and development of vascular lesions by modulating of vascular cell migration, reducing vascular smooth muscle apoptosis and extracellular matrix deposition (Scott-Burden et al. 1990). These effects of Ang II are controlled by complex intracellular signaling pathways containing triggering of the PLC-IP3-DAG cascade, tyrosine kinases, MAP kinases, and RhoA/Rho kinase (Touyz 2005). Intracellular signaling pathways which are augmented afterwards binding of the peptide to its cell-surface receptors, of which two main subtypes have been described, AT1R and AT2R (Murphy et al. 1991). While Ang IV receptor was recognized in recent times, as insulin-controlled aminopeptidase, but, its actions in angiogenesis yet not clarified. AT1R is

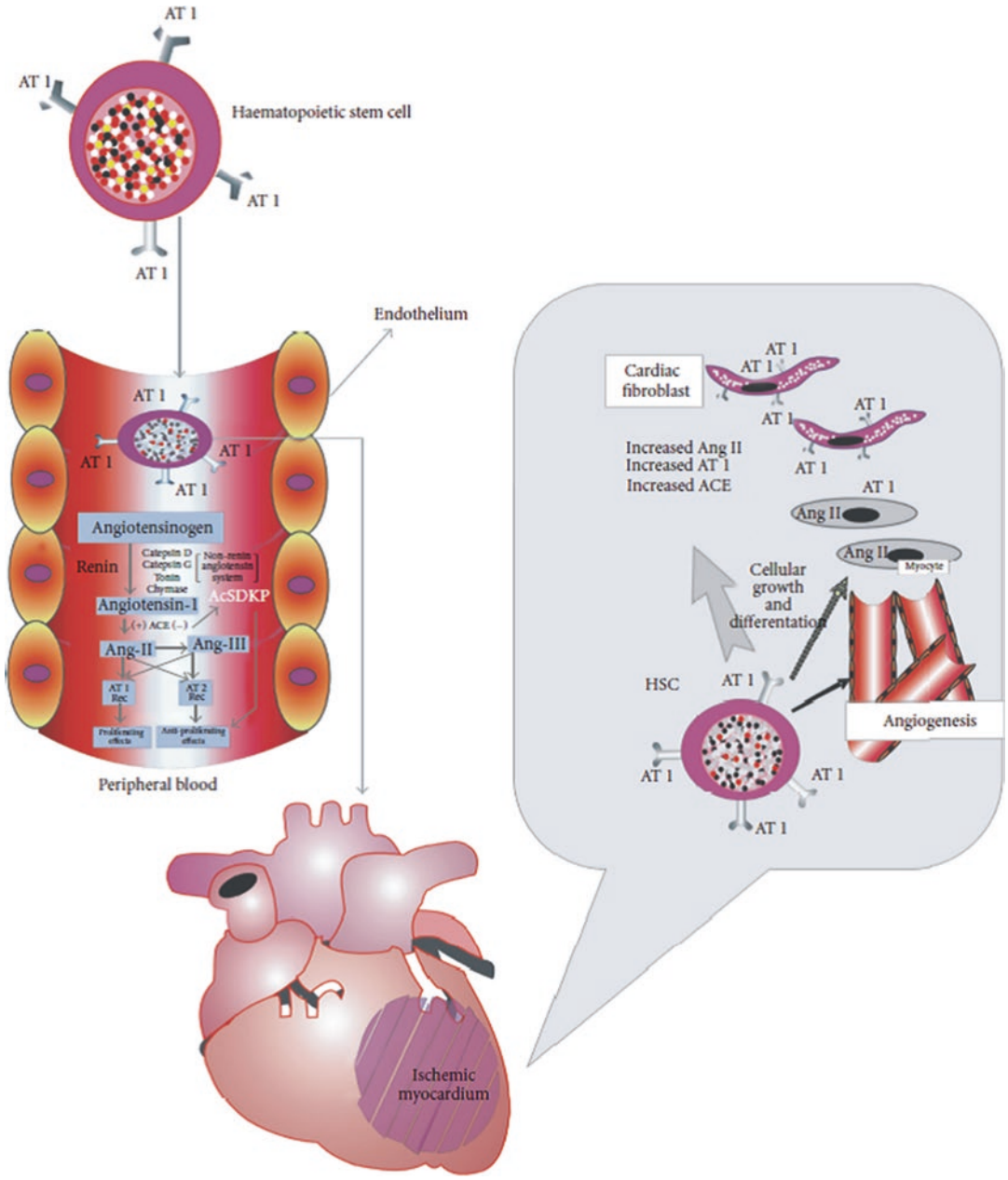

Fig. 20.1 RAS plays role on vascular endothelial cells, myocardial ischemia, angiogenesis, cellular differentiation and development of cardiac fibroblasts (Beyazit et al. 2010)

broadly present in blood vessels, kidney, heart, liver and adrenal glands; however AT2R is expressed mainly in foetal tissue, reducing after birth, with comparatively low quantities normally present in adult tissue. AT1R controls pro-angiogenic actions by boost of inflammation and leukocytes infiltration; however AT2R manages anti-angiogenic actions by control of apoptosis. Presence of AT2R is stimulated in pathological conditions related with cardiac and vascular remodeling or inflammation. Both of the receptors have critic actions in managing VSMC actions, although they differ in their unique effects. The RAS effects on vascular endothelial cells are summarized in Fig. 20.1 (Beyazit et al. 2010).

7 Inflammation and Development of Atherosclerosis

Atherosclerosis is a pathological process that initiates during fetal life. The early lesion of the atherosclerotic plaque, the fatty streak, could be observed in the fetal aorta and formation is stimulated by maternal hypercholesterolemia. This suggests that plasma lipids have an essential role for onset and development of early fetal atherosclerosis and in children. It is possible that unfavorable nutritional features and behavior (overeating, unbalanced lipid-rich diets) alone or in combination with unfavorable alterations in lifestyle such as lack of physical activity will stimulate the development of juvenile obesity, unfavorable alterations in plasma lipids, and the development of atherosclerotic lesion formation. One of the main reasons of atherosclerosis is considered as chronic inflammation. Noticeable cellular elements of atherothrombosis are lymphocytes, platelets, and endothelial cells but in latest studies it was suggested that myeloid leukocytes, specifically monocyte subsets, polymorphonuclear leukocytes, and mast cells play critical role in atherothrombosis (Soehnlein and Weber 2009). These elements are present in the vascular wall and trigger and maintain core mechanisms in plaque development and destabilization. Mesenchymal progenitor cells may also have critical effects in the beginning of myocardial fibrosis (Sopel et al. 2011). Various data suggest a role for oxidative stress in the development of endothelial dysfunction and atherogenesis, apart from the hemodynamic stress of blood pressure (Laursen et al. 1997). Furthermore plenty of data support that vascular reactive oxygen species (ROS) have a critical effect in atherogenesis. In addition to its vasoconstrictive features, novel in vivo and in vitro investigations suggest that Ang II, by the AT1 receptor, stimulates O2- production in endothelial cells, adventitial fibroblasts, vascular smooth muscle cells (VSMC), and mesangial cells by triggering of nicotinamide adenine dinucleotide (reduced form)/NADH phosphate (reduced form) (NADH/NAD(P)H) oxidase resulting in endothelial dysfunction, growth, and inflammation (Lassègue and Clempus 2003). Latest studies have also proven that in endothelial cells in addition to in VSMCs, NAD(P)H-dependent oxidase characterizes the most important O2- source. In a study it was shown that NAD(P)H oxidase is essential in the development of atherosclerosis via investigating the genetically changed mice which are lacking both apolipoprotein E (ApoE) and p47phox, one subunit of NAD(P)H oxidase. Noteworthy decrease in atherosclerotic lesion was detected in the double knockout mice, compared with that of ApoE-lacking mice. Also, ACE inhibitor-related blockade of plaque-infiltrating immune cells are related with suppression of the C-C chemokine receptor 9 (CCR9). The chemokine ligand 25 (CCL25)-CCR9 axis stimulates atherosclerosis, since blocking of CCR9 by RNA interference in hematopoietic progenitors of apoE-lacking mice slowed the atherosclerotic process (Abd Alla et al. 2010). ROS stimulated mitogen activated protein kinase, Akt and JAK/STAT pathways (Schieffer et al. 2000). Pharmacological inhibition of AT1R with Angiotensin receptor inhibitors could not be sufficient to prevent cytokine secretion completely; even though Ang II stimulates NF-kappa B and triggers formation of cytokines such as interleukin-6 and tumor necrosis factor-α, (Han et al. 1999). ROS secreted by Ang II effects the development of vascular diseases by inhibiting nitric oxide, damaging endothelial function, augmenting VSMC growth, and triggering proatherogenic, inflammatory, and adhesion molecule expression (Desideri et al. 2003). Ang II–related increase in O_2- production in the vessel wall is not associated with the hemodynamic activities of Ang II, since norepinephrine-related hypertension did not have parallel activities (Katusic and Vanhoutte 1989). Furthermore, it has been detected that Ang II effects neointimal monocyte infiltration by NF-kappa B stimulation and monocyte chemo attractant protein-1 (MCP-1) expression a significant outcome that is inhibited via angiotensin converting enzyme (ACE) blockers (Hernández-Presa et al. 1997). Ang II controls the presence of vascular cellular adhesion molecule-1 (VCAM-

1), intercellular adhesion molecule-1 (ICAM-1) and P selectin as well as cytokine, chemokine, and growth factor production within the vessel walls (Graninger et al. 2004). Also, RAS may affect the stimulation of complement system in both atherosclerosis and renal damage (Epstein 2001). This inflammatory pathway triggers the vascular inflammatory reaction by increasing the inflammatory cell infiltration to vessel walls. Monocytes convert into macrophages and increase the lipid deposition in the plaque, just after transferring into the vessel wall (Cathcart 2004). Chemokines and MMPs generated from monocytes/macrophages leads to the augmentation of atherosclerotic lesions. Besides, angiotensin II stimulates the intraplaque recruitment of monocytes and lymphocytes and increases the TNF-α, IL-6 and cyclooxygenase-2 expression in atherosclerotic vessels (Cathcart 2004). Moreover, Ang II stimulated increase of transcription factor nuclear factor-kappa B by redox-sensitive cascades, triggers cell adhesion molecules, chemokines MCP-1 and interleukin-8. These molecules augment monocyte and T lymphocyte adherence and accumulation in atherosclerotic plaques (Hernández-Presa et al. 1997). All of these evidences make us to suggest that a local stimulated RAS in vessel walls which triggers infiltration of inflammatory cells into the vessel walls is a significant characteristic of atherosclerosis.

8 RAS, Obesity and Other Mechanisms

The mechanisms by which obesity augments the risk for cardiovascular syndromes, as well as for related diseases such as augmented insulin resistance, diabetes, dyslipidemia, and hypertension is not clarified yet. These risk factors are interaction which are also detected in the "metabolic syndrome X", that includes of augmented insulin resistance, dyslipidemia, and hypertension in conjunction with obesity, and which is provoked by sedentary life style. Adipocyte, that characterizes the main element of fat tissue, not only comprises a functional local RAS, but also elements of the endothelin system such as endothelin receptors. Thus, a rise of body fat mass will lead to a local net rise in actions and/or expression of vasoactive systems. Furthermore, both angiotensin II and endothelin-1 stimulates adipocyte gene expression, comprising leptin and PAI-1 (Ailhaud et al. 2000). Actions and/or expression of genes encoding for vasoactive proteins of the RAS is managed in adipocytes. The actions of renin, that control development of angiotensin I from angiotensinogen, is augmented in plasma of obese cases as well as in obese dogs, make us to think that obesity systemically stimulates RAS (Henegar et al. 2001). In contrast to humans and dogs, obesity in most rodent models is related with decreased plasma renin actions, representing significant species alterations. Experimental obesity in many concerns still parallels pathophysiologic alterations seen in human obesity such as rises in insulin resistance and blood pressure. Like renin stimulation, species alteration exists between humans and rodents with regard to atherosclerosis and dyslipidemia. Therefore, when matching animal data to human disease it should be underlined that rodents are typically resistant to atherosclerosis unless exposed to highly unphysiological diets comprising cholic acid or after genetic manipulation. Natural resistance to atherosclerosis in mice could be somewhat associated to the fact that mice—unlike humans—have high plasma levels of HDL cholesterol relative to LDL cholesterol levels that continue even after high-fat diet treatment. Whether endothelin has effects in development of obesity is not yet clarified. In experimental obesity, there is stimulation in gene and protein expression of endothelin in the cardiovascular system, comprising vasculature and kidney (Barton et al. 2000). In humans, it is like to be a genetic tendency for obesity in some cases, that may position these patients at increased risk for vascular diseases as well. Augmented endothelin-1 plasma levels have been detected in obese normotensive and hypertensive cases (Tiret et al. 1999). As obesity is commonly related with rises in arterial blood pressure, it has been asked whether mutations of the preproendothelin-1 gene could be related with the predisposition to

development of hypertension. Without a doubt, the Lys198Asn polymorphism of the preproendothelin-1 gene was detected to be associated with obesity-related hypertension in Caucasians as well as in Japanese cases of obese patients (Tiret et al. 1999). Therefore, variations of the gene encoding for vasoconstrictor proteins could affect predisposition to hypertension in obese cases. Obesity is associated with the risk of renal disease, in which the RAS has a significant pathogenetic role. It was recently investigated whether obesity locally plays a role regarding expression or stimulation of elements of the RAS in the cardiovascular system. It was proven that diet-related obesity by giving C57 mice with a high fat, low cholesterol diet for 7 months, a treatment period that matches to 20 years in humans by means of maximal lifespan (Surwit et al. 1998). Animals on a high fat-diet were found to be obese and revealed a five times greater rise in body weight as compared to control group (Barton et al. 2000). In normotensive animals obesity was related with augmented ACE actions in the kidney, however not in lung or liver, and likewise with boosted angiotensin II-mediated vascular contractility. Chronic usage of an agent that is orally active antagonist of endothelin ETA receptors completely stopped these alterations, representing that endothelin locally manages RAS actions within the "obese" kidney. Obesity is a part of metabolic syndrome, which is affected by various features of endothelium. The risk for atherosclerotic vascular disease and following cardiovascular events is affected by several risk factors. These risk factors may be evaluated in two major groups which are endogenous and exogenous. Endogenous risk factors include genetic predisposition, hormonal influences, gender, age, and blood pressure. On the other hand exogenous risk groups include modifiable factors such as cigarette smoking, nutritional factors comprising dietary fat, body weight, physical activity, psychological stress and several others. Among variable risk factors, obesity has considered as a major health problem bearing significant social and economic implications. Aldosterone has roles in obesity-related disorders also (Kawarazaki and Fujita 2016). Obese subjects often have hypertension and related cardiovascular and renal diseases, and this has become a serious worldwide health problem. In obese subjects, impaired renal-pressure natriuresis causes sodium retention, leading to the development of salt-sensitive hypertension. Physical compression of the kidneys by visceral fat and activation of the sympathetic nervous system, RAS, and aldosterone/mineralocorticoid receptor (MR) system are involved in this mechanism. Obese subjects often exhibit hyperaldosteronism, with increased salt sensitivity of blood pressure (BP). Adipose tissue excretes aldosterone-releasing factors, thereby stimulating aldosterone secretion independently of the systemic RAS, and aldosterone/MR activation plays a key role in the development of hypertension and organ damage in obesity. In obese subjects, both salt sensitivity of BP, enhanced by obesity-related metabolic disorders including aldosterone excess, and increased dietary sodium intake are closely related to the incidence of hypertension. Some salt sensitivity-related gene variants affect the risk of obesity, and together with salt intake, its combination is possibly associated with the development of hypertension in obese subjects. With high salt levels common in modern diets, salt restriction and weight control are undoubtedly important. However, not only MR blockade but also new diagnostic modalities and therapies targeting and modifying genes that are related to salt sensitivity, obesity, or RAS regulation are expected to prevent obesity and obesity-related hypertension (Kawarazaki and Fujita 2016). Genetic polymorphisms regarding RAS are also blamed for the development of metabolic syndrome. It recent studies the association of polymorphisms within the RAS with metabolic syndrome in a cohort of Chilean subjects is reported (Herrera et al. 2016). Metabolic syndrome (MetS) is associated with hypertension, obesity and dyslipidemia. Thus, genetic variants related with these conditions may modulate its development. The effects of polymorphisms in the renin-angiotensin system (RAS) on metabolic syndrome risk in a cohort of Chilean subjects were evaluated (Herrera et al. 2016). In the study, a total of 152 subjects, 83 with MetS and 69 with-

out MetS of both genders were included, according to the ATP III update criteria (Herrera et al. 2016). The rs4340 Insertion/Deletion (I/D), rs699 (T > C) and rs5186 (A > C) of the ACE, AGT and AGTR1 genes, respectively, were genotyped. After adjusting for age and gender, it was detected that the DD genotype of rs4340 associated with MetS. Specifically, the DD genotype was associated with MetS risk in women. In males, the AA genotype for rs5186 variant was associated with an increased risk for developing MetS when compared with women carrying the same genotype. In subjects without MetS, DD genotype was associated with increased waist circumference while subjects with MetS carrying the rs5186 TT genotype showed higher levels of HDL-cholesterol (Herrera et al. 2016). Therefore it can be concluded that there is a role for RAS polymorphisms in predisposing to metabolic syndrome. Recently, it has been shown that free fatty acids activate RAS in 3T3-L1 adipocytes through Nuclear Factor-kappa B pathway (Sun et al. 2016). RAS stimulation is related with impaired differentiation of preadipocytes and augmented lipolysis and enhanced oxidative stress and inflammatory answer (Sun et al. 2016). Defects in the system are related with obesity, type 2 diabetes, and cardiovascular diseases. It is known that RAS present in a number of tissues, comprising kidneys, heart, and nervous and immune systems. It is also known that elements of RAS, comprising renin, angiotensinogen, ACE, and angiotensins I, II, and III have also been exist in adipose tissue (Sun et al. 2016). It is well established that free fatty acids (FFAs) are stimulators of RAS in leukocytes (Sun et al. 2016). But, whether FFAs has a role in the stimulation of RAS in adipocytes was a debate. It has been shown that the levels of FFAs generating from lipolysis in adipocytes are considerably augmented in peripheral circulation as well as local tissues in obese humans and animals (Sun et al. 2016). It has been proposed that RAS can manage adipocyte differentiation by Ang II and the adipocyte AT1R in mice (Sun et al. 2016). So, FFAs can directly manage RAS stimulation in adipose tissue which might be a stimulating mechanism of glucose and lipid metabolism disorder and obesity-associated diseases. FFA components such as palmitic acid (PA) and lauric acid can bind to Toll-like receptor 4 (TLR4) with the assistance of the endogenous ligand, fetuin A (Fet A), therefore facilitating the stimulation of TLR4 and NF-κB pathways and resulting in the inflammatory cascade (Sun et al. 2016). TLR4 is a member of the family of Toll-like receptors (TLRs) that could stimulate mitogen-activated protein kinase and nuclear factor κB (NF-κB) to regulate inflammatory and immune responses after binding to ligands (Sun et al. 2016). Moreover, recently in a study it was demonstrated that active TLR4 can trigger the stimulation of RAS in hepatocytes and cardiac muscle cells (Sun et al. 2016). Therefore, it has been suggested that palmitic acid (PA) stimulates the TLR4 signaling pathway, resulting in RAS stimulation in adipocytes. Recently in a study, it was detected that palmitic acid (PA), one kind of free fatty acid, stimulates the actions of RAS in 3T3-L1 adipocytes (Sun et al. 2016). In the presence of fetuin A (Fet A), PA upregulated the expression of angiotensinogen (AGT) and angiotensin type 1 receptor (AT_1R) and stimulated the secretion of angiotensin II (ANG II) in 3T3-L1 adipocytes. Moreover, the activation of RAS in 3T3-L1 adipocytes was blocked when Toll-like receptor 4 (TLR4) signaling pathway using TAK242 or NF-κB signaling pathway using BAY117082 is inhibited. So it was identified that there are critical molecular mechanisms linking PA/TLR4/NF-κB signaling pathway to the activity of the local renin-angiotensin system in adipose tissue (Sun et al. 2016). In recent times it was found that Angiotensin II activates different calcium signaling pathways in adipocytes (Dolgacheva et al. 2016). It is well known that Ang II is an important mammalian neurohormone involved in RAS. Ang II is produced both constitutively and locally by RAS systems, including white fat adipocytes. The influence of Ang II on adipocytes is complex, affecting different systems of signal transduction from early Ca(2+) responses to cell proliferation and differentiation, triglyceride accumulation, expression of adipokine-encoding genes and adipokine secretion (Dolgacheva et al. 2016). It is known that white fat adipocytes

express all RAS components and Ang II receptors (AT1 and AT2). In a recent study which was carried out with the primary white adipocytes culture, and Ca(2+) signaling pathways activated by Ang II were investigated using fluorescent microscopy (Dolgacheva et al. 2016). Ca(2+)-oscillations and transient responses of differentiated adipocytes to Ang II were registered in cells with both small and multiple lipid inclusions. Using inhibitory analysis and selective antagonists, it was shown that Ang II initiates periodic Ca(2+)-oscillations and transient responses by activating AT1 and AT2 receptors and involving branched signaling cascades; in these cascades, AT1 receptors play the leading role (Dolgacheva et al. 2016). These studies open a perspective of using Ang II for correction of signal resistance of adipocytes often observed during obesity and type 2diabetes. Recently it was detected that inactivation of adipose angiotensinogen reduces adipose tissue macrophages and increases metabolic activity (LeMieux et al. 2016). The adipose RAS has been linked to obesity-induced inflammation, though mechanisms are not completely understood. In a recent study, adipose-specific angiotensinogen knockout mice (Agt-KO) were generated to determine whether Agt inactivation reduces inflammation and alters the metabolic profile of the Agt-KO mice compared to wild-type (WT) littermates (LeMieux et al. 2016). In this study adipose tissue-specific Agt-KO mice were created using the Cre-LoxP system with both Agt-KO and WT littermates fed either a low-fat or high-fat diet to assess metabolic changes. White adipose tissue was used for gene/protein expression analyses and WAT stromal vascular cells for metabolic extracellular flux assays. No significant differences were observed in body weight or fat mass between both genotypes on either diet. However, improved glucose clearance was observed in Agt-KO compared to WT littermates, consistent with higher expression of genes involved in insulin signaling, glucose transport, and fatty acid metabolism (LeMieux et al. 2016). Furthermore, Agt inactivation reduced total macrophage infiltration in Agt-KO mice fed both diets. Lastly, stroma vascular cells from Agt-KO mice revealed higher metabolic activity compared to WT mice. These findings indicate that adipose-specific Agt inactivation leads to reduced adipose inflammation and increased glucose tolerance mediated in part via increased metabolic activity of adipose cells. In a recent study it was detected that intrarenal renin-angiotensin system mediates fatty acid-induced ER stress in the kidney (Li et al. 2016). It is known that obesity-related kidney disease is related to caloric excess promoting deleterious cellular responses. Accumulation of saturated free fatty acids in tubular cells produces lipotoxicity involving significant cellular dysfunction and injury. In a recent study it was aimed to elucidate the role of RAS activation in saturated fatty acid-induced endoplasmic reticulum (ER) stress in cultured human proximal tubule epithelial cells (HK2) and in mice fed with a high-fat diet (Li et al. 2016). Treatment with saturated fatty acid palmitic acid for 24 h induced ER stress in HK2, leading to an unfolded protein response as reflected by increased expressions of the ER chaperone binding immunoglobulin protein (BiP) and proapoptotic transcription factor C/EBP homologous protein (CHOP) protein as evaluated by immunoblotting. PA treatment also induced increased protein expression of inositol requiring protein 1α (IRE1α), phosphorylated eukaryotic initiation factor-α (eIF2α), and activating transcription factor 4 (ATF4) as well as activation of caspase-3. PA treatment was associated with increased angiotensin II levels in cultured medium. The angiotensin II type 1 receptor (AT1R) blocker valsartan or renin inhibitor aliskiren dramatically suppressed PA-induced upregulation of BiP, CHOP, IRE1α, p-eIF2α, and ATF4 in HK2 cells. In contrast, valsartan or aliskiren did not prevent ER stress induced by tunicamycin. C57BL/6 mice fed with a high-fat diet for 14 weeks exhibited increased protein expressions of BiP and CHOP compared with control mice, which were significantly attenuated by the valsartan treatment. Increased angiotensin II levels in serum and urine were observed in mice fed with a high-fat diet when compared with controls. Therefore it is suggested that the intrarenal RAS activation may play an important role in diabetic kidney injury via mediating ER

stress induced by saturated fatty acid (Li et al. 2016). In a recent investigation it was shown that combined angiotensin receptor modulation is effective in the management of cardio-metabolic disorders (Paulis et al. 2016). It is well-known that cardiovascular and metabolic disorders, such as hypertension, insulin resistance, dyslipidemia or obesity are linked with chronic low-grade inflammation and dysregulation of the RAS. Consequently, RAS inhibition by ACE inhibitors or angiotensin AT1 receptor blockers is the evidence-based standard for cardiovascular risk reduction in high-risk patients, including diabetics with albuminuria. In addition, RAS inhibition reduces the new onset of diabetes mellitus. Yet, the high and increasing prevalence of metabolic disorders, and the high residual risk even in properly treated patients, calls for additional means of pharmacological intervention. In the past decade, the stimulation of the angiotensin AT2 receptor (AT2R) has been shown to reduce inflammation, improve cardiac and vascular remodeling, enhance insulin sensitivity and increase adiponectin production (Paulis et al. 2016). Therefore, a concept of dual AT1R/AT2R modulation emerges as a putative means for risk reduction in cardio-metabolic diseases. The approach employing simultaneous RAS blockade (AT1R) and RAS stimulation (AT2R) is distinct from previous attempts of double intervention in the RAS by dual blockade. Dual blockade abolishes the AT1R-linked RAS almost completely with subsequent risk of hypotension and hypotension-related events, i.e. syncope or renal dysfunction. Such complications might be especially prominent in patients with renal impairment or patients with isolated systolic hypertension and normal-to-low diastolic blood pressure values. In contrast to dual RAS blockade, the add-on of AT2R stimulation does not exert significant blood pressure effects, but it may complement and enhance the anti-inflammatory and antifibrotic/destiffening effects of the AT1R blockade and improve the metabolic profile. Further studies will have to investigate these putative effects in particular for settings in which blood pressure reduction is not primarily desired (Paulis et al. 2016). In a recent study it was proposed that ACE2 deficiency worsens epicardial adipose tissue inflammation and cardiac dysfunction in response to diet-induced obesity (Patel et al. 2016). It is well known that obesity is increasing in prevalence and is strongly associated with metabolic and cardiovascular disorders. RAS has emerged as a key pathogenic mechanism for these disorders; ACE2 negatively regulates RAS by metabolizing Ang II into Ang 1-7. In a recent study the role of ACE2 in obesity-mediated cardiac dysfunction was investigated (Patel et al. 2016). ACE2 null (ACE2KO) and wild-type (WT) mice were fed a high-fat diet (HFD) or a control diet and studied at 6 months of age. Loss of ACE2 resulted in decreased weight gain but increased glucose intolerance, epicardial adipose tissue (EAT) inflammation, and polarization of macrophages into a proinflammatory phenotype in response to HFD. Similarly, human EAT in patients with obesity and heart failure displayed a proinflammatory macrophage phenotype. Exacerbated EAT inflammation in ACE2KO-HFD mice was associated with decreased myocardial adiponectin, decreased phosphorylation of AMPK, increased cardiac steatosis and lipotoxicity, and myocardial insulin resistance, which worsened heart function. Ang 1-7 (24 μg/kg/h) administered to ACE2KO-HFD mice resulted in ameliorated EAT inflammation and reduced cardiac steatosis and lipotoxicity, resulting in normalization of heart failure. Therefore these data make us to think that ACE2 plays a novel role in heart disease associated with obesity wherein ACE2 negatively regulates obesity-induced EAT inflammation and cardiac insulin resistance (Patel et al. 2016). In another study it was shown that oxidative stress causes imbalance of renal renin angiotensin system components and hypertension in obese zucker rats (Luo et al. 2015). It is well-known that oxidative stress plays an important role in the pathogenesis of hypertension, especially in obesity-related hypertension. The natriuretic and antinatriuretic components of the renal renin angiotensin system maintain sodium homeostasis and blood pressure. In a recent study it was aimed to test the hypothesis that increased oxidative stress leads to the imbalance of RAS components and hypertension in

obese zucker rats (Luo et al. 2015). Lean and obese rats received vehicle or tempol, a superoxide dismutase mimetic in the drinking water for 4 weeks. Compared with vehicle-treated lean rats, vehicle-treated obese rats exhibited higher blood pressure and increased renal oxidative stress, accompanied by increased diuretic and natriuretic responses to AT1R antagonist (Candesartan) and AT2R agonist (CGP-42112A) and reduced diuretic and natriuretic response to MasR agonist (Ang-[1-7]). Moreover, obese rats had higher ACE, AT1R and AT2R, lower ACE2 and MasR expressions in the kidney. All of the above-mentioned abnormalities were reversed to some degree by tempol treatment. In primary cultures of renal proximal tubular (RPT) cells from lean and obese rats, tempol treatment also increased AT2R, ACE2, and MasR expressions but decreased AT1R and ACE expressions in obese rats. Taken together, in this recent study it was shown that the imbalance of renal RAS components was associated with increased oxidative stress in obese rats (Luo et al. 2015). Moreover, antioxidant treatment with tempol reversed the imbalance of renal RAS components and led to diuresis and natriuresis, which, at least in part, explains the blood pressure-lowering effect of antioxidant supplementation in obesity-related hypertension. In another study, the inhibitory effect of angiotensin II on BKCa channels in podocytes via oxidative stress had been shown (Gao et al. 2015). It has been well-known that Ang II is an important active substance of the RAS. In a recent study it has confirmed that abnormalities of Ang II may be related with cerebrovascular diseases, endocrine diseases, cardiovascular diseases, liver diseases, such as: cerebral hypoxia, diabetes, obesity, atrial fibrillation, and liver cirrhosis (Gao et al. 2015). However, understanding effects of Ang II on podocytes is not enough. In the study it has been aimed to investigate the effects of oxidative stress on the large conductance, Ca(2+)-activated K(+) channels (BKCa). Results from the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay showed that Ang II induced podocyte death in a concentration-dependent manner (Gao et al. 2015). The measurement of superoxide dismutase (SOD) generation demonstrated that Ang II decreased the total SOD of cellular levels. Meaningfully, pretreatment of a type of ROS scavenger formulations named N-(mercaptopropionyl)-glycine (N-MPG) could inhibit podocyte apoptosis induced by Ang II. Meanwhile, patch-clamp technique was used in this study to detect the effects of Ang II on currents of BKCa channel in podocytes. The results indicated that Ang II inhibited the current amplitude of BKCa channel and decreased the slope of I-V curve. Ang II also made the activation curves of BKCa channel shift to the left. These results may provide a theoretical basis for potential treatment of chronic glomerular disease in the future.

9 Conclusion

Metabolic syndrome constitutes a constellation of findings including central obesity, insulin resistance/type 2 diabetes mellitus (DM), dyslipidemia and hypertension (Karmali et al. 2015). Metabolic syndrome affects 1 in 4 adults in the United States and is rapidly rising in prevalence, largely driven by the dramatic rise in obesity and insulin resistance/DM. Being central to the development of metabolic syndrome and its other related diseases, much focus has been placed on identifying the mitogenic effects of obesity and insulin resistance/DM as mechanistic clues of the link between metabolic syndrome and cancer. Pertinent mechanisms identified include altered lipid signaling, adipokine and inflammatory cytokine effects, and activation of PI3K/Akt/mTOR and RAS/RAF/MAPK/ERK pathways via dysregulated insulin/insulin-like growth factor-1 (IGF-1) signaling (Karmali et al. 2015). Through variable activation of these multiple pathways, obesity and insulin resistance/DM predispose to hematologic malignancies, imposing the aggressive and chemo-resistant phenotypes typically seen in cancer patients with underlying metabolic syndrome. Growing understanding of these pathways has identified druggable cancer targets, rationalizing the development and testing of agents like PI3K inhibitor idelalisib, mTOR

inhibitors everolimus and temsirolimus, and IGF-1 receptor inhibitor linsitinib. It has also led to exploration of obesity and diabetes-directed therapies including statins and oral hypoglycemic for the management of metabolic syndrome-related hematologic neoplasms (Karmali et al. 2015).

The positive regulatory role of plasma LDL on AT1 receptor-mediated hematopoietic stem cell (HSC) differentiation and the production of pro-atherogenic monocytes had been described (Strawn and Ferrario 2008). LDL-regulated HSC function may explain in part hypercholesterolemia-induced inflammation as well as the anti-inflammatory and anti-atherosclerotic effects of AT1 receptor blockers (Ferrario et al. 2004; Ferrario and Strawn 2006). The role of local adipose tissue RAS is directly related to the pathogenesis of metabolic derangements in obesity (Kalupahana and Moustaid-Moussa 2012a). There may be a crosstalk between local BM RAS (Ozturk et al. 2004) and local adipose tissue RAS (Kalupahana and Moustaid-Moussa 2012b) at the genomics and transcriptomics levels (Nehme et al. 2015). In the future regenerative medicine may be used for tissue-specific progenitor cells for vascular repair process. Mononuclear blood cell populations are capable of transforming into endothelial cells and incorporate into ischemic tissue. Most of the clinical investigations on human cell treatment for ischemic vascular disease are constructed on the presence of cell surface antigen like CD34 or VEGFR2 to detect endothelial progenitor cells (Becher et al. 2010). Further studies may clarify functional relations of local BM RAS driving CD34+ stem cells, and distinct local RAS in the vasculature in health and in disease (Zucker and Zimmerman 2011). Studies focusing on genomics and transcriptomics will help us to improve our knowledge regarding these issues.

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MicroRNA and Adipogenesis

21

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Abstract

In obesity, the process of adipogenesis largely determines the number of adipocytes in body fat depots. Adipogenesis is regulated by several adipocyte-selective microRNAs (miRNAs) and transcription factors that modulate adipocyte proliferation and differentiation. However, some miRNAs block expression of master regulators of adipogenesis. Additionally, specific miRNAs have been implicated in adipocyte differentiation and mature adipocyte functions. While, each miRNA targets multiple mRNAs, which may coordinate or antagonize each other's functions, several miRNAs are dysregulated in other tissues during obesity-related comorbidities. In this respect, development of lipid droplets, macrophage accumulation, macrophage polarization, tumor necrosis factor receptor-associated factor 6 activity, lipolysis, lipotoxicity and insulin resistance are effectively controlled by miRNAs.

Keywords

Obesity • microRNAs (miRNAs) • Peroxisome proliferator-activated receptor-gamma (PPAR-gamma) • Fatty acid binding protein 4 (FABP4) • miR-103 • miR-143 • Forkhead box protein O1 (FoxO1) • Triglyceride • Sterol regulatory element-binding protein (SREBP) • miR-34 • miR-33 • Silent information regulator 1 (SIRT1) • miR-155 • Tumor necrosis factor-alpha (TNF-alpha) • miR-223 • Oxidized low density lipoprotein (OxLDL) • Toll-like receptor 4 (TLR4) • Tumor necrosis factor receptor (TNFR)-associated factor 6 (TRAF6) • miR-221 • IL-1 receptor-associated kinase-1 (IRAK-1) • Leptin • CCAAT/enhancer-binding protein (C/EBP) • miR-148a

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1 Introduction

Chronic imbalance of calories consumed versus expended results in the increased storage of the excess energy in the form of triglyceride stores in adipocytes. Eventually, increased intracellular lipids and greater adipocyte size and adipogenesis may cause the increase in fat mass. Adipose hypertrophy and hyperplasia are associated with intracellular abnormalities of adipocyte function, particularly endoplasmic reticulum (ER) and mitochondrial stress. Both of these are associated with changes in circulating adipokines, free fatty acids, and inflammatory mediators (de Ferranti and Mozaffarian 2008). In adipose tissue increased fat mass associated with obesity is characterized by both increased adipocyte size and numbers (Rosen and MacDougald 2006). Nevertheless, limited storage capacity of hypertrophied subcutaneous adipose tissue, coupled with the overstimulation of hormone-sensitive lipase, leads to massive increase in free fatty acid release and subsequent accumulation ectopically (Ge et al. 2014). Further dysfunctional adipose tissue may happen in severe obese subjects with a large and long-lasting fat excess, when fat depots reach the point in which excessive fat storage, cell density, and diminished oxygen availability. Consequently, dysfunctional adipocytes promote decreased lipo/adipogenesis and increased lipolysis and fatty acid release (Ortega and Fernández-Real 2013). Initially, adipogenesis occurs in two stages; firstly, commitment of mesenchymal stem cells to a preadipocyte. At second step, adipogenic stimuli induce terminal differentiation in committed preadipocytes through the epigenomic activation of peroxisome proliferator-activated receptor-gamma (PPAR-gamma) (Cristancho and Lazar 2011). In this respect, multipotent mesenchymal stem cells can develop into lineage committed pre-adipocytes. PPAR and cytidine-cytidine-adenosine-adenosine-thymidine (CCAAT)/enhancer-binding protein (C/EBP) transcription factors coordinate adipogenic gene expression during terminal differentiation into lipid storing mature adipocytes (McGregor and Choi 2011). In obesity, PPAR-gamma and C/EBPs have emerged as master regulators of adipogenesis. Eventually, the process of adipogenesis largely determines the number of adipocytes in body fat depots. Preadipocytes proliferate and can develop into mature adipocytes upon growth arrest, clonal expansion and terminal differentiation (Lefterova and Lazar 2009). On the other hand, white adipose tissue is the primary site for the initiation and exacerbation of obesity-associated inflammation. Hypoxia, endoplasmic reticulum stress, lipotoxicity, and metabolic endotoxemia mediate inflammation in white adipose tissue (Ge et al. 2014). In this context, microRNAs (miRNAs) also play regulatory roles in many biological processes associated with obesity, including adipocyte differentiation and lipid metabolism (Peng et al. 2014). A global survey of the miRNA distribution in human body fluids indicates that the miRNA spectrum in plasma is different from that of most of the other body fluids. This suggests that some miRNAs are selectively exported or retained within the cell (Guduric-Fuchs et al. 2012). Considering the central role of miRNAs in different pathophysiological conditions, circulatory miRNAs may serve as potential biomarkers in obesity and obesity-related comorbidities (Gilad et al. 2008).

2 miRNAs in Adipocyte Proliferation and Differentiation

Adipogenesis is partially regulated by several adipocyte-selective miRNAs and transcription factors that regulate proliferation and differentiation of human adipose-derived mesenchymal stem cells (hMSCs-Ad) (Shi et al. 2015). Actually, miRNAs are class of short non-coding RNAs with 19–22 nucleotides involved in the post-transcriptional regulation of genes (Bartel 2009). Specific miRNAs have been implicated in adipocyte differentiation and mature adipocyte function, including lipolysis, glucose-uptake, and insulin sensitivity (Alexander et al. 2011). miR-422b, -148a, -107, -103, -30c, -30a-5p, and -143 are induced during adipogenesis but are down-regulated in obese adipocytes. Conversely, miR-222 and -221 are decreased during adipogenesis

but are upregulated in obese adipocytes (Xie et al. 2009a). Although ectopic expression of miR-103 or miR-143 has little effect on adipocyte growth, they accelerate the rate of adipocyte differentiation, as measured by triglyceride accumulation. Expression of PPAR-gamma2 is doubled by ectopic expression of either miR-103 or miR-143. However, miR-103 increases the expression level of fatty acid binding protein 4 (FABP4) and adiponectin approximately ninefold and fourfold, respectively (Xie et al. 2009a). Each miRNA targets multiple mRNAs, which may coordinate or antagonize each other's functions. Several miRNAs are also dysregulated in other metabolic tissues during obesity-related diseases (Alexander et al. 2011). In this context, miRNAs target more than 5300 human genes, which represent 30% of human's gene set (Lewis et al. 2005). Indeed, miRNA expression in preadipocytes is altered during adipocyte development and in obesity (McGregor and Choi 2011). Thus, miRNAs can bind to complementary target sites in mRNA genes which can cause translation repression or cause cleavage, deadenylation and degradation of target mRNA genes (Yekta et al. 2004) (Fig. 21.1).

A number of miRNAs have been found dysregulated in white adipose tissue during obesity, and closely associated with obesity-related metabolic disorders (Ge et al. 2014). Fifteen specific circulating miRNAs are significantly deregulated in prepubertal obesity, including the decreased miR-221 and miR-28-3p and increased concentrations of miR-486-5p, miR-486-3p, miR-142-3p, miR-130b, and miR-423-5p. The circulating concentrations of these miRNAs are significantly associated with body mass index and other measures of obesity (Prats-Puig et al. 2013). While morbidly obese patients show a marked increase in miR-140-5p, miR-142-3p and miR-222, the levels of miR-532-5p, miR-125b, miR-130b, miR-221, miR-15a, miR-423-5p, and miR-520c-3p decrease. Surgery-induced weight loss leads to a marked decrease of miR-140-5p, miR-122, miR-193a-5p, and miR-16-1, and upregulation of miR-221 and miR-199a-3p (Ortega et al. 2013). Fifteen overweight individuals who underwent Roux-en-Y gastric bypass surgery are examined for whether gene expression in perioperative omental adipose is associated with gastric bypass-induced weight loss. In these patients, RNA expression profiles from perioperative adipose tissue are correlated with weight loss outcome following bariatric surgery (Kim et al. 2008a). Ortega et al. showed that miR-10a, miR-34a, miR-100, miR-30a, miR-99a and miR-210 are up-regulated in inflamed adipocytes and subcutaneous fat depots from obese when compared to those obtained from lean individuals. Furthermore, mRNA and miRNA expressions in abdominal subcutaneous adipose tissue of 16 morbidly obese women have been assessed before and 2 years after laparoscopic Roux-en-Y gastric bypass. miRNA targeting mRNAs are upregulated in obese adipose tissue and inflamed adipocytes. A total of 5018 different mRNA probe sets and 15 miRNAs are differentially expressed after surgery-induced weight loss when compared with pre-operative samples (Ortega et al. 2010, 2015). Actually, 23 circulating miRNAs; Mus musculus (mmu)-miR-16, mmu-let-7i, mmu-miR-26a, mmu-miR-17, mmu-miR-107, mmu-miR-195, mmu-miR-20a, mmu-miR-25, mmu-miR-15b, mmu-miR-15a, mmu-let-7b, mmu-let-7a, mmu-let-7c, mmu-miR-103, mmu-let-7f, mmu-miR-106a, mmu-miR-106b, mmu-miR-93, mmu-miR-23b, mmu-miR-21, mmu-miR-30b, mmu-miR-221, and mmu-miR-19b are significantly downregulated in diet induced obesity mice. Interestingly, all miRNAs are upregulated in diet-induced obesity with subsequent weight reduction achieved via low-fat diet feeding (Hsieh et al. 2015). In any way whatsoever, changes in metabolism associated with weight reduction achieved either through low-fat diet feeding or surgery-induced weight loss could reverse the obesity-related reductions in the expression of circulating miRNAs.

In adipose tissue, Forkhead box protein O1 (FOXO1) activation suppresses adipocyte differentiation through direct repression of PPAR. Anti-adipogenic effects of FOXO1 result from antagonistic effects of FoxO1 and PPAR signaling as well as insulin signaling (Dowell et al. 2003). Compared with lean mice, FOXO1 accumulates in nuclei of obese adipocytes due to

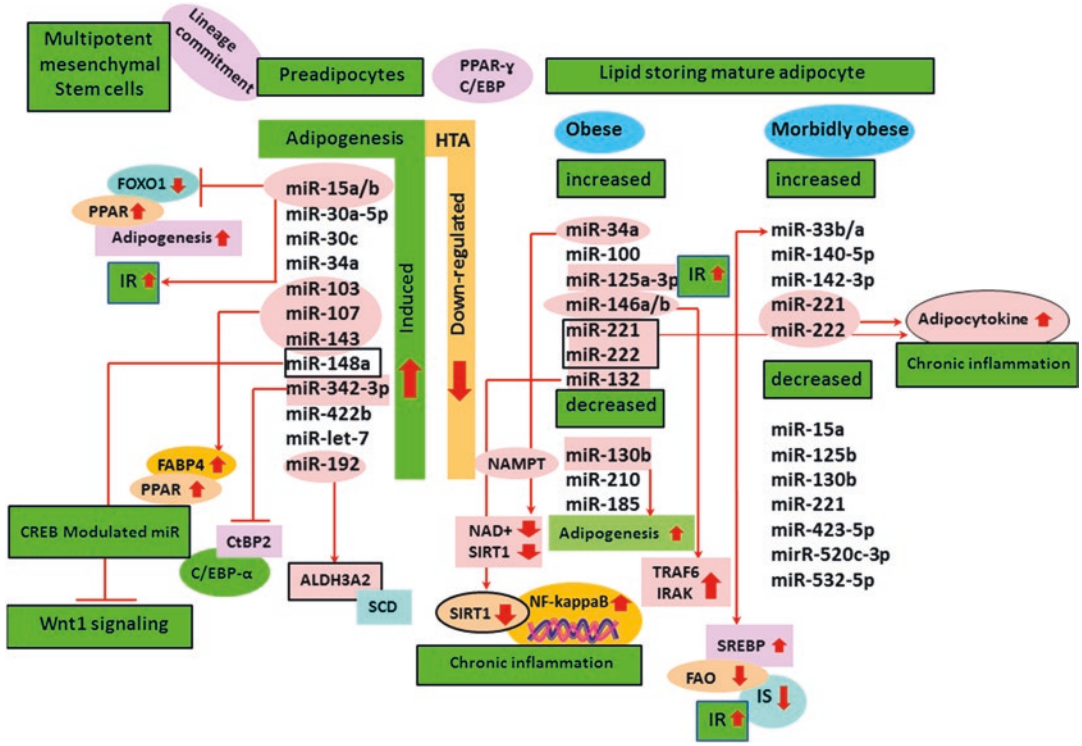

Fig. 21.1 miRNAs play regulatory roles in many biological processes associated with obesity. Adipocyte-selective miRNAs with the master regulators of adipogenesis, PPAR-gamma and CCAAT/enhancer-binding protein (C/EBP) transcription factors, coordinate adipogenic gene expression and determine the number of adipocytes in body fat depots (*HTA* Hypertrophic adipocyte; *C/EBP* CCAAT/enhancer-binding protein; *miR* MicroRNA; *FOXO1* Forkhead box transcription factor 1; *PPAR* Peroxisome proliferator-activated receptor; *FABP4* Fatty acid binding protein 4; *NAMPT* Nicotinamide phosphoribosyltransferase; *NAD+* Nicotinamide adenine dinucleotide; *SIRT1* Silent information regulator 1; *SREBP* Sterol regulatory element-binding protein; *FAO* Fatty acid oxidation; *IS* Insulin signaling; *IR* Insulin resistance; *TRAF* TNF receptor associated factor; *IRAK* Interleukin-1 receptor-associated kinase; *SCD* Stearoyl coenzyme A desaturase-1; *ALDH3A2* Aldehyde dehydrogenase 3 family member A2; *CtBP2* Carboxy-terminal binding protein 2; *NF-kappaB* Nuclear factor-kappa B; *CREB* cyclic adenosine 3′5′ monophosphate (*cAMP*) response element binding protein; *Wnt1* Wingless-type MMTV integration site family member 1)

impaired insulin action in these cells. Nuclear FOXO1 also up-regulates toll-like receptor 4 (TLR4) (Fan et al. 2010). Actually, FOXO1 proteins are direct substrates of protein kinase B (Akt), which controls FOXO1 transcriptional activity by regulating its cytoplasmic/nuclear translocation (Brunet et al. 1999). Over-expression of miR-15a/b in pre-adipocytes is promoted in the early stage of adipocyte differentiation. FoxO1 is the target gene of miR-15a/b. The inhibition of FOXO1 expression caused by miR-15a/b over-expression has a positive effect on adipogenesis (Dong et al. 2014) (Fig. 21.1).

The expression of 40 miRNAs in pre-adipocytes and 31 miRNAs in adipocytes are significantly changed in obesity. While miR-221, miR-125b, miR-34a and miR-100 are up-regulated and miR-130b, miR-210 and miR-185 are down-regulated in obese subjects; miR-130b and miR-210 are both down-regulated during differentiation of adipocytes in subcutaneous fat depots from obese subjects. Contrarily, miR-221, miR-222, miR-100 and miR-125b are down-regulated during adipogenesis and associated with body mass index in human adipose tissue. Only miR-34a is found to be positively

up-regulated during adipogenesis and associated positively with body mass index (Ortega et al. 2010). The miRNA expressions in omental fat and blood from a total of 50 obese patients significantly correlate with body mass index, fasting blood glucose, and glycosylated hemoglobin. Furthermore, miR-17-5p and miR-132 differs significantly between obese and nonobese omental fat (Heneghan et al. 2011). In particular, miR-132 is shown to inhibit silent information regulator 1 (SIRT1) expression through a miR-132 binding site in the 3′-untranslated region of SIRT1. A decrease in SIRT1 activity increases activation of nuclear factor-kappaB (NF-kappaB) and transcription of proinflammatory mediators. Thus, in response to nutritional availability, induction of miR-132 decreases SIRT1-mediated deacetylation of p65 leading to NFkappaB dysregulation contributing to the chronic inflammatory state in adipose tissue. Indeed, circulating levels of interleukin-8 (IL-8) and monocyte chemoattractant protein-1 (MCP-1) are higher in primary human preadipocytes of obese individuals due to miR-132 overexpression (Strum et al. 2009). In fact, SIRT1 deacetylase activity is dependent on nicotinamide adenine dinucleotide (NAD^+) levels. NAD^+ levels are reduced in obesity. Hepatic miR-34a, is elevated in obesity and it decreases SIRT1 expression. At the same time, miR-34a reduces NAD^+ levels and SIRT1 activity by targeting nicotinamide phosphoribosyltransferase (NAMPT), the rate-limiting enzyme for NAD^+ biosynthesis. Eventually, miR-34a reduces both SIRT1 expression and activity in many ways during the obesity. Antagonism of miR-34a in diet-induced obese mice restores NAMPT/NAD^+ levels and alleviates steatosis, inflammation, and glucose intolerance (Choi et al. 2013). On the other hand, miR-33, as an intronic miRNA located within the sterol regulatory element-binding protein (SREBP) genes, is one of the master regulators of cholesterol and fatty acid metabolism. SREBP-1 induction by insulin resistance leads to increased fatty acid and triglycerides synthesis. In humans two miR-33 genes are present; miR-33b, which is located in intron 17 of the SREBP-1 gene on chromosome 17, and miR-33a, which is presented in intron 16 of the SREBP-2 gene on chromosome 22. The activation of SREBPs induces miR-33a and -b expression. Fatty acid oxidation and insulin signaling pathways are inhibited by over expression of miR-33a and -b. Inhibition of endogenous miR-33a and -b increases the activity of these two metabolic pathways (Gharipour and Sadeghi 2013). In fact, miRNAs have important roles on adipocyte differentiation and adipogenesis. miR-33b is induced upon differentiation of human preadipocytes, along with SREBP-1. miR-33b is an important regulator of adipogenesis. Furthermore, inhibition of miR-33b enhances lipid droplet accumulation. Conversely, overexpression of miR-33b impairs preadipocyte proliferation and reduces lipid droplet formation and the induction of PPAR-gamma target genes during differentiation (Price et al. 2016). Eleven miRNAs are identified that alter lipid droplets. In this respect, miR-181d is the most efficacious inhibitor, decreasing lipid droplets by about 60%. Additionally, miR-181d is also confirmed to reduce cellular triglycerides and cholesterol esters (Whittaker et al. 2010) (Fig. 21.1).

Macrophage infiltration, in particular, the number of M1 or classically activated macrophages increase in adipose tissue of obese individuals. Furthermore, macrophages inhibit adipocyte differentiation, potentially leading to adipocyte hypertrophy, altered secretion of adipokines and ectopic storage of lipid within non-adipose tissues (Heilbronn and Campbell 2008). Diet-induced obesity decreases expression of genes that are characteristic of M2 or alternatively activated macrophages while increasing expression of tumor necrosis factor-alpha (TNF-alpha) and inducible nitric oxide synthase (iNOS) that are characteristic of M1 or classically activated macrophages (Lumeng et al. 2007). Collectively, macrophage polarization is a critical component of the inflammatory response in obese adipose tissue. Thereby, miR-223–regulated macrophage polarization is important for adipose tissue function. Macrophage accumulation is significantly higher in adipose tissue of

miR-223 deficient and high fat diet-fed mice. Furthermore, miR-223–deficient macrophages exhibit an increased ability for infiltration. Macrophage polarization directly contributes to the protective effect of miR-223 against diet-induced obesity-associated insulin resistance (Zhuang et al. 2012). Macrophage activation states could potentially alter the balance between adipocyte hypertrophy and hyperplasia through the control of preadipocyte survival and cell number. Thus, human primary monocyte-derived-macrophages exert an anti-apoptotic effect on human primary preadipocytes that is also dependent on the state of macrophage activation (Molgat et al. 2012). Infiltrated and activated macrophages produce extracellular reactive oxygen species (ROS) at high levels in adipose tissue. Extracellular superoxide dismutase (EC-SOD) is an anti-inflammatory enzyme that protects cells from the damaging effects of ROS. EC-SOD expression levels increase after the induction of adipocyte differentiation and then they decline (Adachi et al. 2009). According to another perspective, a higher degree of oxidized low density lipoprotein (oxLDL)-induced lipotoxic cell death within M2 macrophage population could contribute to a decrease in numbers of M2 cells, and thus, a relative increase in proportion of non-M2 cells, within macrophage populations can be seen. This macrophage accumulation is the pro-inflammatory characteristics of a predominantly M1-polarized state in the pathogenesis of obesity-associated inflammatory disorders (Isa et al. 2011). The amount of lipid retained in macrophages also depends on the regulated uptake of oxidized lipoproteins by scavenger receptors. miR-125a-5p can partly regulate oxLDL uptake of macrophages through reducing Lectin-like oxLDL receptor-1 (LOX-1) and CD68 expressions. miR-125a-5p and miR-9 were also significantly up-regulated in monocytes after exposure to ox-LDL. Five microRNAs, microRNA-125a-5p, microRNA-9, microRNA-146a, microRNA-146b-5p, and microRNA-155 are aberrantly expressed after oxLDL treatment of human primary monocytes. miR-125a-5p may be an important regulator of the inflammatory response, lipid uptake in oxLDL-stimulated monocyte/macrophages (Chen et al. 2009). The exposure of human THP-1 macrophages to oxLDL leads to up-regulation of miR-155. Silencing of endogenous miR-155 in THP-1 cells significantly enhances oxLDL-induced lipid uptake, up-regulated the expression of scavenger receptors; LOX-1, CD36 and CD68. Consequently, the release of several cytokines including IL-6, IL-8, and TNF-alpha are promoted. Actually, miR-155 is a negative feedback regulator of oxLDL-stimulated THP-1 inflammatory responses and lipid uptake (Huang et al. 2010). In contrast, expression of miRNA-125a-5p mediates lipid uptake and decreases the secretion of IL-2, IL-6, TNF-alpha and transforming growth factor-beta (TGF-beta) in oxLDL-stimulated monocyte-derived macrophages (Chen et al. 2009). There is a link between fatty acids, TLR4-ER stress pathway and PPAR-gamma, ultimately adipocyte-driven recruitment of macrophages (Nguyen et al. 2012). TLRs and adapter proteins are overexpressed in peripheral blood mononuclear cells from obese subjects, which correlates with increased expression of TNF-alpha and IL-6. This correlation exhibits a potential pathophysiological link between obesity and inflammation leading to insulin resistance (Ahmad et al. 2012). A critical role of TLR and its downstream molecule, TNF receptor (TNFR)-associated factor 6 (TRAF6) has been documented in inflammatory response induced by fatty acid. Saturated fatty acids activate TLR4 signal pathway in human monocyte cells, THP-1. Subsequent to the activation of TLR4, its downstream molecule, TRAF6 is upregulated and eventually induces proinflammatory cytokine production. Actually TRAF6 is a target of miR-194. Overexpression of miR-194 directly decreases TRAF6 expression and attenuates the release of proinflammatory cytokine, TNF-alpha in palmitic acid-activated monocyte during the obesity-induced inflammation (Tian et al. 2015). In contrast, levels of miR-146a/b are positively correlated with TRAF6 mRNA and IL-1 receptor-associated kinase-1 (IRAK-1) mRNA levels (Takahashi et al. 2010). The key inflammatory signaling molecule IRAK-1 is also partially involved in mediating the effects of leptin. Leptin

increases the expression of IRAK-1 of human monocytes in obesity-related inflammation (Vaughan and Li 2010).

During adipogenesis, miRNAs can accelerate or inhibit adipocyte differentiation by acting on transcription factors and regulate signalling pathways related to adipogenesis, or blocking the mitotic clonal expansion stage, thus regulating adipocyte development (Chen et al. 2013). Retroperitoneal adipose tissue expresses five miRNAs that are related to adipocyte differentiation; miR-143, lipid metabolism; miR-103 and -107 and obesity; miR-221 and -222. Moreover, changes in miRNAs expression correlate with several adipocyte gene expressions. In this respect, miR-103 and -107 is correlated with genes involved in fatty acid metabolism, whereas miR-221 and miR-222 is correlated with the expression of adipocytokines (Parra et al. 2010). Moreover, miR-103 and miR-143 are upregulated during adipogenesis and downregulated during obesity. In fact, both miRNAs are highly conserved and are abundant in adipocytes. The level of miR-143 increases more dramatically during adipogenesis, in vivo. However, expression of miR-103 is induced approximately ninefold during adipogenesis (Xie et al. 2009a). Ectopic expression of miR-103 or miR-143 in preadipocytes accelerates the rate of fat cell formation, as measured both by the upregulation of many adipogenesis markers and by an increase in triglyceride accumulation at an early stage of adipogenesis. These changes are linked to the chronic local inflammation environment and enhances TNF-alpha levels in obese adipose tissue, since similar changes in the pattern of miRNA expression occurs after TNF-alpha treatment of differentiated adipocytes (Xie et al. 2009a). In contrast, inhibition of miR-143 prevents adipocyte-specific glucose transporter type 4 (GLUT4), hormone-sensitive lipase (HSL), FABP activating protein 2 (aP2) and PPAR-gamma2 gene expressions in human adipose tissue. Subsequent accumulation of triglycerides in primary sub-cutaneous pre-adipocytes suggests that miR-143 is normally involved in promoting adipocyte differentiation or function (Esau et al. 2004). miR-143 is involved in adipocyte differentiation and may act through target gene extracellular signal regulated kinase5 (ERK5) (Esau et al. 2004) (Fig. 21.1).

miR-192 has an impact on key lipogenic genes. The lipogenic enzymes, stearoyl coenzyme A desaturase (SCD)-1 and the fatty aldehyde dehydrogenase, aldehyde dehydrogenase 3 family member A2 (ALDH3A2) are demonstrated to be direct targets of miR-192. Thus, miR-192 may control adipocyte differentiation and lipid homeostasis (Mysore et al. 2016). Additionally, miR-342-3p is a powerful enhancer of the adipogenesis of human adipose-derived mesenchymal stem cells that acts by inhibiting carboxy-terminal binding protein 2 (CtBP2) and by releasing the key adipogenic regulator C/EBPalpha from CtBP2. Subsequently miR-342-3p activates the expression of adipogenic transcription factors and markers (Wang et al. 2015).

Although a small fraction of known miRNAs is significantly regulated during adipogenesis, miRNAs can adjust protein synthesis from thousands of genes by direct or indirect effects (Selbach et al. 2008). To compare miRNA expression levels in normal and obese states at a genome-wide scale, the expression of more than 370 miRNAs in enriched epididymal adipocytes from leptin deficient ob/ob and diet-induced obese mice have been profiled. A total of 71 miRNAs are expressed at significantly different levels in adipocytes from wild type and leptin deficient mice of the same gender and age, whereas 35 miRNAs are differentially expressed between control and diet-induced obese mice (Xie et al. 2009a). However, comparison of miRNA expressions between subcutaneous and omental fat from human individuals revealed little differences in these two fat depots, overall (Klöting et al. 2009). These changes of miRNA expression profile in adipocyte of ob/ob mice cannot result from leptin deficiency alone, but also is dependent on diet induced obesity (Xie et al. 2009a). Actually, miRNAs play a role in adipocyte differentiation, additionally miRNAs play also a critical regulatory role in adipocyte metabolism. Overexpression of miRNA378/378 during adipogenesis can increase triacylglycerol accumula-

tion due to increased de novo lipogenesis (Gerin et al. 2010). Furthermore, increased miR-335 expressions are associated with an elevated body, liver and white adipose tissue weight, and hepatic triglyceride and cholesterol levels in genetically obese mice. miR-335 levels also closely correlate with the expression levels of adipocyte differentiation markers, PPAR-gamma, aP2, and fatty acid synthase (FAS) (Nakanishi et al. 2009). However, in white adipose tissue, miR-27a is more abundantly expressed in stromal vascular cell fraction than in mature adipocyte fraction. Thus, ectopic expression of miR-27a in preadipocytes represses adipocyte differentiation by reducing PPAR-gamma expression. Interestingly, the level of miR-27a in mature adipocyte fraction of obese mice is more down-regulated than that of lean mice (Kim et al. 2010). The overexpression of miR-27 results in blockade of expression of PPAR-gamma and C/EBPalpha, the two master regulators of adipogenesis. Nevertheless, expression of miR-27 is increased in fat tissue of obese mice and regulated by hypoxia which is an important extracellular stress associated with obesity (Lin et al. 2009). Although PPAR-gamma contains a putative binding site for miR-27, miR-27 does not repress the level of PPARgamma protein in differentiating 3T3-L1 cells (Xie et al. 2009b).

While the levels of miR-143 increase in differentiating adipocytes, inhibition of miR-143 effectively inhibits adipocyte differentiation. Thereby, miR-143 is involved in adipocyte differentiation and may act through target gene Erk5 (Esau et al. 2004). Big Mitogen-activated protein kinase (MAPK) (Bmk1), also known as Erk5, is a member of the MAPK signaling pathway that is required for epidermal growth factor (EGF)-induced cell proliferation. Bmk1 activation occurs through the cell cycle in response to tyrosine kinase signaling (Kato et al. 1998). On the other hand, the miR-27 gene family is downregulated during adipogenic differentiation. Overexpression of miR-27 specifically inhibits adipocyte formation by preventing the expression of PPAR-gamma and C/EBPalpha (Lin et al. 2009). miR-27 is also upregulated by hypoxia, which is characterized in adipose tissue of obese individuals. Interestingly, this miRNA is found to be also upregulated in adipose tissue of genetically obese ob/ob mice, suggesting that the miR-27 gene family is a class of negative regulators involved in the obesity condition (Lin et al. 2009).

Excess lipids can also accumulate ectopically in the kidney, contributing the damage through toxic processes. The kidney is negatively affected by dyslipidemia, lipid accumulation and changes in circulating adipokines that bring about alterations in renal lipid metabolism and promote insulin resistance, generation of ROS and ER stress, ultimately leading to alterations in the glomerular filtration barrier and renal failure. miRNAs and long non-coding RNAs are of relevance for the early detection of lipid-associated kidney disease (Izquierdo-Lahuerta et al. 2016). Moreover, the overexpression of miR-15b suppresses the protein expression of insulin receptor through targeting insulin receptor 3′-untranslated region directly, resulting in an impairment of the insulin signaling in hepatocytes. Finally, the overexpression of miR-15b is related to the pathogenesis of hepatic insulin resistance in saturated fatty acid-induced obesity (Yang et al. 2015). Liver-specific deletion of insulin-induced gene 1 (Insig1) leads to higher hepatic and plasma triglyceride levels by inhibiting the processing of SREBPs. miR-24 deficiency prevents SREBP inhibition, and subsequent expression of lipogenic genes. miR-24 promotes hepatic lipid accumulation and hyperlipidemia by repressing Insig1 (Ng et al. 2014). While both non-alcoholic steatohepatitis and fatty degeneration of hepatocytes correlate negatively with the expression levels of Homo sapiens (hsa)-miR-125b, they are positively associated with the expression levels of primary (pri)-miR-16-2 and pri-miR-7-1 (Sharma et al. 2013).

Lipolysis is the biochemical pathway responsible for the catabolism of cellular triacylglycerol (TG). Lipolytic TG breakdown is a central metabolic process leading to the generation of free fatty acids and glycerol. Adipose triglyceride lipase converts TG to diacylglycerol (DAG). HSL is responsible for the hydrolysis of DAG, and monoglyceride lipase eventually hydrolyzes monoacylglyceride, yielding glycerol and free

fatty acid (Lass et al. 2011). Control of lipolysis is imperative to prevent lipotoxicity in obesity. miR-124a attenuates RNA and protein expression of the major TG hydrolase, adipose triglyceride lipase. Thereby, ectopic expression of miR-124a in adipocytes leads to reduced lipolysis and increases cellular TAG accumulation, lipid storage and plasma fatty acid concentration (Das et al. 2015). miR-145 represses lipolysis by targeting FOXO1 and alpha/beta hydrolase domain 5 (ABHD5) and downregulation of FOXO1 and ABHD5 attenuates lipolysis. Thus, these findings establish KH-type splicing regulatory protein (KSRP) and miR-145 as important negative regulators of lipolysis in lipid droplets of white adipose tissue. Overexpression of KSRP upregulates miR-145 and attenuates lipolysis (Lin et al. 2014).

Kruppel-like factors (KLF) 5 is another important transcription factor participating in adipogenesis that is affected by miRNA. Thus, miR-448 represses the KLF5 mRNA. Overexpression of miR-448 reduces KLF5 together with adipocyte differentiation. In contrast, reduction in miR-448 promotes increased adipocyte differentiation, meanwhile, expression of adipogenic genes and triglyceride accumulation is reduced (Kinoshita et al. 2010). Tryptophan hydroxylase-1, a rate-limiting enzyme for the synthesis of serotonin (5-hydroxytryptamine; 5-HT), is expressed in adipocytes and is required for their differentiation. 5-HT type 2A receptor (5-HT(2A)R) and a 5-HT(2C)R antagonists inhibit adipocyte differentiation. Because 5-HT(2C)R mRNA levels are up-regulated during adipocyte differentiation and miR-448 is located in the fourth intron of 5-HT(2C). miR-448-mediated repression of KLF5 is a negative regulator for adipocyte differentiation (Kinoshita et al. 2010). The inhibitory effect of 5-HT on lipolysis enhances the anti-lipolytic effect of insulin (Hansson et al. 2016). Thus, KLF5 is upregulated and triglyceride concentration is increased when the miR-448 is introduced into preadipocytes (Kinoshita et al. 2010).

miR-130 strongly affects adipocyte differentiation, as overexpressing miR-130 impairs adipogenesis and reducing miR-130 enhances adipogenesis. miR-130 potently represses PPAR-gamma expression by targeting both the PPAR-gamma mRNA coding and 3′-untranslated regions. Adipose tissue from obese women contains significantly lower miR-130 and higher PPAR-gamma mRNA levels than that from non-obese women (Lee et al. 2011).

Another member of the PPAR family, PPAR-alpha is targeted by miR-519d. The treatment of primary human visceral pre-adipocytes with miR-519d or anti-miR-519d results in an increase and decrease in adipogenesis, respectively. miR-519d over-expression with resultant PPAR-alpha repression is related to metabolic dysfunction and adipocyte hypertrophy in subcutaneous fat tissue of severely obese individuals (Martinelli et al. 2010). Adenovirus early region 1-A-like inhibitor of differentiation 1 (EID 1) is a nuclear receptor co-regulator, which is shown to be important for adipogenesis. Its deficiency inhibits adipocyte differentiation in human adipose-derived mesenchymal stem cells. EID 1 is a direct target of miR-138, which is observed to be downregulated during adipogenesis of human adipose mesenchymal stem cells. miR-138 overexpression reduces triglyceride accumulation and inhibits the expression of PPAR-gamma and other adipogenic markers (Yang et al. 2011).

Adipogenesis in mesenchymal stem cells from younger donors is 2.3-fold higher than that of mesenchymal stem cells from older donors. Ninety-five per cent of the changed miRNAs are downregulated with age. hMSCs-Ad have more than 85% of significantly changed miRNAs downregulated with age. hMSCs-Ad from young donors, compared with those from older donors, have significantly elevated potential to differentiate toward adipogenic lineages. The miRNAs directed toward the MAPK/ERK system are expressed at higher levels in cells from older donors. hMSCs-Ad from older donors also exhibit significantly elevated levels of NF-kappaB, in response to downregulation of the MAPK/ERK system to prevent proliferation and inhibit apoptotic stimuli (Pandey et al. 2011).

Forty-two differently expressed miRNAs (meta-signature miRNAs) in mature adipocytes are compared to human stromal vascular cells or

human adipose-derived mesenchymal stem cells. Meta-signature miRNAs are specific for adipogenesis, several of which are correlated with cell differentiation, Wingless-type MMTV integration site family (Wnt) insulin receptor signaling pathway, MAPK signaling and lipid metabolic process. Seventy-nine miRNAs are found to be differentially expressed, most of which are located in obesity related chromosomal regions. In particular, hsa-let-7 family, hsa-miR-15a-5p, hsa-miR-27a-3p, hsa-miR-106b-5p, hsa-miR-148a-3p and hsa-miR-26b-5p have a great importance in adipogenesis. miR-146b-5p, miR-335, miR-424, miR-1275, miR-155, miR-1268, miR-148a, miR-26b, miR-132, miR-365, miR-1908, miR-93 and miR-720 are changed more than threefold in mature adipocyte. More importantly, the highest fold change is observed in miR-146b in mature adipocyte (Shi et al. 2016). Additionally, the levels of miR-148a, miR-26b, miR-30, and miR-199a increase in differentiating human adipose-derived mesenchymal stem cells. Especially, miR-148a expression levels also increase in adipose tissues from obese people. Therefore, miR-148a is used as a biomarker of obesity in human subjects. It represents a cyclic adenosine mono phosphate (cAMP) response element-binding (CREB)-modulated miRNA that acts to repress Wnt1 signaling, thereby promotes adipocyte differentiation. miR-148a acts by suppressing its target gene, Wnt1, which is an endogenous inhibitor of adipogenesis (Shi et al. 2015). In a similar manner, the overexpression of miR-342-3p markedly promotes the differentiation of hMSCs into an adipogenic lineage. Adipogenesis is significantly blocked by miR-342-3p downregulation. Carboxy-terminal binding protein 2 (CtBP2) is a direct target of miR-342-3p in this process. The effects of the inhibition of CtBP2 are similar to those of miR-342-5p overexpression on adipogenic differentiation, promoting the release of C/EBPalpha from CtBP2 binding. miR-342-3p is a powerful enhancer of the adipogenesis of hMSCs-Ad that acts by inhibiting CtBP2 and releasing the key adipogenic regulator C/EBPalpha from CtBP2 (Wang et al. 2015). The level of miR-204-5p is shown to be gradually upregulated during adipocytic differentiation, together with the mRNA expression of the critical adipogenic transcription factors (C/EBPalpha and PPAR-gamma), and the mature adipogenic marker FABP4. miR-204-5p regulates adipogenesis by controlling dishevelled segment polarity protein 3 (DVL3) expression and subsequently inhibiting the activation of the Wnt/beta-catenin signaling pathway (He et al. 2015) (Fig. 21.1).

Adipose lipid storage functions to prevent peripheral lipotoxicity (Kahn et al. 2006). TGF-beta represses Smad3 mRNA expression. Contrarily, Smad3 overexpression mimicks and enhances the TGF-beta response. Smad3 signaling mediates the differentiation-inhibiting and proliferative responses to TGF-beta (Choy et al. 2000). TGF-beta inhibits the transcriptional activity of C/EBPbeta, and this repression may result in decreased adipocyte marker mRNA levels and reduced adipogenesis. TGF-beta signaling and Smad3 inhibit transcription at C/EBP binding sites. Actually, Smad proteins are transcriptional modulators that mediate multiple signaling pathways. Thus, Smad3 cooperates with Smad4 to repress the transactivation function of C/EBPs, without affecting C/EBP binding to the promoter DNA sequences of target genes. TGF-beta blocks adipogenesis by this mechanism (Choy and Derynck 2003). Smad3-deficient mice have reduced adiposity associated with impaired lipid accumulation and adipogenesis. These mice are resistant to high fat diet-induced obesity. The expression of adipose triglyceride lipase and HSL are decreased by approximately twofold. In addition to elevated plasma triglycerides, the reduced free fatty acid and glycerol levels are associated with decreased lipolysis. Fat mass reflects the ratio between lipogenesis and lipolysis. The expression of several antiadipogenic factors and reduced PPAR gamma2 expression, suggests that Smad3 deficiency inhibits adipogenesis (Tan et al. 2011).

miR-21 expression is transiently increased after induction of adipogenic differentiation. Overexpression of miR-21 decreases both protein and mRNA levels of TGF-beta receptor 2 (TGF-betaR2). Decreased expression of TGF-betaR2 during adipogenic differentiation of

hMSCs-Ad is in concordance with an increase in the level of miR-21. Overexpression or inhibition of miR-21 alters Smad3 phosphorylation without affecting total levels of Smad3 protein (Kim et al. 2009). As mentioned above, TGF-beta blocks adipogenesis. Eventually, overexpression of TGF-beta1 in adipose tissue severely reduces both white and brown adipose tissue masses, as adipocytes fail to differentiate (Clouthier et al. 1997). This result also provide a reasonable explanation of how mature adipose tissues can contain high levels of TGF-beta despite its strong inhibitory action on adipogenic differentiation (Kim et al. 2009). While miR-21 significantly promotes adipocyte differentiation, it increases adiponectin mRNA and protein expression and decreases activator protein 1 (AP-1) protein level. The overexpression of AP-1 could absolutely reverse the stimulatory effect of miR-21 on adiponectin. Eventually, miR-21 plays an important role in the regulation of adipocyte differentiation and adiponectin expression by inhibiting AP-1 expression (Kang et al. 2013b). There are 17 microRNAs with upregulated expression; the highest levels are found for miR-21. Meanwhile, the expression of 33 microRNAs is downregulated, among which downregulation of miR-24 is the most extensive one. In this case, miR-24 significantly inhibits adipocyte differentiation and maturity. Additionally, miR-24 significantly decreases the expression of FABP4, while it upregulates AP-1 expression. In contrast, miR-21 has no effect on FABP4 protein and mRNA expression. In brief, miRNA expression profiles change markedly during adipocyte differentiation (Kang et al. 2013a). Due to the ubiquity of endogenous fatty acids and the high intracellular concentration of FABP4, the inhibitor miRNAs need to have greater repressive potency than endogenous fatty acids (Zhang et al. 2014). Generally, adipocyte FABP4 interacts with HSL and modulates its catalytic activity by integrating with several signalling networks. In this process, control of inflammatory responses is potentially through c-Jun N-terminal kinase (JNK)/inhibitor of kappa kinase (IKK) and insulin action in the adipocyte (Furuhashi and Hotamisligil 2008).

miR-138 is significantly down-regulated during adipogenic differentiation. Overexpression of miR-138 could effectively reduce lipid droplets accumulation and inhibits expression of key adipogenic transcription factors, as well as several other adipogenic marker genes, such as FABP4 and lipoprotein lipase (Yang et al. 2011). In this case, miR-138 indirectly regulates PPAR-gamma, an adipogenic transcription factor driving adipogenic gene expression in human mesenchymal stem cells (Rosen and MacDougald 2006).

Hypertrophic obesity is characterized with inflammation and a dysregulated adipose tissue. However, the number of preadipocytes in the abdominal subcutaneous adipose tissue capable of undergoing differentiation to adipose cells is reduced in hypertrophic obesity. This is likely to promote ectopic lipid accumulation that promotes insulin resistance and cardiometabolic risk (Gustafson et al. 2009). Obesity-induced cardiac hypertrophy and contractile reserves are ameliorated by antagonizing cardiomyocyte-specific miR-451 activity in mice via activation of adenosine monophosphate (AMP)-activated protein kinase (AMPK). Loss of miR-451 function reduces palmitate-induced lipotoxicity. In this respect, phosphorylated AMPK are increased and phosphorylated mammalian target of rapamycin (mTOR) is reduced in cardiomyocytes. AMPK decreases myocardial ceramide content. A reduction in ceramide content attenuates the lipotoxic cardiomyopathy (Kuwabara et al. 2015). miRNA targeting is primarily through "seed-matched sites" located within 3′-untranslated regions. Both computational and experimental studies show that each miRNA likely targets approximately 400 mRNAs and that almost half of these in mammalian cells are targeted by one or more miRNAs (Bartel 2009). miRNAs may directly regulate expression of over 30% of human genes (Xie et al. 2005). In total of more than 45,000 miRNA target sites within human 3′-untranslated regions have been maintained. Actually, more than 60% of human protein-coding genes have been under selective pressure to maintain pairing to miRNAs (Bartel 2009; Friedman et al. 2009). In this respect, several miRNAs may induce or decrease adipocyte

differentiation and adipogenesis in obese conditions. miR-301a attenuates saturated free fatty acid-induced activation of PPAR-gamma and production of proinflammatory cytokines in white adipose tissues. miR-301a directly targets the 3′-untranslated regions of PPAR-gamma. Consequently, miR-301a overexpression results in a decrease of PPAR-gamma protein expression, whereas PPAR-gamma overexpression reverses the inhibition of adipocyte differentiation by miR-301a (Li et al. 2016). However, miR-375 expression is increased after induction of adipogenic differentiation. Overexpression of miR-375 subsequently enhances adipocyte differentiation, as evidenced by its ability to increase mRNA levels of both C/EBPalpha and PPAR-gamma2. This is followed by induction of aP2 and triglyceride accumulation. Furthermore, overexpression of miR-375 suppresses phosphorylation levels of ERK1/2. As a consequence, miR-375 promotes adipocyte differentiation, possibly through modulating the ERK-PPAR-gamma2-aP2 pathway (Ling et al. 2011). According to the findings of Kajimoto et al. majority of miRNAs may modulate adipocyte function after differentiation rather than initiating the differentiation. The types of miRNAs involved in adipocyte function may vary between human adipocytes. The antisense inhibition of miR-10b, 15, 26a, 34c, 98, 99a, 101, 101b, 143, 152, 183, 185, 224, and let-7b, all of which are up-regulated during adipogenesis, do not affect adipocyte differentiation in terms of marker gene expression and the accumulation of lipid droplets (Kajimoto et al. 2006). As mentioned above, miRNAs are important regulators of fat cell development. Many miRNAs are differentially expressed in different fat depots and between normal and obese adipose tissue they are likely associated with proper function of adipose tissue. The remarkable inverse regulatory pattern for many miRNAs during adipogenesis and obesity is important in adipose tissue dysfunction of obese humans and during the chronic inflammation in obesity with insulin resistance (Xie et al. 2009b). In this context, miR-193b/-126/-26a increase adiponectin secretion when overexpressed in human adipocytes. However, in human white adipose tissue only miR-193b expression correlates with adiponectin gene expression and homeostasis model assessment of insulin resistance (HOMA-IR) (Belarbi et al. 2015). miR-26b expression is increased in mature adipocytes and is gradually upregulated during adipocyte differentiation, whereas its inhibition effectively suppresses adipocyte differentiation by decreasing lipid droplets and triglyceride accumulation (Song et al. 2014). Furthermore, miR-26b level is reduced in insulin-resistant adipocytes of visceral adipose tissue from obese individuals. Actually, miR-26b promotes insulin-stimulated glucose uptake and increases insulin-stimulated GLUT4 translocation to the plasma membrane in human mature adipocytes. In this respect, miR-26b modulates insulin-stimulated Akt activation via inhibition of its target gene, phosphatase and tensin homolog (PTEN), and significantly increases insulin sensitivity via the PTEN/phosphatidylinositol-3-kinase (PI3K)/Akt pathway. The expression level of miR-26b negatively correlates with increasing body mass index and HOMA-IR in human obese subjects (Xu et al. 2015). Reduced expression of the miR-25-93-106b cluster, or miR-93 alone, increases fat mass and, subsequently causes insulin resistance. A vicious cycle in which pre-diabetic conditions characterized by impaired glucose tolerance and insulin resistance leads to reduced miR-93 levels that in turn accelerates adipogenesis. Suppression of miR-93 augments white adipose tissue by increasing adipocyte precursors through the pluripotency-associated transcription factor Tbx3 and by increasing adipogenesis through SIRT7. Eventually, miR-93 serves as an important regulator for adipogenesis in visceral white fat. About 5% of pre-adipocytes are replicated at any time and 1–5% of adipocytes are replaced each day. This process appears to be tightly controlled by miR-93 (Cioffi et al. 2015). Indeed, SIRT7 (deacetylates histone H3 lysine 18) positively regulates the protein level of testicular receptor 4 nuclear receptor (TR4)/TGF-beta-activated kinase 1 (TAK1), a nuclear receptor involved in lipid metabolism. Activation of TR4 target genes increases fatty acid uptake and triglyceride synthesis or storage. Deficiency of SIRT7 has a pro-

tective effect against high fat diet-induced obesity. Lack of SIRT7 causes the decrease of the expression of genes related to inflammation (Yoshizawa et al. 2014).

In any way, impairment of adipogenesis contributes to the development of obesity-related insulin resistance. Fifty upregulated and 29 downregulated miRNAs are identified in insulin-resistant adipocytes including a 50-fold increase in miR-320 expression. miR-320 increases in insulin-resistant adipocytes. Actually, the p85 subunit of PI3K is a potential target of miR-320 and its inhibition increases insulin sensitivity by improving insulin-PI3K signaling pathways. Insulin sensitivity is increased in insulin-resistant adipocytes, by increasing p85 expression, phosphorylation of Akt and the protein expression of GLUT4 (Ling et al. 2009). miR-29a has strong regulatory functions in obesity as a positive regulator of insulin secretion in vivo by sensitizing beta-cells to overt diabetes. In contrast, in the liver both miR-29a and miR-29c are important negative regulators of insulin signaling via PI3K regulation (Dooley et al. 2016). The increase in miR-29 level causes insulin resistance, similar to that of incubation with high glucose and insulin in combination, which, in turn, induce miR-29a and miR-29b expression (He et al. 2007). On the other hand, miR-125a-3p expressed by abdominal omental adipose tissues is much higher in obese men than women. A negative association of miR-125a-3p with the insulin receptor and PI3K expressions is defined. In this context, increased miR- 125a-3p expression in omental adipose tissues may be a characteristic feature of insulin resistance in obese men (Yeh et al. 2014). Collectively, expression of miR-17-5p, miR-132, miR-134, miR-181a, miR-27a, miR-30e, miR-140, miR-147, miR-155, miR-197, and miR-210 play a role in the link between adipose tissue dysfunction and the development of obesity associated disorders. In particular, miR17-5p and miR-132 have impact in obesity related insulin resistance. Approximately, 7% of the detectable miRNAs are significantly associated with adipocyte size, suggesting that these miRNAs are involved in the development of adipocyte hypertrophy (Klöting et al. 2009). miRNA-132 regulates expression of the CREB protein, which plays a role in glucose homeostasis (Mayr and Montminy 2001). mTOR is a critical regulator of adipogenesis and systemic energy metabolism. Ablation of mTOR in adipose tissues causes insulin resistance. Furthermore, mTOR is required for adipocyte differentiation (Shan et al. 2016). Increases in the expression of IL-13 receptor alpha 1, (IL13RA1), mTOR, IL-20, Semaphorin-4C (SEMA4C) and FAS have been detected and negatively correlated with regulatory miRNA in white adipose tissue which are collected from 24 obese patients undergoing bariatric surgery with biopsy-proven non-alcoholic fatty liver disease (NAFLD) (Estep et al. 2015). miR-451 is involved in cardiomyopathy due to palmitate-induced lipotoxicity through the suppression of the Liver Kinase B1 (LKB1)/AMPK pathway. Palmitate-induced lipotoxicity is ameliorated in cardiomyocyte-specific miR-451-deficient mice compared to controls. In these miR-451-deficient mice, protein levels of Cab39 and phosphorylated AMPK are increased, while phosphorylated mTOR is reduced (Kuwabara et al. 2015).

In subcutaneous white adipose tissue obtained from 56 subjects, 11 miRNAs are present in all subjects and downregulated in obesity. Of these, miR-126 binds directly to the 3′-untranslated regions of chemokine ligand 2 (CCL2) mRNA, miR-193b regulates CCL2 production indirectly through a network of transcription factors. Increased adipocyte CCL2 secretion may initiate adipose inflammation by attracting the migration of inflammatory cells into the tissue. Overexpression of miR-193b and miR-126 in a human monocyte/macrophage cell line attenuates CCL2 production. The levels of the two miRNAs in subcutaneous white adipose tissue are significantly associated with regulation of adipose inflammation through their effects on CCL2 release from human adipocytes and macrophages (Arner et al. 2012).

Freshly isolated mature adipocytes, from obese individuals have an increased expression of mitogen-activated protein 4 kinase 4 (MAP4K4), which is known to inhibit PPAR-gamma induction. Therefore, number of preadi-

pocytes in the abdominal subcutaneous tissue that can undergo differentiation is reduced in obesity with enlarged fat cells (Isakson et al. 2009). Virtually, MAP4K4, a TNF-alpha-activated kinase, is a direct target of miR-30d. Overexpression of miR-30d protects beta-cells against TNF-alpha suppression on both insulin transcription and insulin secretion through the down-regulation of MAP4K4 by the miR-30d. However, miR-30d expression decreases in obesity (Zhao et al. 2012). miR-30 represents 4.9% of the miRNA reads in adipocytes. The miR-30 family is a positive, key regulator of adipocyte differentiation in human adipose tissue-derived stem cell. The up-regulation of miR-30 expression is triggered at early stages of adipocyte differentiation and increases until terminal differentiation (Zaragosi et al. 2011).

The expression of miR-143 in the mesenteric fat is up-regulated in mice fed a high-fat diet. Increased miR-143 expression is associated with an elevated body weight and mesenteric fat weight. Furthermore, miR-143 levels are closely correlated with expression levels of adipocyte differentiation markers such as PPAR-gamma and aP2 as well as plasma levels of leptin (Takanabe et al. 2008). Adipogenic differentiation is impaired by miR-369-5p, whereas it is highly increased by miR-371. FABP4 is a direct target of miR-369-5p. miR-369-5p and miR-371 as antagonistic regulators of adipogenic differentiation (Bork et al. 2011). The miR let-7 is up-regulated after induction of adipogenesis as an important regulator in adipocytes. Therefore, let-7 may have important implications in the amount and function of adipose tissue in human obesity (Sun et al. 2009).

Heme oxygenase-1 (HO-1)–adiponectin regulatory axis can be manipulated to ameliorate the deleterious effects of increased insulin resistance, obesity and the metabolic syndrome (Li et al. 2008). Up-regulation of HO-1 causes adipose remodeling, smaller adipocytes, and increases adiponectin secretion of human bone marrow-derived adipocytes. The anti-obesity effect of HO-1 induction results in an increase in adiponectin secretion, a decrease in TNF-alpha and IL-6, and a reduction in weight gain (Kim et al. 2008b). The induction of HO-1 is effective in suppressing adipocyte differentiation, as evidenced by an increase in the canonical adipocytes-derived regulatory protein Wnt cascade and a decrease in the paternally expressed 1 (Peg-1)/Mesoderm-specific transcript (Mest). HO-1 mediated-increase in Wnt10b, and decrease in Peg-1/Mest results in the maintenance of pre-adipocytes in their undifferentiated state with the slowing of the differentiation process. Wnt signaling cascade modulates the adipocyte phenotype by regulating the transcriptional factors that play a role in adipogenesis (Vanella et al. 2013) (Fig. 21.1). HO-1 activity is reduced in obesity. Increase in HO-1 protein levels ameliorates insulin resistance and compensatory hyperinsulinemia. Incubation of adipocytes with insulin results in a significant decrease in levels of the miRNAs, miR-155, miR-183, and miR-872. Insulin increases HO-1 protein expression in 3T3-L1 adipocytes via PI3K and protein kinase C (PKC)-dependent pathways and miRNAs down-regulation (Chang et al. 2011). Adipocyte-derived microvesicles (ADMs) contain RNA without typical 28S and 18S ribosomal RNA inside the vesicles. ADMs contain approximately 7000 mRNAs and 140 microRNAs. ADMs mediate transport of adiponectin and resistin gene transcripts into macrophages. ADM plays a role as a intercellular communication tool by transporting RNA (Ogawa et al. 2010). Meanwhile, specific miRNAs regulate endothelial cell functions and angiogenesis. Let7-f, miR-27b, and miR-130a are identified as pro-angiogenic miRNAs. In contrast, miR-221 and miR-222 inhibit endothelial cell migration, proliferation, and angiogenesis (Urbich et al. 2008).

The maturation of miRNAs is mediated by the two RNase III endonucleases Dicer and Drosha (Urbich et al. 2008). Dicer-dependent loss of microRNAs in hypothalamic neurons of adult mice causes chronic overactivation of the signaling pathways involving PI3K, Akt, and mTOR. Imbalance in the levels of neuropeptides results in severe hyperphagic obesity (Vinnikov et al. 2014). Twenty-four miRNAs are downregulated more than twofold after Dicer deletion. The

most downregulated miRNAs are mmu-miR-215 and mmu-miR-194. 18 miRNAs are upregulated more than twofold, including mmu-miR-195 and mmu-miR-199b. Consistent with the accumulation of lipids, Dicer deletion causes a marked decrease of microsomal triglyceride transfer protein, long-chain fatty acyl-CoA ligase 5, fatty acid binding protein, and very-long-chain fatty acyl-CoA dehydrogenase (Huang et al. 2012). Precursors of approximately 60–70 nucleotides (pre-miRNA) is cleaved in the cytoplasm by Dicer into the mature 22 nucleotide miRNA. The mature miRNA incorporates into the RNA-induced silencing complex (RISC) which directs the miRNA to the target mRNA leading either to translational repression or degradation of the target mRNA (Bartel 2004; Urbich et al. 2008). One miRNA can cooperatively bind to the same 3′-untranslated regions (Doench et al. 2003).

Computational analysis indicates a relevant role of miRNAs in modulating the expression of genes involved in signaling pathways such as lipid and protein metabolism, circadian rhythm and cell cycle that are well-known to be affected in obesity (Villard et al. 2015). In this respect, miRNAs regulate multiple pathways including insulin signaling, adipokine expression, adipogenesis, lipid metabolism, and food intake regulation. Thereby, miRNA-based therapeutics may be an innovative and attractive treatment modality (Deiuliis 2016). In another aspect, lifestyle interventions and bariatric surgery, known to restore metabolic functions, may also modify the concentrations of circulating miRNAs (Villard et al. 2015).

3 Conclusion

The regulatory role of miRNAs changes in different species or different cells. During adipogenesis, miRNAs can accelerate or inhibit adipocyte differentiation by acting on transcription factors, regulating signalling pathways related to adipogenesis (Chen et al. 2013). In this context, the remarkable inverse miRNA profile reveals a controversy between miRNAs and adipogenesis for human pre-adipocytes and mature adipocytes. Thereby, some miRNAs may represent biomarkers and therapeutic targets for obesity and obesity-related complications (Ortega et al. 2010). miRNA based therapeutics targeting obesity may provide recommendations for future research which could lead to the treatment of obesity (McGregor and Choi 2011). However, the broad-spectrum and redundancy of miRNA–target interactions seems to pose a great difficulty in developing miRNA therapeutics (Alexander et al. 2011).

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The Interactions Between Kynurenine, Folate, Methionine and Pteridine Pathways in Obesity

22

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Abstract

Obesity activates both innate and adaptive immune responses in adipose tissue. Elevated levels of eosinophils with depression of monocyte and neutrophil indicate the deficiencies in the immune system of morbidly obese individuals. Actually, adipose tissue macrophages are functional antigen-presenting cells that promote the proliferation of interferon-gamma (IFN-gamma)-producing CD4+ T cells in adipose tissue of obese subjects. Eventually, diet-induced obesity is associated with the loss of tissue homeostasis and development of type 1 inflammatory responses in visceral adipose tissue. Activity of inducible indoleamine 2,3-dioxygenase-1 (IDO-1) plays a major role under pro-inflammatory, IFN-gamma dominated settings. One of the two rate-limiting enzymes which can metabolize tryptophan to kynurenine is IDO-1. Tumor necrosis factor-alpha (TNF-alpha) correlates with IDO-1 in adipose compartments. Actually, IDO-1-mediated tryptophan catabolism due to chronic immune activation is the cause of reduced tryptophan plasma levels and be considered as the driving force for food intake in morbidly obese patients. Thus, decrease in plasma tryptophan levels and subsequent reduction in serotonin (5-HT) production provokes satiety dysregulation that leads to increased caloric uptake and obesity. However, after bariatric surgery, weight reduction does not lead to normalization of IDO-1 activity. Furthermore, there is a connection between arginine and tryptophan metabolic pathways in the

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generation of reactive nitrogen intermediates. Hence, abdominal obesity is associated with vascular endothelial dysfunction and reduced nitric oxide (NO) availability. IFN-gamma-induced activation of the inducible nitric oxide synthase (iNOS) and dissociation of endothelial adenosine monophosphate activated protein kinase (AMPK)- phosphoinositide 3-kinase (PI3K)-protein kinase B (Akt)- endothelial NO synthase (eNOS) pathway enhances oxidative stress production secondary to high-fat diet. Thus, reduced endothelial NO availability correlates with the increase in plasma non-esterified fatty acids and triglycerides levels. Additionally, in obese patients, folate-deficiency leads to hyperhomocysteinemia. Folic acid confers protection against hyperhomocysteinemia-induced oxidative stress.

Keywords

Dihydrofolate reductase (DHFR) • Endothelial nitric oxide synthase (eNOS) • Folate • Glutathione • Guanosine triphosphate (GTP)-cyclohydrolase 1 (GTPCH1) • Hyperhomocysteinemia • Serotonin (5-hydroxytryptamine, 5-HT) • Indoleamine 2,3-dioxygenase-1 (IDO-1) • Inducible nitric oxide synthase (iNOS) • Insulin resistance • Nitric oxide (NO) • Obesity • S-adenosylhomocysteine (SAH) • S-adenosylmethionine (SAM) • Tetrahydrobiopterine (BH4) • Tetrahydrofolate • Tryptophan 2,3-dioxygenase (TDO2) • Tryptophan • Neopterin • Kynurenine • Tumor necrosis factor-alpha (TNF-alpha)

1 Introduction

Actually diet-induced obesity is associated with the loss of tissue homeostasis and development of type 1 inflammatory responses in visceral adipose tissue. A key event is a shift of adipose tissue macrophages toward an M1 phenotype. Consequently, obesity-induced adipocyte hypertrophy results in upregulated surface expression of stress markers (Wensveen et al. 2015b). Thereby interferon-gamma (IFN-gamma), as a prototypical T-helper (Th) 1 cytokine, accompanies obesity-related inflammatory response (Rocha et al. 2008). In addition to the innate immune response, adaptive immunity may also participate in this process (Rocha et al. 2008). In obese individuals, plasma tryptophan concentrations have been shown to decrease and to be independent of weight reduction or dietary intake. Furthermore, decreased plasma tryptophan levels despite the increased kynurenine concentrations, reduces serotonin (5-hydroxytryptamine, 5-HT) production. Deprivation of tryptophan favors immunosuppression. Clinical manifestations of these metabolic alteration are mood disturbances and depression, however impaired satiety ultimately leads to increased caloric intake and obesity (Brandacher et al. 2007). Sustained increase of kynurenine and decrease of 5-HT production encourages the formation of "vicious cycle" (Oxenkrug 2010). The higher kynurenine to tryptophan ratio reflects the higher indoleamine 2,3-dioxygenase-1 (IDO1) activity in obese compared to lean individuals. There are no differences between obese and lean subjects with respect to tryptophan 2,3-dioxygenase (TDO2) and indoleamine 2,3-dioxygenase-2 (IDO2) activities (Wolowczuk et al. 2012). Furthermore, phosphorylated guanine nucleoside to guanosine triphosphate (GTP) is catalyzed by guanosine triphosphate cyclohydrolase I (GTPCH1), the first and rate-limiting step of pteridines biosynthesis. IFN-gamma selectively stimulates the early steps of pterin biosynthesis in macrophages, thereby neopterin

reflects IFN-gamma-inducible pro-inflammatory immune status in clinical studies (Murr et al. 2002; Schoedon et al. 1986). On the other hand, the IFN-gamma-inducible IDO gene is expressed synergistically in response to IFN-gamma and tumor necrosis factor-alpha (TNF-alpha) (Robinson et al. 2005). IFN-gamma inducible-IDO/GTPCH inflammation cascade might be characterized by IFN-gamma polymorphism, kynurenine to tryptophan ratio (IDO activity) and tetrahydrobiopterine (BH4) to neopterin ratio (GTPCH activity) (Oxenkrug et al. 2011). Actually, BH4 is required for nitric oxide (NO) synthesis and inhibition of superoxide release from endothelial NO synthase (eNOS). The oxidation products of BH4, 7,8-dihydrobiopterin (7,8-BH2), is recycled back to BH4 by dihydrofolate reductase (DHFR) (Whitsett et al. 2013). In this case DHFR or GTPCH1 deficiency attenuates vascular endothelial growth factor (VEGF)-induced eNOS activity and NO production (Sugiyama et al. 2009). In obese children, the deficiency of folate leads to hyperhomocysteinemia. In this context, folate is an important cofactor in re-methylation of homocysteine to methionine (Iamopas et al. 2014). Furthermore, plasma levels of S-adenosylmethionine (SAM), S-adenosylhomocysteine (SAH), and homocysteine are either independently or in combinations associated with obesity (Ming et al. 2009) (Fig. 22.1).

In this chapter involvement of obesity-related chronic immune activation focusing on kynurenine, unconjugated pteridine, folate and methionine pathways are summarized. In this context, this review attempts to briefly comment on the various reasonable explanations that have been proposed for the IFN-gamma-inducible pro-inflammatory immune status and related pathways in obesity.

2 Innate and Adaptive Immune Responses in Obesity

Both adipocytes and immune cells are implicated to induce adipose inflammation in obesity. IFN-gamma stimulates adipocyte major histocompatibility complex class II (MHC II), NOD-like receptor family pyrin domain containing 3 (NLRP3) and caspase-1 expression, while adipocyte MHCII mediates CD4+ T cell activation (Yin et al. 2014). Obesity activates both innate and adaptive immune responses in adipose tissue. Neutrophil activation is a fundamental process in the innate immune response. In addition to circulating myeloperoxidase and calprotectin levels, membrane expression of CD66b on circulating neutrophils are significantly increased in severely obese subjects as compared to healthy controls (Nijhuis et al. 2009) Elevated levels of eosinophils, monocyte CD14, and monocyte CD14+/CD16+ subsets, with depression of monocyte and neutrophil CD62L indicate the deficiencies in the immune system of morbidly obese individuals. These abnormal levels reverse rapidly with Roux-en-Y gastric bypass (Cottam et al. 2002). The number of CD4+ lymphocytes in visceral adipose tissue correlates with body weight. CD4+ lymphocytes can either differentiate into Th1-cells, releasing pro-inflammatory cytokines like IFN-gamma and TNF-alpha, or to anti-inflammatory Th2-cells with the expression of interleukin (IL)-10 or IL-4 in diet-induced obesity (Kintscher et al. 2008).

CD40/CD40L signaling provides bidirectional costimulatory signals between antigen-presenting cells and CD4+ T cells. CD40 expression is positively correlated with CD80 and CD86 expression in obese patients. In this case, CD40 signaling in adipose tissue macrophages regulates MHC II and CD86 expression to control the expansion of CD4+ T cell-mediated adaptive immune response (Morris et al. 2016). Deletion of MHC II expression in macrophages leads to an adipose tissue-specific decrease in the effector/memory CD4+ T cells, attenuation of CD11c+ adipose tissue macrophage accumulation and obesity-induced meta-inflammation (Cho et al. 2014). Immune processes contribute to the development of obesity and its complications. The interaction between the costimulatory protein CD40 and its downstream adaptor protein tumor necrosis factor receptor-associated factor 6 (TRAF6) promotes adipose tissue inflammation. Conversely, inhibition of the CD40-TRAF6 interaction causes a significant decrease in the number of adipose tissue CD4+ and CD8+ T

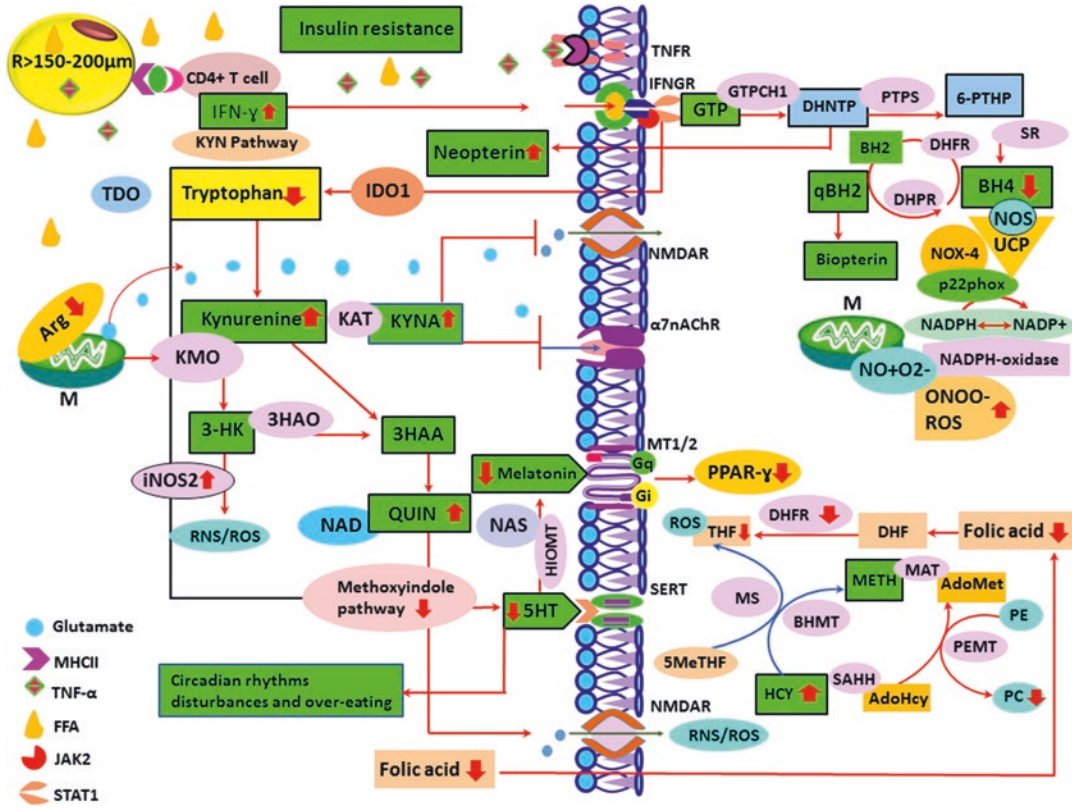

Fig. 22.1 Tryptophan depletion is a persistent metabolic disorder in morbidly obese patients despite different weight reduction strategies. Impaired satiety due to decrease in 5-HT production ultimately leads to increased caloric uptake and obesity. In addition to cellular immune activation, oxidative stress and folate depletion could be involved in the development of hyperhomocysteinemia. Increased homocysteine levels are related with abdominal obesity. Despite normal dietary intake, persistent folate deficiency is inversely correlated with body mass index in obese individuals (*FFA* Free fatty acid; *IFN-gamma* Interferon gamma; *KYN* Kynurenine; *MHCII* Class II major histocompatibility complex; *IDO* Indoleamine 2, 3-dioxygenase; *TDO* tryptophan-2,3-dioxygenase; *IFNGR* Interferon-gamma receptor; *STAT1-alpha* Signal transducer and activator of transcription 1; *JAK2* Janus kinase 2 *TNF-α* Tumor necrosis factor alpha; *M* Mitochondria; *Arg* Arginine; *KMO* Kynurenine 3-monooxygenase; *3HK* 3-hydroxykynurenine; *iNOS* Inducible nitric oxide synthase; *ROS* Reactive oxygen species; RNS Reactive nitrogen species; *3HAO* 3-hydroxyanthranilic acid oxidase; *NAD* Nicotinamide adenine dinucleotide; *NADPH* Nicotinamide adenine dinucleotide phosphate, reduced; *KAT* Kynurenine aminotransferase; *KYNA* Kynurenic acid; *3HAA* 3-hydroxyanthranilic acid; *QUIN* Quinolinic acid; *5HT* Serotonin; *HIOMT* hydroxyindole-O-methyltransferase; *NAS* Nacetylserotonin; *SERT* Serotonin transporter; *MT1/MT2* Membrane bound melatonin receptors; *Gi* inhibitory G-protein; *Gs* stimulatory G-protein; *TNFR* TNF receptor-1; *GTP* Guanosine triphosphate; *GTPCH I* Guanosine triphosphate cyclohydrolase I; *DHNTP* Dihydroneopterin triphosphate; *PTPS* Pyruvoyl-tetrahydropterin synthase; *6-PTHP* 6-Pyruvoyl-tetrahydropterin; *BH2* 7,8-dihydrobiopterin; *DHFR* Dihydrofolate reductase; *DHPR* dihydropteridine reductase; *BH4* Tetrahydrobiopterin; *SR* Sepiapterin reductase; *qBH2* Quinonoid dihydrobiopterin; *UCP* Uncoupling; *NOX4* NADPH oxidase 4; *p22phox* p22phox Protein, human neutrophil cytochrome b-light chain (*CYBA*); *NOS* Nitric oxide synthase; *NMDAR* Nmethyl-D-aspartate receptor; *α7nAChR* Alpha7nicotinic acetylcholine receptor; *ONOO-* Peroxynitrite; *PPAR-γ* Peroxisome proliferator-activated receptor-gamma; *THF* Tetrahydrofolate; *DHF* Dihydrofolate; *5Me-THF* 5-methyl-THF; *HCY* homocysteine; *MS* Methionine synthase; *METH* Methio nine; *MAT* AdoMet: S-adenosylmethionine; AdoHcy S-adenosylhomocysteine; PE Phosphotidylethanolamine; *PC* Phosphotidylcholine; *PEMT* Phosphotidylethanolamine N-methyltransferase; *MAT* Methionine adenosyltransferase; *SAHH* S-adenosylhomocysteine hydrolase; *BHMT* Betaine: homocysteine methyltransferase)

cells, as well as macrophages (van den Berg et al. 2015). Thus, dietary obesity is shown to activate the proliferation of effector memory CD4+ T cells in adipose tissue. Actually, adipose tissue macrophages are functional antigen-presenting cells that promote the proliferation of IFN-gamma-producing CD4+ T cells in adipose tissue. These macrophages can promote proliferation and IFN-gamma production from antigen-specific CD4+ T cells (Morris et al. 2013). On the other hand, dendritic cells (DCs) critically bridge innate and adaptive immunity through their capacity to drive antigen-specific T cell activation (Merad et al. 2013). Furthermore, DCs are controlled by both cytokines and transcription factors (Belz and Nutt 2012). In addition to loss of weight, the mean percentage of CD4+ and CD8+ T lymphocytes significantly decrease postoperatively in morbidly obese patients who underwent bariatric surgery (Fathy and Morshed 2014). As mentioned above, obesity is associated with T-cell abnormalities in adipose tissue. Thus, morbidly obese individuals have a selective increase in peripheral blood CD4+ naive, memory, natural CD4+ CD25+ FoxP3+ regulatory T (Treg), and Th2 cells. Although peripheral blood CD8+ T-cell numbers are not increased, adipose tissue CD8+ T cells are elevated due to selective redistribution. Hence, CD4+ and CD8+ T-cell proliferation is increased in adipose tissue. Rising CD4+ T cell population with propensity toward a Treg- and Th2-dominated phenotype suggests a more anti-inflammatory set point (van der Weerd et al. 2012). According to findings of Nishimura et al., obese adipose tissue activates CD8+ T cells, which, in turn, promote the recruitment and activation of macrophages. These results reveale that CD8+ T cells have an essential role in the initiation and propagation of adipose inflammation. In this context, a vicious cycle is evident between CD8+ T cells, macrophages and adipose tissue (Nishimura et al. 2009). Hence IFN-gamma produces direct effects on metabolic pathways in adipocytes (Rocha et al. 2008). IFN-gamma expression in adipose tissue increases with diet-induced obesity. IFN-gamma stimulation significantly increases expression not only of the T-cell chemoattractants, monokine induced by IFN-gamma (MIG), IFN-gamma inducible protein-10 (IP-10), and IFN-inducible T cell-alpha chemoattractant (I-TAC), but also of monocyte chemoattractant protein-1 (MCP-1), MCP-2, and "regulated upon activation normal T cell expressed and secreted" (RANTES) (Rocha et al. 2008). Signal transducer and activation of transcription (STAT) proteins become activated by tyrosine phosphorylation and form homodimers and heterodimers. These dimers are translocated to the nucleus, and promote the transcriptional activation of cytokine-inducible genes. The IFN signaling pathway, including STAT1, is the predominant pathway that leads to the physiological induction of cytokine-inducible genes during the development of host defense responses (Meraz et al. 1996). Although mean percentages of IL-2- and IL-4- secreting CD4+ cells in obese children are not significantly different from those found in lean children, the mean percentage of CD4+ T cells secreting IFN-gamma is significantly higher in the obese. Therefore in obese children, a shift to Th1-cytokine profile which is dominated by the production of IFN-gamma, is related to insulin resistance (Pacifico et al. 2006). IFN-gamma might also play an important role in growing adipose tissue, where adipocytes respond to inflammatory products derived from infiltrating macrophages such as TNF-alpha (Wellen and Hotamisligil 2003). Actually adipose stress is simultaneously detected by natural killer (NK) cell and CD8+ T cell. IFN-gamma secreted by these cells promotes M1 adipose tissue macrophage polarization (Wensveen et al. 2015b). In this context, tissue-resident NK cells represent a crucial link between obesity-induced adipose stress and visceral adipose tissue inflammation. In this regard, NK cells are key regulators of macrophage polarization and insulin resistance in response to obesity-induced adipocyte stress. Thereby, obesity drives the upregulation of ligands of the NK cell-activating receptor (NCR1) on adipocytes. Actually, NK cell proliferation and IFN-gamma production is stimulated through these receptors, which in turn trigger the differ-

entiation of pro-inflammatory macrophages (Wensveen et al. 2015a). Indeed, when stimulated by IFN-gamma, differentiated adipocytes secrete various inflammatory mediators (Rocha et al. 2008). Expression of leptin, IL-6, IL-8, TNF-alpha, MCP-1, IP-10, macrophage inflammatory protein-1beta (MIP-1beta), granulocyte colony stimulating factor, IL-1 receptor antagonist (IL-1ra), and adiponectin is positively correlated with adipocyte size. However, differential expression of pro- and anti-inflammatory factors with increasing adipocyte size result in a shift toward dominance of pro-inflammatory adipokines (Skurk et al. 2007). Thus, obese IFN-gamma-deficient animals have significantly reduced adipose tissue expression of mRNA-encoding inflammatory genes such as TNF-alpha and MCP-1. In these cases, adipose tissue inflammatory cell accumulation decreases (Rocha et al. 2008).

3 Tryptophan Metabolism and Obesity

Immune activation is inextricably linked with dysregulation of the tryptophan metabolism, shifting catabolic routes towards oxidative breakdown along the kynurenine axis. Several enzymes are able to metabolize tryptophan, but activity of IDO-1 plays a major role under pro-inflammatory, IFN-gamma dominated settings. Deprivation of the essential amino acid tryptophan restricts T cell proliferation, which favors immunosuppression (Strasser et al. 2017). About 95% of tryptophan is metabolized by the tryptophan-kynurenine pathway (Gál and Sherman 1980; Schwarcz and Pellicciari 2002). One of the two rate-limiting enzymes which can metabolize tryptophan to kynurenine is IDO, the other enzyme that is able to break tryptophan's indole ring is TDO2 (Dang et al. 2000; Schwarcz and Pellicciari 2002). While TDO is activated by stress hormones, IDO is activated by pro-inflammatory cytokines (Oxenkrug 2010). Kynurenine to tryptophan ratio disequilibrium concomitant with immune system activation is the hallmark of IDO1 rather than TDO2 activation in obesity (Schröcksnadel et al. 2006). IDO1 is expressed in white adipose tissue. Furthermore, TNF-alpha is correlated with IDO1 in adipose compartments suggesting that TNF-alpha could be the inductor of IDO1 in adipose tissue (Wolowczuk et al. 2012). IDO suppresses adaptive immunity by inducing T-cell differentiation to Treg through tryptophan depletion and/or kynurenine pathway products. Additionally, IDO induces the production of immunosuppressive cytokine, IL-10 by stimulated lymphocytes (Eleftheriadis et al. 2012). Two different mechanisms have been proposed in tolerance induction mediated by tryptophan catabolism. Firstly, tryptophan breakdown suppresses T cell proliferation by critically reducing the availability of this amino acid from local tissue microenvironments (Mellor and Munn 2004). Secondly, inhibition of CD8+ T cell-mediated cytotoxic function is an important mechanism behind the immune-modulating property of IDO-high environment (Liu et al. 2009). Thus, strategies to supplement tryptophan while dieting could be useful in treating uncontrolled weight gain (Strasser et al. 2015).

Actually, IDO mediated tryptophan catabolism due to chronic immune activation is the cause of reduced tryptophan plasma levels and be considered as the driving force for food intake in morbidly obese patients (Brandacher et al. 2007). One of the factors influencing availability of tryptophan as a substrate of methoxyindole and kynurenine pathways is plasma free tryptophan concentration. Therefore kynurenine is formed in the mammalian brain by 40%, and 60% is taken up from the periphery (Németh et al. 2005). The methoxyindole pathway leads to formation of 5-HT and 5-methoxy-N-acetyltryptamine (melatonin). Less than 5% of tryptophan is metabolized along this pathway (Gál and Sherman 1980). The rate-limiting step of melatonin biosynthesis is 5-HT-N-acetylation resulting in the formation of N-acetyl-serotonin with subsequent methylation into melatonin (Oxenkrug 2007). Artificial light-at-night emerge as a statistically significant and positive predictor of overweight and obesity. Together with other factors, artificial light-at-night is a contributing factor to excessive body mass in 70% of obese humans in more than

80 countries (Rybnikova et al. 2016). A deficiency of melatonin, one of the consequences of sleep deprivation, has also been demonstrated to correlate with obesity. Melatonin is a pineal secretory product involved in the regulation of internal biological clocks and energy metabolism (Szewczyk-Golec et al. 2015). Melatonin significantly increases the expression of peroxisome proliferator-activated receptor-gamma (PPAR-gamma), a master regulator of adipogenesis, and promotes differentiation into adipocytes. Melatonin-treated cells also form smaller lipid droplets and abundantly express several molecules associated with lipolysis, including adipose triglyceride lipase and perilipin (Kato et al. 2015). Melatonin supplementation by regulating inflammatory infiltration ameliorates obesity-induced adipokine alteration (Favero et al. 2015). Actually kynurenic acid may contribute to cross talk between the melatonin and kynurenine pathways by inhibiting 5-HT-N-acetylation. The N-methyl-D-aspartate (NMDA) receptor-mediated glutamatergic neurotransmission is a step necessary for serotonin N-acetyltransferase induction and melatonin biosynthesis (Zawilska et al. 1997) (Fig. 22.1).

As mentioned above, the immunomodulatory enzyme IDO is widely distributed in mammals and is induced preferentially by IFN-gamma. IDO degrades tryptophan to form N-formyl kynurenine (Brandacher et al. 2007). In case of obesity, activation of IDO shifts tryptophan metabolism from 5-HT synthesis to the formation of kynurenines. Increased concentration of kynurenine might contribute to development of metabolic syndrome (Brandacher et al. 2006). Also decrease in plasma tryptophan levels and subsequent reduction in 5-HT production provoke satiety dysregulation that leads to increased caloric uptake and obesity (Brandacher et al. 2007). The tryptophan/kynurenine pathway is the main route of tryptophan degradation and generates several neuroactive and immunomodulatory metabolites. Besides fat, muscle and liver, pancreatic islet tissue itself are sites of inflammation during obesity. Only some tryptophan/kynurenine pathway genes are constitutively expressed, both in beta-cells as well as non beta-cells. The regulatory enzyme IDO1 of tryptophan/kynurenine pathway is not constitutively expressed. Thereby, IDO1 and kynurenine 3-monoxygenase (KMO) expression are potently activated by IFN-gamma, IL-1beta and gluco-lipotoxicity, respectively, rather in beta-cells than in non beta-cells. Thus islet kynurenine to kynurenic acid ratio is enhanced following IFN-gamma stimulation and gluco-lipotoxicity. However, acute exposure to kynurenine potentiates glucose-induced insulin secretion by normal islets (Liu et al. 2015). Almost 99% of the dietary tryptophan, not used in protein synthesis, is metabolized along the kynurenine pathway to produce nicotinamide adenine dinucleotide (NAD) (Gál and Sherman 1980; Han et al. 2010). KMO is a flavin adenine dinucleotide (FAD)-dependent enzyme that catalyzes the 3-hydroxylation of kynurenine in the presence of nicotinamide adenine dinucleotide phosphate, reduced (NADPH) and molecular oxygen. KMO localizes to the outer membrane of mitochondria and is highly expressed in peripheral tissues. The levels of kynurenine, kynurenic acid, and anthranilic acid, are substantially, but differentially, elevated in the liver, brain, and plasma of KMO-deficient animals (Giorgini et al. 2013; Heyes et al. 1992).

Kynurenic acid is a metabolite in the kynurenine pathway and the overall process leading to the formation of kynurenic acid includes oxidation of tryptophan to formylkynurenine, hydrolysis of formylkynurenine to kynurenine, and transamination of kynurenine to a side chain keto acid intermediate, and intramolecular cyclization of the intermediate to kynurenic acid (Han et al. 2010). Kynurenic acid is the end product of this pathway and the only known endogenous NMDA receptor antagonist (Schwarcz and Pellicciari 2002). NMDA receptors typically consist of the obligatory GluN1 subunit and different GluN2 subunits. GluN2B-containing NMDA receptors in hypothalamic agouti-related peptide (AgRP) neurons play a critical role in the central control of body weight homeostasis and blood glucose balance. Loss of GluN2B from AgRP neurons reduces body weight, fat mass, and food intake, whereas GluN2B in pro-opiomelanocortin neurons is not required for normal energy balance

control. Mice lacking GluN2B in AgRP neurons are also more sensitive to anti-obesity actions of leptin (Üner et al. 2015) (Fig. 22.1). Diet-induced obesity produces overexpression of dopamine D4 receptor (D4R) mRNA in the ventromedial hypothalamic nucleus (VMH). Activation of D4R in the paraventricular nucleus (PVN) induces inhibition of glutamate release and subsequently blocks stimulated food intake by inhibiting satiety (Tejas-Juárez et al. 2014). Preference for high fat diet is not observed with low doses of the NMDA receptor antagonists (Buttigieg et al. 2014). High fat diets trigger neurochemical changes, leading to a desensitization of NMDA receptors and reduces density of the NR2B subunit of NMDA receptors within the hippocampus, which might account for cognitive deficits. All of these changes are compatible with the development of leptin resistance within the hippocampus (Valladolid-Acebes et al. 2012). In the hippocampus, leptin facilitates NR2B-NMDA receptor-mediated Ca^{2+} influx in cerebellar granule cells via a mitogen-activated protein kinase (MAPK)-dependent pathway (Irving et al. 2006). The affinity of the binding site for the NMDA antagonists is significantly higher in obese mice than lean mice (Li et al. 1999).

The expression of several kynurenine pathway enzyme genes including IDO1, kynureninase, KMO, and kynurenine aminotransferase III is significantly increased in the omental adipose tissue of obese women compared to lean subjects. In fact, the levels of kynurenine, kynurenic acid, and quinolinic acid are associated with higher body mass index. The expression of these metabolites are induced by proinflammatory cytokines in human primary adipocytes. The expressions of IDO1, kynureninase, KMO and kynurenine aminotransferase III are also higher in proinflammatory M1 than in anti-inflammatory M2 macrophages (Favennec et al. 2015). Eventually, plasma tryptophan concentrations are decreased in obese subjects independent of weight reduction or dietary intake (Brandacher et al. 2007). Actually, serum concentrations of kynurenine to tryptophan ratio is an indirect indicator of IDO activity (Brandacher et al. 2006). However, after bariatric surgery weight reduction does not lead to normalization of kynurenine to tryptophan ratio. Thus, tryptophan depletion in morbidly obese patients persists in spite of significant weight reduction following bariatric surgery (Brandacher et al. 2006). This might thereby be responsible for diminished 5-HT functions, leading to unchanged satiety dysregulation and a reward-deficiency-syndrome (Brandacher et al. 2006).

Tryptophan metabolites, including kynurenine, 3-hydroxyanthranilic acid, and picolinic acid, are key mediators of immunosuppression. In contrast to kynurenine and 3-hydroxyanthranilic acid, exposure of CD4+ T cells with picolinic acid does not affect cell viability, whereas proliferation and metabolic activity are suppressed in a dose-dependent manner (Prodinger et al. 2016). This means that IDO is able to inhibit proliferation of CD4+ T lymphocytes, CD8+ T lymphocytes, and NK cells through the formation of L-kynurenine, picolinic acid and quinolinic acid. Consequently, IDO enhances own inhibitory potential by depriving the extracellular tryptophan (Frumento et al. 2002). Selective association of the IDO-competent phenotype with down-modulation of the TYRO protein tyrosine kinase binding protein gene encodes the signaling adapter, DAP12 of cell surface. Down-modulation of DAP12 involves in IFN consensus sequence binding protein (ICSBP), a transcription factor also known as IFN regulatory factor 8 (IRF-8). IRF-8 is required in tolerogenic DCs for the positive regulation of IDO and the negative regulation of DAP12 (Orabona et al. 2006).

4 Serotonin and Obesity

Serotonin (5-hydroxytryptamine, 5-HT) is a neurotransmitter that is synthesized through the actions of two different tryptophan hydroxylases, TpH1 and TpH2, which are found, respectively, in enterochromaffin cells and neurons. 5-HT is inactivated by the 5-HT reuptake transporter (SERT)-mediated uptake into enterocytes or neurons (Gershon and Tack 2007). 5-HT cannot cross the blood-brain barrier, thereby the central and peripheral serotonergic systems are function-

ally separated. The functions of 5-HT in energy homeostasis range from central control of food intake to direct regulation of adipose tissue activity in the periphery (Namkung et al. 2015). The serum 5-HT level is elevated in animals fed a high fat diet compared to controls fed a low fat diet (Kim et al. 2011). In the over-fed state, 5-HT level increases in white adipose tissue, leading to the augmentation of lipogenesis via 5-HT receptor 2A (HTR2A). 5-HT also suppresses thermogenesis in the brown adipose tissue via HTR3. When 5-HT signaling is inhibited, lipogenesis decreases and thermogenesis increases in adipocytes (Oh et al. 2015) (Fig. 22.1).

5 Cross-Talk Between Unconjugated Pteridine, Homocysteine and Folate Pathways in Obesity

Neopterin formation by human monocyte-derived macrophages and DCs is induced by the pro-inflammatory cytokine IFN-gamma, which is released by activated T-lymphocytes. Like other pro-inflammatory cytokines, IFN-gamma also triggers the formation and release of reactive oxygen species (ROS). Chronic ROS-production leads to the depletion of antioxidants with a consequence that oxidative stress develops (Schroecksnadel et al. 2006). Diet-induced obesity is associated with the loss of tissue homeostasis and development of type 1 inflammatory responses in visceral adipose tissue, characterized by IFN-gamma. A key event is a shift of adipose tissue macrophages toward an M1 phenotype (Wensveen et al. 2015b). Actually, neopterin concentrations are significantly higher in patients with elevated body mass index and increased glucose concentrations (Ledochowski et al. 1999). IFN-gamma stimulated neopterin production in human monocytes/macrophages reflects the degree of Th1-type immune activation. Eventually, the increased formation of neopterin and degradation of tryptophan may result in a decreased T cell responsiveness and leads to immunodeficiency (Widner et al. 2000). In 113 subjects with clinical markers of metabolic syndrome have been followed for 6 years, neopterin concentrations of more than 16 nmol/L at baseline are found to be associated with the increased risk of mortality. In these cases, neopterin concentrations correlate with abdominal obesity, insulin resistance and plasma pyridoxal-5′-phosphate deficiency. Since pyridoxal-5′-phosphate is a cofactor of IFN-gamma-induced key enzymes of tryptophan-kynurenine metabolism, its deficiency is associated with the increased production of xanthurenic acid through kynurenine pathway. Therefore, assessment of neopterin concentrations may be utilized as a tool for monitoring IFN-gamma-inducible inflammation in obesity (Oxenkrug et al. 2011). Indeed, pyridoxal-5-phosphate deficiency diverts kynurenine-NAD metabolism from production of NAD to the excessive formation of xanthurenic acid. The fall in NAD^+ inhibits cellular functions including insulin synthesis and secretion, and thus the beta-cell ultimately dies (Okamoto 2003). In this respect, monitoring kynurenine, pyridoxal-5-phosphate and xanthurenic acid levels in obesity might help to identify the subjects at risk for insulin resistance (Oxenkrug 2013).

Actually in obese subjects, the ratio of kynurenine to tryptophan, which reflects IDO1 activation, is higher than in lean subjects. Inflammation is associated with a T-cell infiltration in obese adipose tissue, with predominance of Th17 in the omental compartment and of Treg in the subcutaneous depot (Wolowczuk et al. 2012). Macrophage activation is modified by T-cells. IFN-gamma-secreting Th1 as well as IL-17-secreting T-cells enhance macrophage pro-inflammatory functions by inducing the release of IL-1, IL-6, and TNF-alpha (Winer et al. 2009). In contrast, anti-inflammatory IL-4 and IL-13-secreting Th2 as well as CD4+ Foxp3+ T-cells regulate macrophage function by differentiating macrophages into anti-inflammatory IL-10 secreting, M2 macrophages (Tiemessen et al. 2007). The Th17/Treg balance is decreased in subcutaneous fat and correlates with IDO1 activation in obesity. In contrast, in the omental compartment, despite IDO1 activation, the Th17/Treg balance control is impaired. This means that

IDO1 activation is a local compensatory mechanism to limit obesity-induced inflammation (Wolowczuk et al. 2012).

The levels of kynurenine, kynurenic acid, and quinolinic acid are associated with higher body mass index. The expression of several kynurenine pathway enzymes including IDO1, kynureninase, KMO and kynurenine aminotransferase III are increased in the omental adipose tissue of women with obesity compared to lean. Expression of these enzymes is induced by pro-inflammatory cytokines in human primary adipocytes, except for KMO that is expressed in pro-inflammatory macrophages. (Favennec et al. 2015).

The major unconjugated pteridine, BH4 is a key redox-active cofactor in eNOS catalysis and is an important determinant of NO-dependent signaling pathways. DHFR deficiency increases ROS production. In endothelial cells 7,8-BH2 is rapidly recycled to BH4 via DHFR (Sugiyama et al. 2009). BH4 is a required cofactor for the synthesis of NO by eNOS, and endothelial BH4 bioavailability is a critical factor in regulating the balance between NO and superoxide production. Biosynthesis of BH4 is determined by the activity of GTPCH1. However, BH4 levels may also be influenced by oxidation, forming BH2, which promotes eNOS uncoupling. Inhibition of DHFR in BH4-deficient states leads to eNOS uncoupling. DHFR plays key role in regulating the BH4 vs. BH2 ratio and eNOS coupling under conditions of low total biopterin availability (Crabtree et al. 2011). Compared with healthy controls, the obese children have significantly increased concentrations of nitrosative and oxidative stress markers. Additionally, increased NO production in obese children is associated with metabolic risk factors (Codoñer-Franch et al. 2011). Indeed, pro-inflammatory cytokine generation is a major mechanism in obesity, which is associated with a reduced NO availability due to excess peroxynitrite synthesis. Therefore, abdominal obesity is associated with vascular endothelial dysfunction and reduced NO availability is secondary to enhanced oxidative stress production (Virdis 2016). In this respect, malondialdehyde (MDA) is generated as an end product from oxidative degradation of polyunsaturated fatty acids in obesity. It has been recognized as an important indicator of lipid peroxidation for inflamed abdominal visceral adipose tissue (Horton and Fairhurst 1987; Lee et al. 2015). Collectively, endothelial dysfunction caused by high-fat diet which is related to dissociation of endothelial adenosine monophosphate (AMP) activated protein kinase (AMPK)- phosphoinositide 3-kinase (PI3K)-protein kinase B (Akt) -eNOS pathway. Reduced endothelial NO availability correlates with the increase in plasma non-esterified fatty acids and triglycerides levels (García-Prieto et al. 2015). Furthermore, IFN-gamma-induced activation of the inducible nitric oxide synthase (iNOS) might be mediated by kynurenine derivatives of tryptophan (Oxenkrug 2007). IL-4 significantly decreases IFN-gamma plus picolinic acid-induced NOS mRNA expression and NOS transcription. These data indicate that there is a connection between arginine and tryptophan metabolic pathways in the generation of reactive nitrogen intermediates (Melillo et al. 1994). Increase in homocysteine levels in addition to ROS generation impairs endothelial functions by inhibiting endothelial protein S-nitrosylation (Chen et al. 2014) (Fig. 22.1). Hyperhomocysteinaemia decreases phosphorylation of eNOS at serine-1177 and phosphorylation of Akt at serine-473. Furthermore, L-methionine administration induces a significant increase in plasma homocystein and decrease in plasma NO (Yan et al. 2010). Indeed, obese patients have statistically higher homocysteine levels than lean individuals. In these patients increased homocysteine levels are related mostly with abdominal obesity (Vayá et al. 2012). Thus, activation of PI3K and its downstream pathways phosphoinositide-dependent kinase (PDK)/Akt and eNOS improve hyperhomocysteinemia-induced vascular endothelium dysfunction (Sharma et al. 2013).

A positive association between homocysteine and long interspersed nucleotide 1 (LINE-1) methylation is found. During the DNA methylation reaction, universal methyl-donor SAM is demethylated to SAH, which is subsequently hydrolyzed to homocysteine. Under optimal conditions, homocysteine is re-methylated to methi-

onine, which is converted to SAM to provide the methyl group for subsequent reactions. A deficiency in methyl-donor micronutrients leads to an increase in homocysteine (Yi et al. 2000). Hyperhomocysteinemia also involves cellular immune activation. Existing positive correlation between homocysteine concentrations and the degree of immune activation is indicated by simultaneously increasing neopterin concentrations. Cellular immune activation and oxidative stress could be involved in the development of hyperhomocysteinemia (Widner et al. 2002). On the other hand, homocysteine concentrations not only correlate inversely with folate levels, but they also show a positive relationship with neopterin concentrations (Schroecksnadel et al. 2003). Thus, macrophages stimulated by IFN-gamma produce ROS, which oxidize antioxidants, lipoproteins and oxidation-sensitive B-vitamins. Thereby Th1-type immune response could contribute importantly to the development of hyperhomocysteinemia, and may also be a major determinant of disease progression (Schroecksnadel et al. 2004). Plasma homocysteine levels negatively correlate with liver and spleen apolipoprotein AI and positively correlate with IFN-gamma. SAM/SAH ratios are reduced in folate-deficiency (Mikael et al. 2013). Thus, in obese children folate-deficiency also leads to hyperhomocysteinemia. In these cases, folic acid supplementation shows a homocysteine lowering effect (Iamopas et al. 2014). However, serum folate concentration is significantly lower in obese patients with non-alcoholic fatty liver disease (NAFLD) than in those with normal liver. An inverse correlation has been found between serum folate concentration and body mass index of these patients, (Hirsch et al. 2005). Actually tetrahydrofolate is an essential cofactor for the conversion of homocysteine to methionine. In this respect, folate depletion causes the develop of hyperhomocysteinemia. Because the tetrahydrofolate is very susceptible to oxidation, under oxidative stress conditions oxidative degradation of tetrahydrofolates is increased. In this manner, folate deficiency may develop despite normal dietary intake (Widner et al. 2002). Fuchs et al. asserted that immunologically induced oxidative stress could lead to folate depletion resulting in hyperhomocysteinemia (Fuchs et al. 2001). Fasting baseline serum folate is lower in the obese group; in contrast, red blood cell folate is higher. Area-under-the-curve for the absorption phase and peak serum folate concentrations are lower in obese versus normal-weight women. Furthermore, overall serum folate response is lower in obese versus normal-weight women (da Silva et al. 2013; Tinker et al. 2012).

Macrophages show a twofold to threefold increase in expression of the inflammatory mediators, IL-1beta, IL-6, TNF-alpha and MCP-1 at the RNA and protein level under conditions of folate deficiency. Decrease in intracellular folate levels reduce growth rate of monocyte-macrophage lineage (Kolb and Petrie 2013). Hepatic oxidative stress has been associated with enhanced expression of NADPH oxidase in obesity. Folic acid confers protection during hyperhomocysteinemia-induced oxidative stress. Folic acid supplementation has a protective effect against high-fat diet induced hepatic oxidative stress and liver injury. Antioxidant effect of folic acid appears to be dependent on transcriptional regulation of NADPH oxidase (Sarna et al. 2012).

Furthermore, increased utilization of betaine for homocysteine re-methylation during folate deficiency may lead to steatosis by disrupting choline metabolism, because folate and choline metabolite betaine independently serve as methyl donors for homocysteine re-methylation to methionine (Christensen et al. 2010). On the other hand, dietary methionine restriction reduces adiposity but does so through a paradoxical increase in both energy intake and expenditure. The increase in energy expenditure fully compensates for increased energy intake and effectively limits fat deposition (Orgeron et al. 2014). The primary pathways affected by methionine restriction in white adipose tissue involve phagocyte and macrophage migration, and the majority of genes within these pathways are downregulated by methionine restriction (Wanders et al. 2014).

Tetrahydrofolate plays a crucial role in a number of reactions that generate methyl groups. These methyl groups are used for the re-

methylation of homocysteine thereby supporting S-adenosylmethionine (AdoMet) synthesis, S-adenosyl-L-homocysteine (AdoHcy) removal, and hence maintaining methylation capacity (da Silva et al. 2014). Fatty liver and changes in AdoMet and folate status are observed in obesity and metabolic syndrome (da Silva et al. 2014) (Fig. 22.1).

Conversely high doses of folic acid supplementation are capable of protecting endothelial cells through reducing levels of homocysteine and increasing BH4 and NO production. In this case no significant differences have been observed in neopterin levels (Zhang et al. 2014). Positive correlations are found between kynurenine/tryptophan and neopterin and homocysteine concentrations (Frick et al. 2004).

Protein and lipid oxidative damage increases while glutathione levels, the antioxidant byproduct of methionine metabolism, decrease via the transsulfuration pathway in obese diabetic patients (Valle et al. 2012). Glutathione synthesis is regulated by the rate limiting enzyme, gamma-glutamyl cysteine synthetase and its substrates L-cysteine (Griffith 1999). Cysteine is synthesized by transsulfuration from homocysteine, a product of the methionine (Brosnan and Brosnan 2006). Trans-sulfuration is regulated by stimulation of cystathionine beta-synthase and inhibition of methylene tetrahydrofolate reductase in response to changes in the level of S-adenosylmethionine, and this promotes homocysteine degradation when methionine availability is high. Thus, desulfuration reactions dominate when cysteine is deficient, whereas oxidative catabolism dominates when cysteine is in excess (Stipanuk and Ueki 2011). Cystathionine beta-synthase catalyzes the first irreversible step that commits homocysteine to trans-sulfuration and cysteine synthesis. S-adenosylmethionine is an allosteric activator of cystathionine beta-synthase (Jhee and Kruger 2005). The trans-sulfuration pathway displays a reciprocal sensitivity to pro- and antioxidants. The upstream half of the glutathione biosynthetic pathway leading to cysteine biosynthesis is redox sensitive while the downstream half is leading from cysteine to glutathione (Vitvitsky et al. 2003). A cross-sectional study using data from 1550 subjects recruited from nine European countries in the COMAC project demonstrated that plasma cysteine is a strong independent positive predictor of fat mass, body mass index and obesity (Elshorbagy et al. 2009). Unlike most plasma amino acids, cysteine levels do not decrease with gastric bypass-induced weight loss, further supporting the concept that elevated cysteine may be a cause, not a consequence of obesity (Elshorbagy et al. 2012).

6 Conclusion

Tryptophan depletion is a persistent metabolic disorder in morbidly obese patients despite different weight reduction strategies. Impaired satiety due to decrease in 5-HT production ultimately leads to increased caloric uptake and obesity. Folate status have been associated with changes in the expression of genes involved in lipid metabolism, obesity, and metabolic syndrome. This vicious cycle may be improved by folic acid supplementation.

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Eligibility and Success Criteria for Bariatric/Metabolic Surgery

23

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Abstract

Obesity is a worldwide main health concern, with a high treatment failure. This chapter focuses on the definition of obesity, based on excessive fat accumulation and thus underscores the importance of body composition, and the clinical tools currently used to diagnose it, mainly body mass index that is only a proxy measure of body composition. It also highlights the importance of the personal commitment to comply to a healthy diet and physical activity recommendations since surgery is most effective when accompanied by lifestyle modifications. Additionally, it addresses the description of types of patients who could benefit most from surgical management of excessive body fat percentage and metabolic derangements, as well as on the indications for surgery that are currently valid.

Keywords

Bariatric surgery • Metabolic surgery • Candidate • Indications • Comorbidities • Body composition • Adiposity • Fat-free mass • Detailed phenotyping

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1 Introduction

1.1 Obesity as a Worldwide Healthcare Concern

Obesity is a major public health challenge particularly in urban settings, and has negative clinical implications on almost every organ system (Bray et al. 2016; Frühbeck et al. 2013; Grieve et al. 2013). The World Health Organization (WHO) also offers some critical key facts:

- Worldwide obesity has more than doubled since 1980.
- In 2014, more than 1.9 billion adults, 18 years and older, were overweight. Among them, over 600 million were obese.
- 39% of adults aged 18 years and over were overweight in 2014, and 13% were obese.
- Most of the world's population lives in countries where overweight and obesity kills more people than underweight.
- 42 million children under the age of 5 years were overweight or obese in 2013.

Morbid obesity, in particular, is a rapidly growing segment of the obesity epidemic (Kral et al. 2012). The detrimental effects of obesity can be especially prevalent in these patients, taxing not only individuals, but health-care systems and society at large (Frühbeck et al. 2016).

In 1995 at the *"27th Bethesda Conference"* (1996) obesity was classified as a category II risk factor, which means that the proper management of obesity is likely to lower the incidence of cardiovascular events. Consistently, in 1997 the *American Heart Association* identified obesity as an independent risk factor (Eckel et al. 1998). It is noteworthy that there is a significantly increased risk of cardiovascular disease (CVD) independent of other traditional risk factors (age, sex, physical activity, smoking, blood pressure and cholesterol levels adjusted RR) for patients who fulfill body mass index (BMI) criteria of moderate overweight, and increases with the diagnosis of obesity (Prospective Studies Collaboration 2009). Additionally, BMI is directly associated with a linear increase in the risk of vascular events. Furthermore, overweight and obesity are major risk factors for type 2 diabetes (T2D), hypertension, and atherogenic dyslipidemia, among others. These diseases, when clustered, form the metabolic syndrome (MS) that is a condition with exponential risk for CVD as compared with its isolated components (Ärnlöv et al. 2010). The MS and its components belong to the group of non-communicable diseases (NCDs) that share the characteristic of being non-infectious or non-transmissible medical conditions (NCD Risk Factor Collaboration (NCD-RisC) 2016; Alleyne et al. 2013; Clark 2013). The WHO emphasizes that raised BMI is a major risk factor for NCDs such as CVD (mainly heart disease and stroke), which were the leading cause of death in 2012 (Ng et al. 2014). Furthermore, NCDs are expected to be even more prevalent in the upcoming decades. Obesity also seems to be associated with several other serious health problems, ranging from non-alcoholic fatty liver disease, osteoarthritis, obstructive sleep apnea (OSA) and other respiratory alterations to gastrointestinal disorders, renal problems, infertility and cancer (Acioglu et al. 2010; Acosta and Camilleri 2014; D'Agati et al. 2016; De Pergola and Silvestris 2013; Hassan et al. 2014; Jungheim et al. 2013; Wluka et al. 2013).

2 Concerns and Limitations of the Application of the Definition of Obesity

The WHO defines obesity as *"an abnormal or excessive fat accumulation that presents a risk to health"*. Despite being universally accepted, this definition is not universally applied, since the degree of fat accumulation is not routinely measured to diagnose obesity (Blundell et al. 2014). Tools to measure accurately the total amount of fat are not widely available. The BMI, based on a person's weight (in kilograms) divided by the square of his or her height (in meters), is the most common way to evaluate the degree of obesity. A person with a BMI between 25.0 and 29.9 kg/m^2 is considered overweight, while a person with a

BMI of 30.0 kg/m^2 or above is considered obese. Although useful and practical, the BMI does not include the evaluation of the body composition and, in the era of precision medicine, has mayor limitations for diagnosing obesity (Gómez-Ambrosi et al. 2011, 2012a). In fact, individuals with class I obesity (BMI 30.0 kg/m^2) may exhibit significantly increased total and intra-abdominal adipose depots that are similar to those of patients with 34.9 kg/m^2 or even morbid obesity (BMI 40.0–44.9 kg/m^2), which partly explains the pattern of comorbidities observed in these individuals (Gómez-Ambrosi et al. 2014). Despite this, it is worth mentioning that the BMI has also advantages; it is easy to measure and provides a better estimate of total body fat than body weight alone. In adults, BMI has shown to be in itself a strong predictor of overall mortality (Flegal et al. 2013, 2015). The progressive excess mortality above 30 kg/m^2 is mainly due to vascular disease. Some studies suggest that at 30–35 kg/m^2 median survival is reduced by 2–4 years, while at 40–45 kg/m^2 it is reduced by 8–10 years (Kitahara et al. 2014). This effect is comparable with smoking. Similar results have been reported in adolescents, where a BMI in the 50th to 74th percentile, within the accepted normal range during adolescence has been associated with increased cardiovascular and all-cause mortality during 40 years of follow-up (Twig et al. 2016).

3 Beneficial Effects of Bariatric and Metabolic Surgery

Strictly speaking, the term 'bariatric surgery' is applied to all surgical procedures that aim to reduce excess weight (Frühbeck 2015). Currently, the two procedures that are most frequently performed all around the world are sleeve gastrectomy (SG), and Roux-en-Y gastric bypass (RYGB). SG has gained popularity in recent years because of its low complication rate and good weight loss results (Angrisani et al. 2015). Almost 50% of all bariatric surgeries performed nowadays in the United States were sleeve gastrectomies, which makes it the most popular weight loss surgery according to the *American Society for Metabolic and Bariatric Surgery* (ASMBS). The RYGB has been considered the 'gold standard' of weight loss surgery, because of its good results in terms of weight loss and resolution of comorbidities.

The mechanisms of action of bariatric surgery are complex and involve multiple neuroendocrine signals that exert effects at the central nervous system as well as in peripheral organs (Batterham and Cummings 2016; Knop and Taylor 2013; Koshy et al. 2013). The type of bariatric procedure performed depends on the patient's characteristics and the surgeon's preferences. Long-term effects remain favorable after 3–5 years (Fig. 23.1) or even after 10 years of surgery with rates of remission of T2D, improvement of circulating levels of triglycerides and uric acid, together with diastolic blood pressure remaining significantly better (Sjöström 2013). Levels of HDL cholesterol and systolic blood pressure were only improved during the first 2 years after surgery, before returning to preoperative levels. These outcomes translate into the reduction in the risk of CVD and mortality that are the rationale for the prescription of metabolic surgery (Rubino 2013, 2016).

These findings highlight that the favorable modulation of comorbidities does not take place uniformly or gradually for all comorbidities (Frühbeck 2015). Three main pillars of action should be considered in relation to the temporal pattern of response elicited by surgery in ameliorating obesity and its comorbidities. These pillars encompass weight-loss-independent, weight-loss-dependent and adiposity-dependent effects. Most cardiovascular risk factors and mechanically-related comorbidities (such as respiratory alterations, orthopedic problems and gastro-esophageal reflux disease [GERD]) ameliorate slowly, and mainly in parallel with body weight and fat loss. By contrast, T2D seems to have a more complex pathophysiology (Batterham and Cummings 2016). On the one hand, after surgery, T2D tends to improve soon and rapidly, even before major reductions in body weight occur, which implicates mechanisms independent of weight loss that involve the modulation of intrinsic gut hormones via the

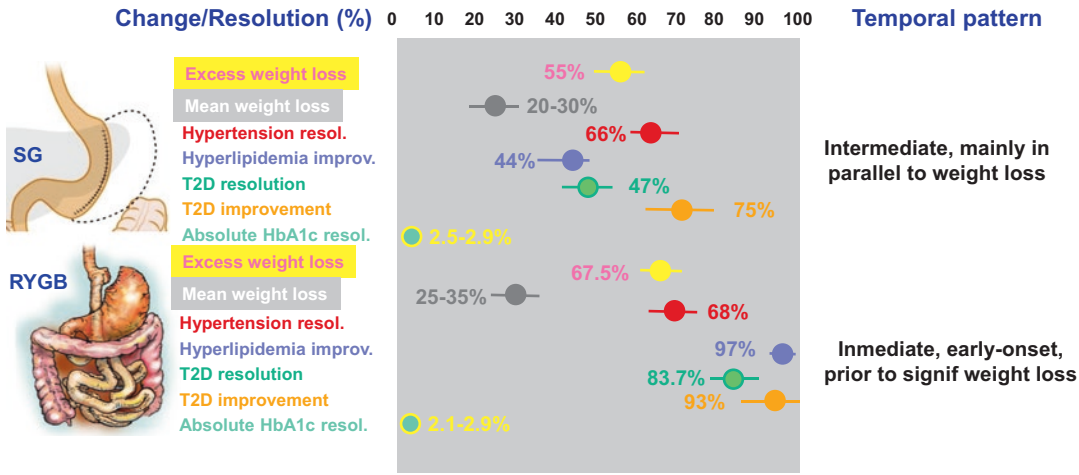

Fig. 23.1 Summary of the effects (expressed as mean data for efficacy and 95% confidence intervals) of sleeve gastrectomy (SG) and Roux-en-Y gastric bypass (RYGB) on weight loss (reported as excess and mean weight loss) and principal comorbidities (hypertension, hyperlipidemia and T2DM) at 3–5 years. The last column on the right indicates the temporal pattern of the effects on T2DM. *Modified from Frühbeck 2015*

gastro-entero-insular axis. Bariatric operations, especially those incorporating duodenal exclusion, can improve insulin sensitivity two to three-fold within days after surgery. On the other hand, gastric banding which does not induce changes in levels of incretins or gut hormones induces improvements in T2D directly related to weight loss.

3.1 Weight-Loss-Independent Effects

The immediateness of the favorable effect of surgery on T2D improvement shows that weight-loss-independent mechanisms in the control of glucose metabolism are occurring from the very beginning. The RYGB, a procedure that involves an anatomical-physiological rearrangement of the gastrointestinal tract, has a superior effect on T2D remission compared to the SG, a technique reducing the stomach size (Makaronidis and Batterham 2016). Furthermore, duodenal exclusion alone is reportedly sufficient to reduce fasting blood levels of glucose in patients with T2D independently of weight loss. Thus, signals originating in the proximal small intestine seem to exert a direct effect on the physiological regulation of glucose homeostasis. T2D usually starts to improve in the first few days after surgery, before any significant weight loss has occurred, which indicates that an important part of this effect depends on gut hormones and neuroendocrine changes that are initiated soon after surgery and independently of excess body fat (Frühbeck 2015). However, it is also true that the degree of weight loss is a predictor of whether a patient will experience sustained remission of T2D (Ribaric et al. 2014). Similarly, long-term weight gain is a determinant of T2D recurrence. Of note, T2D remission also depends on the severity of the pancreatic alteration at baseline and particularly the preservation of the capacity of beta cells to respond to a stimulation to produce more insulin. The severity of the pancreatic alteration depends mainly on the time the patient has suffered obesity, and genetic factors. At the same time, the amount and trajectory of weight loss, as well as remission of T2D, is hugely variable. These findings illustrate that some individuals are able to maintain T2D remission in the long-term even if they regain the weight lost (probably those in whom the gut-dependent effects are dominant), whereas in others, T2D recurrence takes place when weight regain occurs (patients in whom the gut-dependent effects disappear and

are lost with the potential refunctionalization of the gastrointestinal tract over time). This variability in both weight change and improvements in comorbidities probably relies on the potentially variable degree to which the different mechanisms of action operate in different individuals. These findings underscore the complexity and multiple factors that influence T2D remission or resolution and amelioration of insulin resistance.

3.2 Weight-Loss-Dependent Effects

The weight-loss-dependent effects of bariatric surgery are mainly evident in the psychosocial sphere and the mechanical comorbidities tightly related to the effects of excess body weight, such as GERD, OSA and other respiratory alterations as well as osteoarthritis and joint problems (Frühbeck 2015). Bariatric surgery considerably improves psychological and cognitive characteristics of individuals with symptoms that are mostly the result of increased body weight, that consequently improve with weight loss.

The prevalence of GERD in individuals with morbid obesity is as high as 45%, with obesity increasing the risk of GERD becoming symptomatic, progressing to erosive esophagitis and esophageal adenocarcinoma. Markers of visceral and general obesity are independent determinants of esophageal inflammation, which correlates with endoscopic findings and symptoms of GERD. Bariatric procedures as RYGB improve GERD symptoms because of the disconnection of most of the acid production portion of the stomach from the esophagus and the weight loss achieved. Thus, the greater the excess weight loss (EWL), the greater the improvement in the GERD score.

In the majority of individuals with morbid obesity, bariatric surgery also improves or resolves OSA. Factors that predispose patients to OSA include a small upper airway lumen, unstable respiratory control, a low arousal threshold, small lung volume and dysfunctional dilator muscles in the upper airway. In addition to the mechanical weight-dependent effects, the surgical approach also exerts a metabolic weight-independent effect that results in improved insulin resistance and systemic inflammation that decrease the cardiovascular risk. Concurrently, cardiorespiratory complications such as hypoxemia, pulmonary hypertension, hypercapnia, and even OSA-associated atrial fibrillation, might also improve.

Obesity has been identified as an independent and modifiable risk factor for osteoarthritis and joint problems (Wluka et al. 2013). Weight loss decreases the risk of incident knee osteoarthritis, ameliorates symptoms in established disease, improves function and reduces disease progression. These improvements are based on the biomechanical relationship between increased loads on articular cartilage, subsequent wear and cartilage breakdown. However, osteoarthritis also has a multifactorial etiology with its systemic effects being related to the adipokine profile and metainflammation.

3.3 Adiposity-Dependent Actions

During surgically-induced weight loss, it should not be taken for granted that adiposity will improve with total body weight loss (Frühbeck 2015). In fact, EWL, BMI and adiposity have quite diverse trajectories over time (Fig. 23.2). While a dramatic EWL takes place during the first few months (generally until the first 12–18 months after surgery), the decrease in BMI and body fat follows a less steep trajectory. Moreover, the decline in BMI is not paralleled by the decrease in adiposity. Particularly during the first month, the marked decrease in body weight corresponds to the fat-free mass (FFM), with a low effect on total adiposity. Of note, even if a BMI in the range of overweight or normal weight is reached, it does not necessarily mean that body fat has decreased to normal limits. BMI neither discriminates between fat mass and FFM, nor distinguishes between body shape and fat distribution. Thus, during surgery-induced weight loss, particular consideration should be given to the fat mass to FFM ratio to also value the contribution of the FFM to physiological functioning, pathology and well-being. In fact, left ventricular volume, stroke volume and cardiac output are

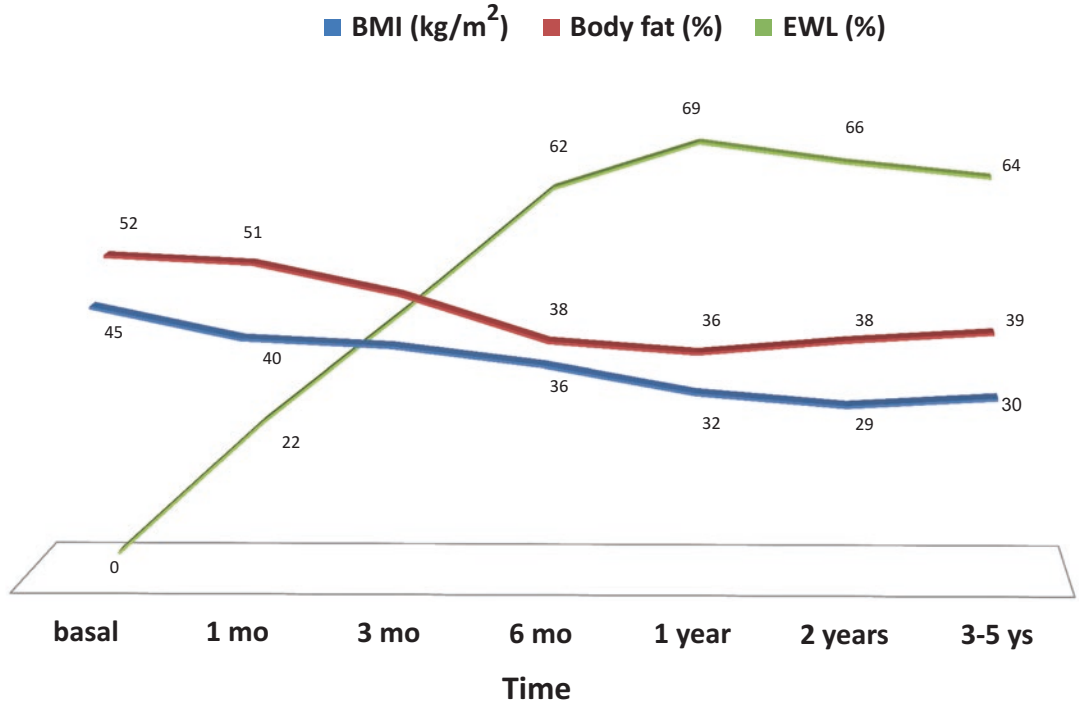

Fig. 23.2 Short, medium and long-term trajectories of excess weight loss (EWL), BMI and total adiposity following bariatric surgery. EWL, BMI and adiposity exhibit diverse trajectories over time. While a dramatic EWL takes place during the first few months after surgery, the decrease in BMI and body fat follows a less steep trajectory. The figure further illustrates that the rapid decline in BMI is not paralleled by the decrease in adiposity, in particular during the first month. Moreover, even if a BMI in the range of overweight or normal weight is reached after several years, body fat percentage does not necessarily decrease to normal limits (normal limits are 10–20% in men and 20–30% in women). *Modified from Frühbeck 2015*

primarily associated with FFM, whereas blood pressure, heart rate and variables reflecting cardiac dysfunction tend to be related to total body fat and visceral adiposity. Moreover, FFM can be used to predict the diameter of the carotid artery lumen, while visceral adiposity has emerged as the main determinant of premature carotid artery atherosclerosis.

As skeletal muscle is the body's largest glucose buffering system, a conserved large muscle mass promotes insulin sensitivity and protects against the metabolic syndrome. Moreover, FFM, but not fat mass or BMI, is positively associated with self-determined meal size and daily energy intake in humans (Blundell et al. 2012). Thus, a rapid weight loss mainly due to a reduction of FFM entails the subsequent loss of protective muscle strength and its metabolic benefits on the regulation of energy homeostasis.

In addition, in the long term, adiposity rebounds more easily after bariatric surgery than does BMI, even in patients who have maintained the weight loss. This feature is particularly important in relation to the adipose tissue secretion profile for proinflammatory and prothrombotic factors that will return to increased circulating concentrations, thereby reversing the beneficial effects attained via the decrease in adipokines after the surgery-induced reduction in adiposity.

Taken together, these findings may explain why some cardiovascular comorbidities re-emerge after surgery (such as hypertension and hypercholesterolemia) and the lack of a reduced incidence of cancer in male patients with obesity. Interestingly, the incidence of cancer and cancer-related deaths occur in a setting that provides a unique adipose tissue microenvironment with concomitant systemic endocrine alterations that favor

both tumor initiation and progression (Frühbeck 2015). Low-grade chronic inflammation, dysregulation of growth signaling pathways, hyperinsulinemia and hypoxia associated with increased adiposity are widely accepted as important factors that link obesity and cancer pathogenesis (Catalán et al. 2013; Pérez-Hernández et al. 2014). White adipose tissue constitutes a relevant source of growth factors, adipokines and stromal progenitor cells. Given that adipose tissue surrounds organs with a high predisposition to become malignant (for example, the prostate, the mammary gland or the colon), recruitment of stromal progenitor cells to the tumor microenvironment favors the formation of supportive tumor stroma. Excess adiposity modifies the expression profile of adipose tissue genes to ultimately foster growth of fat mass via an upregulation of genes related to cellular activity (increased cell proliferation or differentiation, cell cycle activation and inhibition of apoptosis) and mild immunoinflammatory processes (reduced immunosurveillance). Adipocytes and infiltrating immune cells secrete proinflammatory adipokines and cytokines, growth factors, metalloproteinases, and reactive oxygen species, which can induce DNA damage and chromosomal instability, thereby favoring carcinogenesis.

Conversely, growing tumor cells frequently extend beyond the primary organ in which they developed towards surrounding fat depots, thereby contributing to tumour progression and metastasis. Taken together, the rebounding of adipose tissue several years after undergoing bariatric surgery might explain the lack of reduced incidence of cancer in some patients.

3.4 Beyond the Semantics

The finding that the benefits of bariatric surgery extend well beyond weight loss provided the clinical rationale for the emergence of metabolic surgery, which is primarily intended to treat T2D and is offered to patients with obesity who have a lower BMI than those who are eligible for bariatric surgery (Rubino et al. 2014). This implies that a new model of care is being developed that is distinct from traditional bariatric surgery (Rubino et al. 2016). Although patients undergoing metabolic surgery tend to have a lower BMI than those undergoing bariatric surgery, body composition does not differ much between both patient groups. The two groups have similarly high total and visceral adiposity (Fig. 23.3), and thus associated cardiometabolic comorbidities, which blurs the strict BMI-centric cut-off limits (Frühbeck 2015).

Importantly, as aforementioned, bariatric surgery entails not only weight-reducing effects but also metabolic improvements. Thus, strictly using the terminology, the dichotomy between bariatric surgery for weight-reduction procedures and metabolic surgery for techniques aimed at improving T2D does not match the actual practicalities of clinical care and therapeutic effects. When offering surgery to treat metabolic diseases or T2D rather than simply as a weight-reduction therapy, clinicians need to consider that most of these patients have levels of dysfunctional adipose tissue similar to those of patients with morbid obesity (Fig. 23.3). Moreover, weight and fat loss is also generally reported among patients undergoing metabolic surgery, which makes it difficult, if not impossible, to determine the relative contribution of gastrointestinal alterations in anatomy and physiology from weight loss itself.

Interestingly, so far both surgical approaches differ in their stated therapeutic goals. Offering the same surgical interventions to treat diabetes mellitus and metabolic diseases as for weight-reduction purposes reportedly changes demographical and clinical characteristics of surgical candidates (Cefalu et al. 2016). In general, more men undergo metabolic surgery than bariatric surgery and patients who undergo metabolic surgery are usually older than those undergoing bariatric surgery. Although patients undergoing metabolic surgery have a lower BMI than patients undergoing bariatric surgery, they have more comorbidities, in particular, T2D (in terms of both frequency and severity, with increased HbA1c levels and percentage of insulin use), hypertension, dyslipidemia and other CVD (Moncada et al. 2016).

Similarly, patients with a BMI <35.0 kg/m^2 without relevant metabolic alterations (below the

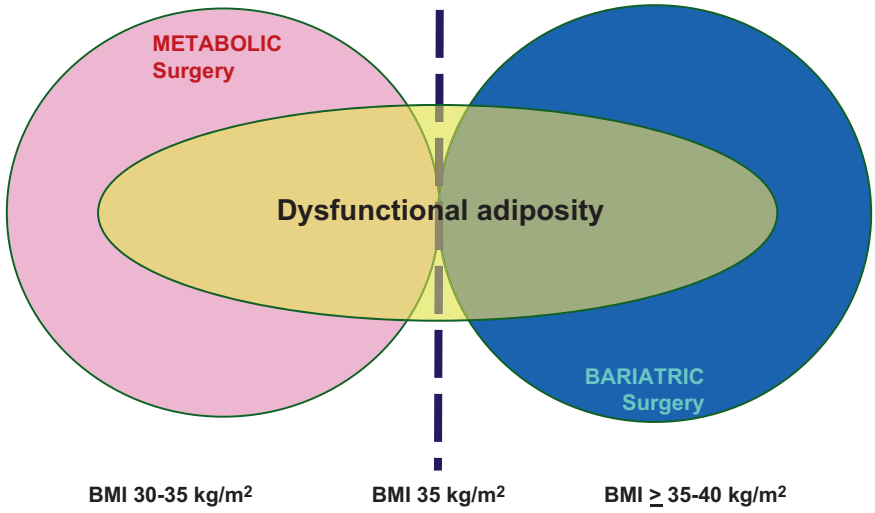

Fig. 23.3 The strict separation of bariatric and metabolic surgery based on current BMI eligibility criteria is blurred by the common feature of a high degree of dysfunctional adiposity. *Dashed lines* indicate the overlap between metabolic surgery and bariatric surgery. *Modified from Frühbeck 2015*

traditional cut-off point for bariatric surgery) can have osteoarthritis and joint problems, which can increase the difficulties of losing weight by conventional means. Therefore, bariatric surgery could be a useful and effective approach to alleviate the pain and functional impairment of these conditions also in patients with lower BMIs (Frühbeck 2015). In addition to their involvement in the mechanical, weight-dependent effects of bariatric surgery, adipokines also have a critical role in the pathophysiologic features of osteoarthritis. Thus, patients with orthopedic problems and who are not eligible for bariatric surgery would also benefit from the metabolic aspects of the surgical approach that are related to inflammation. These reflections should have relevant implications in clinical care to support the consideration of bariatric–metabolic surgery as an effective therapeutic tool for a wider spectrum of patients than traditionally thought. Thus, the strict application of semantics obviates the broader pathophysiological circumstances of the variety of existing patients, thereby limiting the selection of the candidates who would benefit equally from the multifactorial effects of the surgical approach.

4 Currently Accepted Indications and Contraindications for Bariatric and Metabolic Surgery

Indications for the surgical management of severe obesity were first outlined by the *National Institutes of Health (NIH) Consensus Development Panel* in 1991 and reviewed by the *American Bariatric Society* in 2004. European and International Societies have agreed on general guidelines for the indication of bariatric and metabolic surgery recently (De Luca et al. 2016; Fried et al. 2014). It should be noted that bariatric surgery programs encourage patients to participate in lifestyle changes prior to surgery to demonstrate their commitment.

4.1 Candidates for Bariatric Surgical Procedures

- Adults with a BMI ≥40 kg/m^2 regardless of comorbid illness.
- Adults with a BMI 35.0–39.9 kg/m^2 with at least one serious comorbidity, including:

- Type 2 diabetes (T2D)
- Hypertension
- Hyperlipidemia
- Obstructive sleep apnea (OSA), obesity-hypoventilation syndrome (OHS) or the combination of both.
- Nonalcoholic fatty liver disease (NAFLD) and/or nonalcoholic steatohepatitis (NASH)
- Pseudotumor cerebri
- Gastroesophageal reflux disease
- Asthma
- Venous stasis disease
- Severe urinary incontinence
- Debilitating arthritis
- Impaired quality of life
- Disqualification for other surgeries as a result of obesity (i.e., surgeries for osteoarthritic disease, ventral hernias, or stress incontinence)

4.2 Contraindications

The only major contraindication for bariatric surgery is the presence of an unacceptable risk-to-benefit balance, which is mostly due to a severe cardiac disease with life-threatening anesthetic risk as well as lack of understanding of the need of close follow-up. Importantly, there are other conditions that require a holistic approach to determine whether the expected benefits are worth in the clinical condition of the patient such as the following:

- Diagnosis of T1DM, unless surgery is indicated for other reasons, such as severe obesity.
- Untreated major depression or psychosis.
- Uncontrolled and untreated eating disorders.
- Current drug and alcohol abuse.
- Severe coagulopathy.
- Inability to comply with nutritional requirements including life-long vitamin and mineral replacement.

These possible conditions should be detected in the preoperative work out of the multidisciplinary team so that they may be improved and corrected before the elected procedure. Most of them could be corrected in order to identify the optimal moment for the surgery.

4.3 Candidates for Metabolic Surgery

Indications for metabolic surgery in the treatment algorithm for T2D have been recently updated by a Joint Statement by International Organizations (Rubino et al. 2016). Patient selection for metabolic surgery should be based on balancing surgical and other long-term risks with potential long-term benefits to individual patients, as with any operation.

This trade-off needs to take into account factors such as baseline CVD risk due to metabolic disease and hyperglycemia that does not adequately respond to non-surgical treatments, as well as conditions that could contraindicate any elective operation, as aforementioned. In addition, preoperative indicators other than BMI should be established to make patient selection for metabolic surgery diabetes relevant. There are no data showing that baseline BMI predicts metabolic surgery success. Instead, strong evidence indicates that preoperative BMI, at least within the obese range, does not predict the benefits of gastrointestinal surgery with regard to diabetes prevention, remission, relapse after initial remission, or the magnitude of its effects on CVD events, cancer, or death. Of note, evidence shows that the rate of remission of T2D is equivalent among patients with preoperative BMI $\geq$35 kg/m^2 compared to those with mean preoperative BMI <35 kg/m^2 (71% versus 72%, respectively) (Sjöholm et al. 2013). Overall, the surgical value seems to be more related to improved glucose homeostasis than weight loss per se. This observation could be due to the aforementioned limitation of BMI, since it does not take into account the BF%. Of note, even if a BMI in the range of overweight or normal weight is reached, it does not necessarily mean that body fat has decreased to normal limits. BMI neither discriminates between fat mass and FFM, nor distinguishes between body shape and

fat distribution. Accordingly, it has been reported that a high percentage of subjects classified as lean and as overweight according to BMI (up to 29% and 80%, respectively) could have BF% within the obesity range (Gómez-Ambrosi et al. 2011, 2012b). Furthermore, in the long term, adiposity rebounds more easily after bariatric surgery than does BMI, even in patients who have maintained the weight loss. Although baseline BMI per se does not predict outcomes in metabolic surgery, available evidence, including all existing RCTs, is based on studies that have included BMI ranges among their primary criteria for eligibility.

4.4 Defining Goals and Success of Bariatric and Metabolic Surgery

The classic concept of success of bariatric surgery, the loss of 50% of excess body weight, is a somewhat arbitrary metric. As opposed to weight loss, expressed as kg or pounds depending on the countries or as a percent of baseline weight, EWL was introduced to complement the use of actual weight loss as it provides an improved estimate of the amount of weight loss that has been achieved relative to a defined goal level (Frühbeck 2015). A drawback to using EWL is that the more obese a patient is, the less likely they are to achieve a 100% reduction independently of how 'normal weight' is defined. Moreover, since one of the main targets of any weight loss program is loss of excess fat, the amount of body fat should be determined directly. In this line, work towards a definition of success that examines pathophysiological components of unhealthy adiposity associated with all-cause mortality would be more useful, as would the potential addition of factors that might even identify metabolic frailty or subclinical disease (Frühbeck 2015). These components include common carotid artery intima–media thickness, coronary artery calcium, markers of endothelial dysfunction, inflammation and fatty liver. Functional tests such as brachial artery macrovascular flow-mediated vasodilation and microvascular reactive hyperemia might provide more dynamic and clinically relevant information. Relying only on BMI misses a precise representation of the metabolic profile and can disguise an increased risk of CVD. Going a step further, the role of the diverse procedures relative to rigorously defined end points of heart failure, atherothrombotic state, hepatic, pancreatic and renal function should be assessed, with its systematic application aiding inter-study comparisons.

Current trends move to a less steady concept of success that takes into account the improvement in T2D, OSA, GERD, and resolution of other comorbidities. T2D for example, describes a continuum of hyperglycemic states associated with an increased morbidity and mortality. Currently, the most effective strategy to control refractory T2D and thus, even in the case of relapse, temporary normalization of glycemic control or major long-term improvement of glycemia without remission confers benefits for patients with T2D. In some cases, even just halting the progressive trend towards an increase in weight and adiposity, together with its often subsequent clustering of mechanical, metabolic and psychosocial comorbidities, should be considered a success. Signals that optimize the regulation of stress should be positively evaluated. The profound changes that translate into improved mood and self-esteem, as well as the marked reduction in hedonic thoughts and psychosocial circumstances, might also be considered a success in some patients (Frühbeck 2015). The mere reduction of metabolic neuroendocrine signals that optimize the regulation of stress should be also positively evaluated. In addition, obesity is a known risk factor for infertility in women (Edison et al. 2016; Sharma et al. 2015). Thus, improving the reproductive health of women should be also considered a success (nearly 50% of patients undergoing bariatric surgery are women of reproductive age, with fertility in obese women improving after these surgical interventions (Chor et al. 2015). Health-related quality of life and health-care costs are also important and merit equal consideration.

4.5 Eligibility and Success Criteria

A main goal that defines success after surgery is the remission of T2D. Due to its relevance it is critical to try to predict whether a patient is likely or not to succeed under this point of view. A preoperative tool that describes the probability of diabetes remission after RYGB, namely the DiaRem score (Table 23.1), has been described (Still et al. 2014). The DiaRem score stratifies the risk based on a weighted system of punctuation (range 0–22) that stratifies subjects into five groups based on scoring for age, glycated hemoglobin and the medication used: a score of 0–2 has the highest likelihood of T2D-remission followed by the subsequent groups (3–7, 8–12, 13–17, 18–22) with a decreasing probability of remission of T2D.

Eligibility for bariatric/metabolic surgery should be based on a more dynamic, comprehensive and functional evaluation of the patient's current global health and on a more reliable prediction of future morbidity and mortality (Frühbeck 2015). A clear dissociation exists between the wealth of scientific advances and the incorporation of this information into the clinical practice. This disconnect derives in missed opportunities to understand, diagnose and treat obesity with increased precision, and to make better use of knowledge and resources to inform health-care decisions. A mentality change in this field is urgently needed. The validation and application of algorithms or scoring systems that quantify the actual and future health burden induced by obesity in the individual patient represents the way ahead where the development of tools for precise phenotyping beyond BMI as well as for guiding therapeutic approaches should be demanded. The Edmonton Obesity Staging System (EOSS) is a 5-point ordinal classification (stage 0–4), that considers comorbidity and functional status. It assesses obesity drivers, complications and barriers using a framework that addresses the mental, mechanical, metabolic and monetary aspects of obesity development and weight management to determine how healthy a patient is. The highest EOSS scores identify individuals with obesity who have the greatest mortality risk, since EOSS is reportedly a better predictor of mortality than BMI grading. It is debatable though whether these patients are the ones who will benefit the most from the surgical approach. A high EOSS staging score suggests the patient will have an increased anaesthetic risk. Moreover, increased severity of some comorbidities related to high EOSS scores might result in a reduced likelihood of improvement once end-organ damage or established end-stage disabilities have been reached. In this context, prioritization of patients with high rather than low EOSS scores might mean that an opportunity to use the surgical approach as a measure in patients who could really attain full recovery is missed. Currently, it is also challenging to identify which patients will indeed progress to higher EOSS stages and which patients will remain stable. Thus, although the EOSS has certain advantages, it is not a completely objective system and it does not discriminate between patients who have different number of comorbidities (e.g. a patient with one comorbid condition is staged at the same level as a patient with three, four or five comorbidities, when the pathophysiological cir-

Table 23.1 DiaRem score

Prediction factor	Score
Age (years)	
If age <40	0 points
If age 40–49	1 point
If age 50–59	2 points
If age >60	3 points
HbA1c (%)	
If HbA1c <6.5	0 points
If HbA1c 6.5–6.9	2 points
If HbA1c 7.0–8.9	4 points
If HbA1c >9.0	6 points
Other diabetes medications	
If not using sulfonylureas or not using insulin-sensitizing agents	0 points
If on sulfonylureas and insulin-sensitizing agents	3 points
Treatment with insulin	
If not using insulin	0 points
If using insulin	10 points
DiaRem score → (sum of individual components)	

Taken and modified from Still et al. (2014)

cumstances are certainly remarkably different). Although the EOSS might be a useful tool to redefine indications for bariatric or metabolic surgery in patients with severe obesity, further research is needed to determine its cost-effectiveness, optimal ways of incorporation into clinical practice and improved quantification of objectivity. In addition, other relevant aspects in the development of comorbidities, such as body composition and fat distribution, should be considered.

5 Conclusion

Although BMI is universally accepted to indicate bariatric surgery, the observation that its benefits go beyond the loss of excess weight implies that BMI is not an optimal criterion for the selection of candidates. It is also remarkable that BMI does not predict the metabolic effectiveness of the intervention. The severity of the pancreatic alteration at baseline and the preservation of the capacity of islets to respond to a stimulation to produce more insulin should be specifically studied. As mentioned above, it is necessary to delineate meaningful definitions of goals and successful treatment not only circumscribed to weight loss but also to treat diseases associated with obesity or merely metabolic alterations. A standardized evaluation of comorbidities and outcomes, including a uniform acceptance of the definition and terminology of each disease state and specific biomarkers to be used, should be universally applied for surgical referral and follow-up. Improvement in the accuracy of prognoses and the identification of at-risk individuals who could benefit from early intervention should be pursued. A more aggressive management of patients in risk groups could be attained with regular screening. Orienting our goals away from a 'weight-centric' approach towards a more functional holistic approach with extensive evaluation of comorbidities is required. More robust, proactive approaches are urgently needed. Detailed phenotyping of key features (such as body composition, fat distribution and immunometabolism, hunger and satiety, energy homeostasis, metabolic flexibility, sleep patterns, stress factors and environmental clues) would yield much more personalized approaches than focusing on BMI. Clarifying the long-term effects of the surgical interventions on these features should be pursued.

A composite measure that includes all relevant outcomes would be ideal. However, with so many inter-related variables, developing the optimal composite is not easy. Realistic expectations should be agreed on an individual basis with the patient, and success evaluated in a personalized frame of reference. This means that the definitive definition of success should be customized for every single patient in response to the individual balance between benefit and risk. Consequently, the definition of success after bariatric/metabolic surgery should be tailored to the individual characteristics and circumstances of each patient and should be discussed with the patient before the surgery is performed. Table 23.2 summarizes the key points of the chapter.

Table 23.2 Key points of the chapter

Bariatric surgery has a proven role in achieving sustained weight loss, improving obesity-related comorbidities and reducing mortality.
Bariatric surgery is considered to address mainly weight loss, whereas metabolic surgery focuses mainly on improving type 2 diabetes mellitus.
Bariatric and metabolic surgery cannot be viewed as dichotomic procedures, as most of the clinical benefits of both approaches have a multifactorial origin derived from a combination of effects.
Detailed patient phenotyping shows that the BMI cut-off points for determining eligibility for surgery are blurred when considering total adiposity and fat distribution, as BMI often does not tally with these factors.
A more functional, individualized and holistic approach with extensive evaluation of comorbidities will yield improved patient selection that does not have a merely 'weight-centric' focus.
The separation of bariatric and metabolic surgery based on current BMI eligibility criteria should be revised.
Success after bariatric surgery depends on previously defined goals, which should be tailored for each patient before the intervention.

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Does Bariatric Surgery Improve Obesity Associated Comorbid Conditions

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Atilla Engin

Abstract

Obesity is a constantly growing health problem which reduces quality of life and life expectancy. Bariatric surgery for obesity is taken into account when all other conservative treatment modalities have failed. Comparison of the multidisciplinary programs with bariatric surgery regarding to weight loss showed that substantial and durable weight reduction have been achieved only with bariatric surgical treatments. However, the benefits of weight loss following bariatric procedures are still debated regarding the pro-inflammatory and metabolic profile of obesity.

Keywords

Obesity • Bariatric surgery • Weight loss • Sleeve gastrectomy • Roux-en-Y gastric bypass • Insulin resistance • Anemia • Homocysteine • Leptin • Oxidative stress • Total cysteine (tCys) • Glutathione • Endothelial nitric oxide synthase (eNOS) • Inducible nitric oxide synthase (iNOS) • Resistin • C-reactive protein • Adiponectin • Interleukin 6 (IL-6) • Ghrelin • Triglycerides • Lysyl oxidase (LOX) • Visfatin • Apelin • Vaspin • Retinol-binding protein-4 (RBP4)

1 Introduction

Available treatments of obesity range from diet, exercise, behavioral modification, and pharmacotherapy to surgery, with varying risks and efficacy. Nonsurgical modalities achieve only relatively short-term and limited weight loss in most patients. However surgical therapy is the most effective option in terms of extent and duration of weight reduction with acceptable operative risks (Mun et al. 2001). Since it is claimed that bariatric operations are associated with the improvements in certain comorbidities linked to obesity, these surgical interventions have become increasingly popular in recent years due to growing rates of morbid obesity and metabolic

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syndrome (Andreelli et al. 2009). A total of 5386 references from 17 electronic databases analyzed by Picot et al. revealed that bariatric surgery is both clinically and economically effective intervention for obese people compared with non-surgical treatment (Picot et al. 2009). Indeed, surgery appears to be more effective than conservative modalities for weight loss and control of some comorbid conditions in patients with a body mass index of 40 kg/m^2 or greater (Maggard et al. 2005). But there are some contradictions for bariatric surgery such as poor myocardial reserve, significant chronic obstructive airways disease or respiratory dysfunction, in addition to the non-compliance of medical treatment or psychological disorders of a significant degree (Colquitt et al. 2009). Based on findings of 14 clinical studies, surgeons working in higher volume centers have lower mortality and complication rates in weight loss surgery (Padwal et al. 2011). Thus, the overall in-hospital mortality rate is 0.15% (5 of 3692 patients) but no significant difference has been found between the 3401 patients with body mass index less than 60 kg/m^2, and 291 super-super obese patients with body mass index more than 60 kg/m^2, regarding the post-operative mortality rate of 0.12% and 0.34%, respectively (Stephens et al. 2008).

While bariatric surgery rates increased significantly between the years 2002 to 2008, the rates did not change between the years 2009 to 2012 (Buchwald and Oien 2013; Johnson et al. 2016a). However, during the time period of 2002–2012, average obesity and diabetes rates steadily increased from 30.7% to 39.2% and from 8.0% to 12.7%, respectively. Although obesity and diabetes rates continuously increased, bariatric surgery rates did not correspondingly increase (Johnson et al. 2016a). Even so, the mean surgery rate per 100,000 population increased from 45.31 in 2002 to 67.95 in 2012 (Johnson et al. 2016b). In detail, the proportion of vertical sleeve gastrectomies increased from 3.0% to 54% from 2008 to 2014 whereas Roux en Y gastric bypass decreased from 52% in 2008 to 32% by 2014 (Abraham et al. 2016). Thus, in 2012, there was a sharp reduction in the number of gastric bypass and gastric banding operations and they were replaced by an increase in the number of sleeve gastrectomy operations (Nguyen et al. 2016). In this context, laparoscopic sleeve gastrectomy is the most commonly performed bariatric procedure. Utilization of laparoscopic sleeve gastrectomy increased from 23.7% of all bariatric procedures during the fourth quarter of 2011 to 60.7% during the second quarter of 2014 while laparoscopic gastric bypass decreased from 62.2% to 37.0%, respectively. Utilization of laparoscopic sleeve gastrectomy surpassed that of laparoscopic gastric bypass in the second quarter of 2013 (50.6% versus 45.8%) (Esteban Varela and Nguyen 2015).

According to a retrospective cohort study, using data from the United Kingdom Clinical Practice Research Data-link, in 3882 patients with an average body mass index of 44.7 kg/m^2, dramatic reductions in weight and body mass index are sustained over a 4-year period. This weight loss is accompanied by substantial improvements in pre-existing type-2 diabetes and hypertension as well as a reduced risk of incident type-2 diabetes, hypertension, angina, myocardial infarct, and obstructive sleep apnea (Douglas et al. 2015). Actually, quality of life improvements occurs within the first 2 years following bariatric surgery (Hachem and Brennan 2016). Resolution or improvement ratio of type 2 diabetes in meta-analytic means is 86.0%. Additionally, meta-analysis and weighted means analysis revealed that hyperlipidemia, hypercholesterolemia, hypertriglyceridemia and hypertension is improved significantly by all type of bariatric procedures (Health Quality Ontario 2005). Despite the negative association between obesity and the cardiovascular disease risk factors, surprisingly, obese patients with established cardiovascular disease might have better short- and long-term prognosis, suggesting an “obesity paradox” (Artham et al. 2009; Jousilahti et al. 1996). Hence, the prognosis of obese patients with coronary heart disease is generally equal to or even superior to that in leaner coronary heart disease patients (Todd Miller et al. 2008). Bariatric surgery significantly improves hepatic insulin sensitivity index and decreases hepatic triglyceride, total cholesterol, and fatty acyl-CoA content,

without an improvement in peripheral insulin sensitivity. The increased insulin sensitivity after Roux-en-Y gastric bypass occurs earlier in the liver than in the muscle and afterwards both may contribute to long-term remission of type 2 diabetes (He et al. 2014). The preoperative glucose disposal rate predicts the acute effect of bariatric surgery on peripheral insulin sensitivity. Obese individuals who are the insulin resistant before surgery improve postoperatively, whereas those with preoperative insulin sensitivity are worsened after bariatric surgery (Dunn et al. 2012). Although, homeostatic model assessment-insulin resistance (HOMA-IR) has been used in many studies for the metabolic effects of bariatric surgery, weight loss is a critical component for improvement of glucose homeostasis in the morbidly obese with insulin resistance (Campos et al. 2010). Additionally, obesity surgery has generated some controversies with reported benefits in terms of inflammation control (Compher and Badellino 2008). In this chapter, the effects of updated bariatric procedures on the pathophysiological consequences of lipotoxicity will be discussed.

2 Effect of Bariatric Surgery in Obesity-Related Comorbidities

Bariatric surgery is performed by using two major types of techniques: Purely restrictive and malabsorption-based procedures. Purely restrictive methods intend to limit the volume of food that can be ingested, and involved two different surgical techniques. The first; reversible and adjustable gastric bands are used to reduce the volume of the upper part of the stomach but it is obsolete. The second is a newly developed sleeve gastrectomy technique which involves resection of the greater curvature of the stomach only (Andreelli et al. 2009). Alternatively, two different malabsorption-based methods are currently used; Roux-en-Y gastric bypass and biliopancreatic diversion techniques. The principle of gastric bypass is to create a small and isolated gastric pouch which is then anastomosed to the upper jejunum bypassing the rest of the stomach duodenum and proximal jejunum (Andreelli et al. 2009). Biliopancreatic diversion consists of a distal gastrectomy with a long Roux-en-Y reconstruction, where the entero-enterostomy is placed 50 cm proximal to the ileocecal valve (Scopinaro et al. 2005). A total of 13,273 patients underwent bariatric surgery between 1980 and 2006, have been compared with 132,730 subjects from the general population. Of the bariatric surgery cohort, patients had undergone primary gastric bypass surgery, restrictive surgery and another procedure, 31.4%, 59.2% and 9.4%, respectively. Restrictive surgery has been converted to a gastric bypass during follow-up in 12.7% of patients. In patients who had undergone gastric bypass, however, the risk of myocardial infarction and diabetes was similar to that of the reference cohort. Despite this, the risk of death after bariatric surgery remained higher than in the general population (Plecka Östlund et al. 2011). Taking into consideration of confounding factors in morbid obesity, predominantly malabsorptive approaches is more effective than their restrictive alternatives (Muscelli et al. 2005; Rubino et al. 2004). In this respect, the most recently performed surgical procedures are sleeve gastrectomy, Roux-en-Y gastric bypass and biliopancreatic diversion with duodenal switch. Thus, for more than 14-years follow-up revealed that Roux-en-Y gastric bypass achieves a permanent and significant weight loss in more than 90% of patients (Mun et al. 2001). However, increased body mass index is associated with greater incidence and severity of obesity-related comorbidities and inadequate post-bariatric surgery weight loss. In comparison to Roux-en-Y gastric bypass, duodenal switch provides superior resolution of diabetes, hypertension, and dyslipidemia in three hundred fifty super-obese patients with body mass index more than 50 kg/m^2, independent of weight loss (Prachand et al. 2010). Overall, bariatric surgery reduces body mass index significantly. In this context subcutaneous fat, visceral abdominal fat and epicardial fat volume decrease by 32%, 44%, and 28%, respectively, whereas no significant change occurres in myocardial triglyceride content after

bariatric surgery (Gaborit et al. 2012). Furthermore, surgical-induced weight loss leads to a larger decrease in paracardial than epicardial ectopic fat deposition (van Schinkel et al. 2014). Ectopic fat mobilization, particularly the absolute loss of visceral adipose tissue, may play a major role in type 2 diabetes resolution following biliopancreatic diversion with duodenal switch surgery (Auclair et al. 2016). Pancreatic fat drastically decreases after the bariatric surgery. This suggests that decreased pancreatic fat contributes to improved beta cell function and type 2 diabetes resolution after the bariatric surgery (Gaborit et al. 2015).

A high prevalence of nutritional deficiencies is found amongst bariatric surgery candidates suffering from morbid obesity. The prevalence of pre-operative nutritional deficiencies is 35% for iron, 24% for folic acid, 24% for ferritin, 3.6% for vitamin B12, 2% for phosphorous, and 0.9% for calcium. Additionally, hemoglobin and mean corpuscular volume of these patients is measured 19% lower as compared to the healthy one. Moreover, high levels of parathyroid hormone are found among 39% of these patients (Schweiger et al. 2010). Anemia is present in 6.5% of obese subjects prior to the surgery and increases to 33.5% at 36 months of bariatric surgery. The levels of total cholesterol, low-density lipoprotein cholesterol, triglycerides, and glycemia are reduced, while high-density lipoprotein cholesterol is increased (Blume et al. 2012). In fact, prevalence of absolute- and functional-iron deficiency is 8.7% and 52.5%, respectively among 947 consecutive bariatric surgery candidates. Anemia is found in 11.2% of the cohort, 80% of which are associated with iron deficiency. Among patients with functional-iron deficiency, less than 20% transferrin saturation is common (Careaga et al. 2015). After bariatric surgery, anemia is detected in 17%, low ferritin in 15%, low vitamin B12 in 11%, and low red blood cell folate in 12% of patients (Toh et al. 2009). Retrospective analyzes of 5909 patients receiving Roux-en-Y gastric bypass revealed that anemia is 12.2% at baseline, which, respectively, increased to 20.9% and 25.9% at 12 and 24-months follow-up. Although the serum iron level did not change substantially after surgery, the frequency of patients with ferritin deficiency increased from 7.9% at baseline to 13.4% and 23.0% at 12 and 24 months, respectively. Vitamin B12 deficiency increased from 2.3% at baseline to 6.5% at 12 months after surgery (Weng et al. 2015). Hemoglobin concentration is not correlated with vitamin B12 or folate concentrations but it is related to iron status (Coupaye et al. 2009). Iron deficiency develops after gastric bypass for several reasons including intolerance to red meat, diminished gastric acid secretion, and exclusion of the duodenum from the alimentary tract. Bariatric surgery patients require lifelong follow-up of hematological parameters and iron levels since iron deficiency and anemia may develop many years after surgery (Love and Billett 2008). Among 431 patients who underwent Roux-en-Y gastric bypass and were followed for 10-years, 27% had anemia and related deficiencies; iron, folic acid, and vitamin B12 were seen in 20%, 12%, and 2%, respectively (Karefylakis et al. 2015). Actually, absolute iron deficiency is more common in patients undergoing bariatric surgery. In these patients, preoperative anemia may cause the increase of overall length of hospital stay (Khanbhai et al. 2015). High-fat diet leads to systemic iron deficiency which is traced back to reduced duodenal iron absorption. The mRNA and protein expressions of the duodenal iron transporters, divalent metal transporter 1 and transferrin receptor protein 1 (CD71) are significantly higher in high-fat diet-animals. In this case iron deficiency is the consequence of diminished intestinal iron uptake (Sonnweber et al. 2012). In Roux-en-Y gastric bypass, the mechanisms that result in iron deficiency are mainly related to a lack of gastric acidity and the exclusion of the duodenum and a part of the jejunum (Love and Billett 2008). Actually, duodenal cytochrome b is highly expressed in duodenal brush-border membrane and is implicated in dietary iron absorption by reducing dietary ferric iron to the ferrous form for transport via natural resistance-associated macrophage protein-2 (Nramp2)/divalent-cation transporter 1 (DCT1)/divalent metal-transporter 1 (DMT1) (McKie et al. 2002). In patients undergoing Roux-en-Y gastric bypass, DMT1 expres-

sion of enterocytes locate in the proximal jejunum. Amount of these cells increases 6 months after surgery, whereas quantity of the receptor in the same area decreases (Marambio et al. 2014). This mechanism maintains iron reserve at normal levels despite all the side effects of the Roux-en-Y gastric bypass. Iron absorption from both a standard diet and from a standard dose of ferrous ascorbate decreases significantly after 6 months of bariatric surgery to 32.7% and 40.3% of their initial values, respectively (Ruz et al. 2009). Most of the morbidly obese patients undergoing malabsorptive procedures will develop some nutritional deficiency. Nutrient deficiency is proportional to the length of absorptive area and to the percentage of weight loss (Alvarez-Leite 2004; Bloomberg et al. 2005). Actually, in morbidly obese patients, bacterial overgrowth prevalence is higher than in healthy subjects and is associated with more frequent severe hepatic steatosis (Sabaté et al. 2008). Thus, more than one quarter of morbidly obese patients undergoing bariatric surgery have advanced liver disease including 7% steatohepatitis, 16% fibrosis, and 4% cirrhosis (Kroh et al. 2007). Trace elements, essential minerals, water-soluble and fat-soluble vitamins deficiencies are common in obese individuals and often persist after bariatric surgery, despite multivitamin and mineral supplements. Thereby small intestinal bacterial overgrowth also promotes micronutrient deficiencies after Roux-en-Y gastric bypass (Bal et al. 2012). Thus, abdominal symptoms are common after bariatric surgery, almost 81% of these individuals have upper gut bacterial overgrowth and glucose malabsorption (Andalib et al. 2015). Eighteen percent of 151 patients underwent to Roux-en-Y gastric bypass meet the criteria for thiamine deficiency. In these patients an abnormal glucose-hydrogen breath test has been observed supporting the presence of small intestinal bacterial overgrowth (Shah et al. 2013). The normal stomach is virtually sterile but frequent colonization of aerobes, anaerobes and fungi in the used very small proximal pouch and unused large bypassed gastric chambers are detected. In clinical evaluation, gastric pH, as well as bacterial count is higher in the functioning proximal stomach and breath test is positive in 40.5% of the postoperative patients (Ishida et al. 2007).

Increase in serum heme levels is around 100-fold compared to their respective baseline levels following surgery. The increase in heme and biliverdin following surgery represents an increase in heme oxidation by heme oxygenase leading to greater antioxidant protection and insulin sensitivity (Sarosiek et al. 2016). The heme oxygenase system reduces inflammation through the suppression of macrophage-infiltration and abrogation of oxidative/inflammatory transcription factors like nuclear factor-kappa B (NF-kappaB), c-Jun N-terminal kinase (JNK), and activating protein-1 (Ndisang 2010). Before bariatric surgery, obese patients display approximately 50% higher serum protein carbonyl groups concentration and increased serum homocysteine levels than healthy individuals. After surgery, serum homocysteine does not change in contrast to protein carbonyl groups. However higher body mass index, HOMA-IR, serum leptin, triacylglycerols, low density lipoprotein/high density lipoprotein (LDL/HLD) cholesterol ratio, insulin, and glucose concentrations decrease after bariatric surgery (Sledzinski et al. 2009). This suggests that serum homocysteine concentration is not directly associated with oxidative stress in obese patients following bariatric surgery (Sledzinski et al. 2009). In particular, hyperhomocysteinemia has been described as an important vascular risk factor after bariatric surgery, however a high dose of folate supplement prevents hyperhomocysteinemia (Ledoux et al. 2011). Roux-en-Y gastric bypass and caloric restriction is sufficient to significantly reduce elevated oxidative/nitrative stress and genomic damage in obese animals (Bankoglu et al. 2016). Obese patients have approximately 17% higher mean serum total cysteine (tCys), and more than twofold higher glutamate concentrations. Gastric bypass patients have no change in tCys concentrations, while duodenal switch patients show a significant decrease in tCys. Total homocysteine concentrations increase in duodenal switch patients but not in gastric bypass patients. Independent of surgery type, serum concentrations of methionine and cystathionine decrease, while serum glutathione

and taurine remain stable. Eventually, despite 30% weight loss, and decreases in methionine, cystathionine and glutamate, there is no significant change in serum tCys in patients after gastric bypass surgery. The decrease in tCys in patients undergoing duodenal switch could be related to malabsorption (Aasheim et al. 2011). In contrast, a greater potential availability of substrates for glutathione production is observed after bariatric surgery. Glutathione is a tripeptide comprised of glutamate, cysteine, and glycine. These amino acids along with the recycling intermediates Cys-Gly and 5-oxoproline are increased in all groups following surgery. Furthermore, the mixed heterodimer cysteine-glutathione and glutathione homodimer, oxidized glutathione, GSSG, are elevated following surgery. Weight-loss surgery shifts glucose usage away from glycolytic pyruvate production to the pentose phosphate pathway. Reduced glutathione is regenerated via reduced nicotinamide adenine dinucleotide phosphate (NADPH) (Sarosiek et al. 2016).

Improvement of insulin resistance, lipidemia, and blood pressure as well as reduction of systemic inflammation after bariatric surgery are associated with the increase of serum nitric oxide (NO) concentration (Sledzinski et al. 2010). Although, at baseline, NO production of the obese patients is not different from that of the healthy control subjects, after bariatric surgery with a significant reduction in body mass index, NO production is decreased compared to the healthy controls. This apparent paradoxical observation may be explained by an overexpression of inducible nitric oxide synthase (iNOS) in the obese patients instead of endothelial nitric oxide synthase (eNOS), and after weight-reducing surgery, most probably iNOS is down-regulated to reverse the overproduction of NO (Lin et al. 2007). Actually, Noronha et al. showed that obesity is characterized by overexpression of iNOS in the vascular wall. Consistent with increased production of NO in obese subjects, plasma nitrite is significantly higher. In contrast to eNOS, iNOS mRNA is also significantly higher in aortas of obese mice. Because of the impairment of NO-mediated vasorelaxation, increased generation of the reactive oxygen species (ROS) may act as an endothelial derived hyperpolarizing factor. This finding is supported with the evidence of ROS-mediated vasodilation which prevents the emergence of endothelial dysfunction in iNOS-deficient obese mice (Noronha et al. 2005). Although surgical intervention does not lead to any difference in mRNA expression of NADPH oxidase, paraoxonase, superoxide dismutase 2, glutathione peroxidase and catalase, higher nitrite/nitrate availability is observed compared to preoperative NO levels. Inhibition of inflammation and enhanced availability of NO after bariatric surgery is a beneficial effect of weight loss (Blum et al. 2015). Thus, 6 months after bariatric surgery, serum NO concentration is approximately 40% higher than that of the pre-operative values. Surprisingly, serum NO concentration in non-obese controls is essentially similar to that of obese patients before surgery. In contrast, serum L-arginine concentration is higher in obese patients than in controls and decrease significantly after surgery. The increase in serum NO concentration contributes to diverse beneficial effects of weight loss after bariatric surgery especially in the context of risk of atherosclerosis (Sledzinski et al. 2010). After 1 year of a multidisciplinary program of weight reduction in all obese women weight loss is at least 10% of their original weight. Compared with baseline, sustained weight loss is associated with reduction of cytokine and adhesin concentrations and with improvement of vascular responses to L-arginine (Ziccardi et al. 2002). Loss of weight due to a short-term, intensive diet and exercise may improve the lipid and metabolic profile, decrease oxidative stress and increase NO production and decrease endothelial cell activation (Roberts et al. 2006). Roux-en-Y bypass leads to decreased proinflammatory parameters together with increased nutritional antioxidants, catalase, and thiobarbituric acid reactive substances, and decreased reduced glutathione 6 months after surgery (Boesing et al. 2010). In the preoperative period, the obese individuals show higher oxidation and inflammation levels and lower indices of antioxidant defense than those of the control

group. One year after Roux-en-Y gastric bypass, an improvement in antioxidant protection is associated with a reduction in inflammatory and oxidative markers (João Cabrera et al. 2010). Actually, Roux-en-Y gastric bypass in diet-induced obese rats, improves NO bioavailability resulting from higher endothelial Akt/NO synthase activation, reduces JNK phosphorylation, and decreases oxidative stress. In these animals, Roux-en-Y gastric bypass rapidly reverses obesity-induced endothelial dysfunction and restores the endothelium-protective properties of HDL via a glucagon-like peptide-1 (GLP-1)-mediated mechanism (Osto et al. 2015). Virtually, Roux-en-Y gastric bypass preserves respiratory chain complex I activity of mitochondria and restores adenosine triphosphate (ATP) levels, thereby restoring energy output, probably by limiting the amount of oxidative stress and tumor necrosis factor-alpha (TNF-alpha) (Verbeek et al. 2015).

Furthermore, after gastric partition surgery, the NO production significantly decreases which might be reflecting the usual status of NO production in obese subjects. The down-regulation of serum NO levels after weight reduction surgery positively associate with the changes of body mass index and serum triglyceride levels (Lin et al. 2007). The low grade inflammation is improved due to the weight-losing effect of bariatric surgery, and this improvement has been strongly related to insulin sensitivity and adiposity. TNF-alpha receptors are positively related to adiposity and endothelial dysfunction markers. Moreover, the correlation between the increases in vascular cell adhesion molecule 1 (VCAM-1) and tumor necrosis factor receptor 2 (TNFR2) levels after surgery suggests that the persistence in the activation of this low grade inflammation pathway might be involved in the lack of decrease in VCAM-1 levels. In morbidly obese patients, although interleukin (IL)-6 and TNF-alpha remains activated four-months after bariatric surgery, weight loss, insulin sensitivity, endothelial function, as well inflammatory response improves in parallel over the medium term (Vázquez et al. 2005). Bariatric surgery causes a significant decrease in mRNA expression of E-selectin and IL-6 (Blum et al. 2015). Within the first 6 months after bariatric surgery, soluble E-selectin (sE-selectin) levels decrease. Despite substantial weight loss, soluble cell adhesion molecule-1 (sICAM-1) and soluble VCAM-1 (sVCAM-1) plasma levels do not decrease significantly. After 24 months, sICAM-1 levels significantly decrease, whereas sE-selectin levels further decrease. However, sVCAM-1 levels remain elevated. Adiponectin levels do not change significantly during the first 6 months after bariatric surgery, whereas resistin levels increase. After 24 months, adiponectin levels are similar with the normal-weight controls, but resistin levels remain still high. Although not all endothelial activation markers normalize after bariatric surgery, bariatric surgery can reduce endothelial activation in the long term (Nijhuis et al. 2007). Six months after Roux-en-Y gastric bypass, weight loss leads to significant improvements in clinical parameters indicative of cardiovascular disease or risk, including brachial artery diameter, endothelial independent vasodilation, and Framingham cardiovascular risk score (Brethauer et al. 2011). Bariatric surgery has been shown to resolve or improve cardiovascular disease risk factors, to varying degrees. C-reactive protein decreases, endothelial function improves, and a 40% relative risk reduction for 10-year coronary heart disease risk is observed, as determined by the Framingham risk score (Heneghan et al. 2011). Bariatric surgery results in significant improvements in inflammatory, structural, and functional markers of coronary atherosclerosis in morbidly obese subjects. While the mean body mass index, the mean carotid intima-media thickness and mean high-sensitivity C-reactive protein decreases 37%, 40% and 47%, respectively, the mean flow-mediated dilation increases by 148% (Habib et al. 2009). At 6-months post-surgery, subcutaneous and visceral adipose tissue volumes significantly reduce by 34.7% and 44.1%, respectively and insulin sensitivity is improved by 160.9%. Significant longitudinal correlations are found between insulin sensitivity and plasma C-reactive protein. These findings offer insights that link obesity and insulin resistance via the activity of inflammatory mediators (Gletsu et al.

2005). Virtually, weight loss represents an effective method for downregulating the inflammatory state and ameliorating endothelial dysfunction in obese individuals. A 30% reduction in fat mass, fasting insulin and HOMA index of obese subjects may cause a decrease in plasma levels of TNF-alpha and IL-6 by 25–30% (Bruun et al. 2003). Bariatric surgery produces improvements in markers of inflammation, oxidative stress and several adipokines among adolescents with severe obesity. IL-6, leptin and oxidized low-density lipoprotein cholesterol significantly decrease, whereas adiponectin significantly increases up to 12-months following elective laparoscopic Roux-en-Y gastric bypass or vertical sleeve gastrectomy (Kelly et al. 2016). With the exception of adiponectin, circulating concentrations of adipocyte-derived hormones, leptin and acylation-stimulating protein (ASP), as well as visfatin (also known as pre-B-cell colony-enhancing factor 1/nicotinamide phosphoribosyltransferase—PBEF/NAMPT) are usually increased in obesity and insulin-resistant states, and subsequently decline during weight loss (Faraj et al. 2003). Decrease in leptin levels and elevation in adiponectin concentrations in adipoinsular axis correlate with weight loss and improvement in insulin concentrations following bariatric surgery (Ballantyne et al. 2005). Preoperative adiponectin level is 1.5-fold lower in diabetic versus nondiabetic patients. Morbidly obese patients overall show a 3.12-fold downregulation of adiponectin expression versus control group. Interestingly, following bariatric surgery adiponectin levels are upregulated 2.79-fold, which is close to the level of the normal control group (Hindle et al. 2010). In these cases, plasma lipids, insulin resistance, leptin, soluble TNFR1 (sTNFR1), and IL-6 decrease while adiponectin and ghrelin levels increase significantly. Insulin resistance improves after weight loss and correlates with high adiponectin levels (Vendrell et al. 2004). However, after bariatric surgery, plasma ghrelin levels remain unchanged in the weight-stable subjects, but increase by approximately 60% in weight-reducing subjects due to negative energy balance. Thus, the postoperative average ghrelin level in the weight-reduced group is higher than that in weight-stable subjects, despite the fact that their average postoperative body mass index and gastric pouch size were similar to those in weight stable patients. In this respect, energy balance may be a more important determinant of postsurgical ghrelin levels after gastric bypass than the body weight (Faraj et al. 2003). Adiponectin production may be determined primarily by adipocyte size and insulin sensitivity. Indeed, larger insulin-resistant adipocytes produce less adiponectin (Swarbrick and Havel 2008). Bariatric surgery raises circulating adiponectin levels by elevating gene expression of adiponectin from omental adipocytes. Gastric bypass results in resolution of the leptin resistance status that characterizes obese humans. Eventually, this procedure up-regulates the adiponectin gene expression in the visceral fat. These patients have a significant correlation between omental adiponectin and serum leptin (Chen et al. 2012a). Furthermore, also a significant correlation between increased adiponectin and lower plasma triglyceride levels and adipose insulin resistance indicate that elevated systemic adiponectin levels may serve as a biomarker for metabolic health following bariatric surgery independent of weight loss (Malin et al. 2014). After Roux-en-Y gastric bypass, adenosine monophosphate (AMP)-activated protein kinase (AMPK) activity increases 3.5-fold and oxidative stress decreases by 50% in subcutaneous adipose tissue. In addition, malonyl-CoA levels are reduced by 80%. Furthermore, patients have improvements in their body mass index and insulin sensitivity (HOMA) and have increased circulating high-molecular weight adiponectin and decreased fasting plasma insulin levels (Xu et al. 2015). A20 is a mediator of adiponectin for anti-inflammatory action in white adipose tissue. Adipose A20 expression in obese human exhibits a negative correlation with insulin sensitivity. Bariatric surgery-induced weight loss is accompanied by enhanced white adipose tissue A20 expression, which is positively correlated with increased serum adiponectin (Hand et al. 2015). Seven days after Roux-en-Y gastric bypass, significant reductions in the insulin resistance are observed, while triglyceride and adiponectin lev-

els remain unchanged after meal. However, 90 days after surgery, triglycerides decrease at fasting, and postprandial, adiponectin, GLP-1, and insulin increase significantly after meal (Umeda et al. 2013). Postprandial GLP-1 levels increase as early as 2 days after Roux-en-Y gastric bypass (le Roux et al. 2007). GLP-1 suppresses endogenous glucose production, promotes glucose uptake, and importantly, reduces glucagon secretion (Salehi et al. 2008). While GLP-1 stimulating insulin secretion, inhibits glucagon secretion, thereby contributes to limit postprandial glucose excursions. It also inhibits gastrointestinal motility and gastric emptying. In this respect, decreased secretion of GLP-1 may contribute to the development of obesity (Holst 2007). Sleeve gastrectomy has repeatedly produced an exaggerated postprandial increase in GLP-1 comparable to that of Roux-en-Y gastric bypass (Peterli et al. 2012; Ramón et al. 2012). Actually, the decrease in fasting glucagon concentrations is specific for Roux-en-Y gastric bypass. Furthermore, bariatric surgery reduces concentrations of the adipocyte-derived hormones and inflammatory molecules such as, leptin, high-sensitivity C-reactive protein (hs-CRP), acylation-stimulating protein, IL-6 and sICAM-1 (Swarbrick et al. 2008).

Actually, bariatric surgery leads to an improvement in fasting and postprandial lipemia after 14 days. Additionally, the fall in fasting triglycerides is associated with an improvement of insulin resistance. The reduction of postprandial lipemia most probably is likely related to reduced intestinal lipid absorption consequent to bariatric surgery (Griffo et al. 2014). Twelve months after bariatric surgery, a significant increase in plasma levels of adiponectin and HDL cholesterol and a significant decrease in levels of IL-6, hs-CRP, cholesterol, triglycerides, LDL cholesterol, glucose, insulin and HOMA index occur in morbidly obese patients (Illán-Gómez et al. 2012). Visceral fat contains approximately 15-fold higher resistin mRNA compared with subcutaneous fat. In fact, the amount of visceral fat plays a major role in modulating insulin action and glucose tolerance (Gabriely et al. 2002). Furthermore, abdominal adipose tissues and plasma are obtained from 25 subjects undergoing bariatric surgery, 15 non-obese subjects, and 12 subjects after gastric bypass at the average follow-up of 19 months after the surgical procedure. Omental adiponectin gene expression increases fivefold in morbidly obese subjects after bariatric surgery when compared with matched morbidly obese subjects and reaching levels equal to age and gender matched non-obese controls (Chen et al. 2012b). Gastric bypass surgery results in resolution of the leptin resistance status that characterizes obese subjects. There is an inverse correlation between serum leptin levels and omental adiponectin gene expression (Chen et al. 2012a). In this respect, omentectomy added to Roux-en-Y gastric bypass surgery results in statistically significant improvements in short-term glucose levels, lipid levels, and the ratio of high molecular weight adiponectin/total adiponectin. These effects are not seen in Roux-en-Y gastric bypass alone. However, the long-term clinical significance of these changes found at 90-days after omentectomy is likely negligible (Dillard et al. 2013). Thus, the proposed advantages of omentectomy added to bariatric surgery regarding to weight loss and improvement of obesity-related disorders are not confirmed (Sdralis et al. 2013).

In Roux-en-Y gastric bypass, many of the changes in gastrointestinal hormones, adipokines and cytokines as well as in hypothalamic neuropeptides and neurotransmitters resemble the changes observed in the anorexia, suggesting that this surgical procedure attenuates appetite and triggers a catabolic state responsible for prolonged weight loss (Guijarro et al. 2006). Surgical treatment of super obesity by laparoscopic sleeve gastrectomy or by laparoscopic Roux-en-Y gastric bypass provides a statistically significant reduction in the leptin levels. In this series of patient, a significant reduction in the ghrelin level is noted for the laparoscopic gastric bypass, comparable to the laparoscopic sleeve gastrectomy. By this procedure, the main fraction of the ghrelin producing cells which are located in the bypassed part of the stomach is deactivated (Major et al. 2015). Actually, the relationship between bariatric procedure and the plasma levels of ghrelin are very complex. Thus, laparoscopic sleeve gastrectomy significantly reduces

the production of ghrelin in 90% of patients in a durable fashion (Bohdjalian et al. 2010). It is probably, the result of resecting the gastric fundus where the majority of ghrelin production takes place (Frühbeck et al. 2004).

Secreted protein acidic and rich in cysteine (SPARC) is the first extracellular matrix (ECM) protein described in adipose tissue. Matrix metalloproteinases (MMPs) also play a role in ECM remodeling, and MMP-2 and MMP-9 may be associated with abnormal ECM metabolism. The serum SPARC and the MMP-2 concentrations decrease significantly after bariatric surgery. Changes in the serum SPARC concentration are significantly correlated with HOMA-IR changes, while decrease in the serum MMP-9 concentration are found to be inversely correlated with serum adiponectin levels (Lee et al. 2014). Reduced MMP-7 levels in obesity might be restored approximately 1 year after bariatric surgery by significant weight loss. This indicates that the reorganization of adipose tissue in obesity might be partially reversible by weight reduction (Ress et al. 2010). The lysyl oxidase (LOX) family of amine oxidases, including LOX and LOX-like (LOXL) isoenzymes, controls ECM maturation. Upregulation of LOX activity is essential in adipose tissue fibrosis. Thus LOX is the main isoenzyme expressed in human adipose tissue and that its expression is strongly upregulated in samples from obese individuals (Halberg et al. 2009; Henegar et al. 2008). However, inhibition of LOX activity with beta-aminopropionitrile attenuates insulin resistance, reverses the decrease in glucose transporter 4 (GLUT4) and adiponectin levels, and the increases in both suppressor of cytokine signaling 3 (SOCS3) and dipeptidyl peptidase 4 expression in obese animals (Miana et al. 2015). Lacking SOCS3 protein specifically in leptin receptor (LepR)-expressing cells (LepR SOCS3 KO) exhibit increased leptin sensitivity in the hypothalamus. LepR SOCS3 deficient subjects show attenuated food intake and weight regain after a 2-day period of fasting (Pedroso et al. 2016). Expression levels of genes encoding ECM, cross-linking enzymes, metalloproteinases, and their inhibitors are modified 1 year after bariatric surgery. LOX expression and protein are significantly decreased and associated with decreased fat mass as well as other cross-linking enzymes (Liu et al. 2016).

Improvements in glucose metabolism and insulin resistance following bariatric surgery in the short-term result from decreased stimulation of the entero-insular axis by decreased caloric intake and in the long-term appears from the decreased fat mass and resulting changes in release of adipocytokines (Gumbs et al. 2005). The preoperative profile with high levels of pro-inflammatory adipocytokines in obese individuals is linked to improvements in glucose homeostasis and lipids postoperatively. After bariatric surgery, the levels of TNFR1, TNFR2, visfatin, and CRP are significantly lower than its baseline levels, whereas high molecular weight adiponectin is higher. On the other hand, surgical intervention markedly decreases fasting glucose, insulin, and HOMA2-IR levels. HDL moderately increase, whereas triglyceride levels sharply decrease (Auguet et al. 2014).

In the surgically treated morbidly obese patients, weight loss is best predicted by preoperative resistin concentrations (Vendrell et al. 2004). Resistin circulates at increased levels in obesity, and has effects on glucose homeostasis that oppose those of insulin (Steppan and Lazar 2002). It activates NFkappaB-dependent cytokine release and adhesion molecule expression including TNF-alpha and IL-6 (Singla et al. 2010). Eventually increased plasma resistin level impairs glucose tolerance and insulin action, thereby glucose uptake by adipocytes is reduced (Steppan et al. 2001). Furthermore, resistin inhibits adipocyte differentiation and may function as a feedback regulator of adipogenesis, and also functions as a regulator of glucose homeostasis and a physiologic antagonist to hepatic insulin action (Wolf 2004). Actually, in morbidly obese patients, high resistin expression in serum is associated with hepatic steatosis, inflammation, and fibrosis (Edwards et al. 2013). Resistin levels do not change after bariatric surgery. Although there is a positive association between basal resistin and fat

mass, resistin concentrations do not change after massive weight loss with biliopancreatic diversion in morbidly obese patients without diabetes mellitus (de Luis et al. 2011). In diabetic-obese patients, downregulation of resistin and leptin gene expression after bariatric surgery may play a role in normalizing obesity-associated insulin resistance (Edwards et al. 2011). Indeed, both leptin and resistin mRNA levels are elevated in the obese-diabetic group but decreased after bariatric surgery to levels near those of the nondiabetic group. Greater downregulation of resistin and leptin expression occurs in patients who lost more excess body weight, while patients who lose less than 10% excess body weight have a mean increase in expression of these two genes (Edwards et al. 2011). Thus, a 54.5% loss of excess body mass index in morbidly obese patients through restrictive bariatric surgery is associated with significant improvement in leptin, resistin, and IL-6 levels, and an increase in adiponectin levels, in addition to improving insulin sensitivity (Marantos et al. 2011). The percentage of total weight loss correlates positively with adiponectin levels and negatively with leptin levels. However, weight loss itself, rather than the procedure type, is responsible for hormonal variation. Nevertheless, the leptin levels reflect the body weight changes best, after bariatric therapies (Wroblewski et al. 2016). Serum visfatin levels in morbidly obese women following bariatric surgery and after losing at least 15% of the initial weight, increase in relation to the amount of weight lost. This increase is higher in diabetic patients (Botella-Carretero et al. 2008). In contrast, Caron-Cantin et al. showed that 37% weight loss induced by biliopancreatic diversion with duodenal switch did not influence the overall plasma visfatin and apelin levels in severely obese patients (Caron-Cantin et al. 2013). In fact, visfatin has shown to be increased in obesity and in type 2 diabetes. Accordingly, plasma visfatin level in the severely obese patients are increased but only when accompanied by high glucose levels, even in the range of impaired fasting glucose. Following bariatric surgery visfatin levels are significantly increased independent of the type of bariatric surgery in severely obese patients with normal fasting glucose (García-Fuentes et al. 2007). In this context, massive weight loss after bariatric surgery is accompanied by an increase in circulating concentrations of visfatin. This increase correlates with the decrease in plasma insulin concentrations and HOMA-IR (Krzyzanowska et al. 2006).

Apelin mRNA is detectable in nondifferentiated preadipocytes and its production increases fourfold upon differentiation of the fat cells, together with adiponectin and leptin. Apelin expression in fat cells is strongly inhibited by fasting and recovered after refeeding, in a similar way to insulin. A direct regulation of apelin expression by insulin is observed in human adipocytes and clearly associated with the stimulation of phosphatidylinositol 3-kinase (PI3K), protein kinase C (PKC) , and mitogen activated protein kinase (MAPK) (Boucher et al. 2005). Apelin plasma levels are significantly increased in diabetic patients. Apelin and apelin receptor expression in humans is regulated according to the severity of insulin resistance (Dray et al. 2010). Bariatric surgery results in a significant decrease in apelin levels only in the morbidly obese subjects with impaired fasting glucose or diabetes (Soriguer et al. 2009). In addition, apelin is increased in relation to the worsening of insulin-resistance/insulin-secretion. After bariatric surgery, apelin levels significantly decrease together with an improved metabolic profile and independently from weight loss (Cavallo et al. 2012). Actually, vaspin has potential insulin sensitizing property. Changes in serum vaspin concentrations are significantly correlated with the body weight of obese subjects with higher HOMA-IR (Chang et al. 2010). Vaspin levels in severely obese subjects display a biphasic profile. High preoperative fasting vaspin levels are associated with lower insulin, HOMA-IR, and triglyceride, despite higher HDL-cholesterol, ASP and IL-6 levels. After bariatric surgery, both high and low preoperative vaspin groups display similar weight loss. But patients with high vaspin levels maintain their high vaspin levels in addition to better glucose, insulin, HOMA-IR, HDL-

cholesterol, and triglyceride profile, while in the low vaspin group their vaspin levels gradually increase with weight loss (Lu et al. 2014). Following bariatric surgery, increase in serum vaspin concentrations correlate significantly with the reduction of circulating leptin, insulin, and C-peptide levels and with the amelioration of insulin sensitivity (Handisurya et al. 2010). In this context, higher vaspin is better in respect to severely obese patients pre-and post-bariatric surgery (Lu et al. 2014).

Adipocyte-derived signals contribute to the pathogenesis of type 2 diabetes (Yang et al. 2005). Thus, an adipocyte-secreted molecule, serum retinol-binding protein-4 (RBP4) concentrations are elevated in overweight or obese humans and positively correlated with body mass index and insulin resistance (Graham et al. 2006). In obese subjects, serum RBP4 is increased two to threefold and correlates positively with adipose RBP4 mRNA and intra-abdominal fat mass and inversely with insulin sensitivity. Furthermore, RBP4 mRNA also correlates positively with adipocyte size and visceral fat, which is a major source of RBP4 in insulin-resistant states (Klöting et al. 2007). RBP4 concentrations decrease by nearly 30% one month following Roux-en-Y gastric bypass surgery. On the other hand, at 12 months no significant decrease is observed when compared to pre-surgical levels, despite marked weight loss and improved insulin resistance (Swarbrick et al. 2008). Nevertheless, lowering serum levels of RBP4 and IL-6 are important contributors of the improved insulin sensitivity after bariatric surgery (Mitterberger et al. 2010). Substantially, enhanced levels of serum RBP4 is the signal for the development of systemic insulin resistance in humans (Wolf 2007). Hence, a marked decrease of RBP4 levels after bariatric surgery correlates with reduction in visceral-fat mass (Tschoner et al. 2008). Actually, a decrease in RBP4 levels is only observed after surgically induced weight loss accompanied by relevant reductions in body fat. RBP4 might be considered as a dynamic marker of negative energy balance and it is reduced during weight loss when a negative energy balance threshold is reached. Furthermore, variation of RBP4 within the first month after Roux-en-Y gastric bypass may be a predictor of weight loss success (Gómez-Ambrosi et al. 2008).

Free fatty acid levels are raised in obesity as a consequence of increased production and reduced clearance of fatty acids. Biliopancreatic diversion intensely reduces circulating lipid levels and improves insulin resistance in addition to changes in the pattern of leptin peaks in plasma (Raffaelli et al. 2015). Indeed, a marked decrease in fasting leptin levels, and an increase in the orexigenic hormone, ghrelin levels, after bariatric surgery-induced weight loss, independent of the type of surgery is performed (Terra et al. 2013). Thus, anti-inflammatory effects of adiponectin and the pro-inflammatory effects of leptin and C-reactive protein, reduction in chronic inflammation associated with less visceral fat after surgery may contribute to the reduction in cardiovascular disease risk factors (Appachi and Kashyap 2013).

After bariatric surgery in morbidly obese individuals, decreased visceral adiposity is accompanied by reduced accumulation of CD3+ T-lymphocytes, Mac-3 or F4/80 positive macrophages, as well as reduction in the expression of interferon-gamma (IFN-gamma) and other inflammatory cytokines in the mesenteric adipose tissue. Furthermore, surgery improves endothelium-dependent vasorelaxation in small mesenteric arteries. Surgery also reduces mesenteric adipose tissue/small mesenteric arteries superoxide production (Zhang et al. 2011). In addition to decrease in hs-CRP, TNF-alpha, IFN-gamma, IL-1 receptor antagonist, IL-6, and IL-13, C3 and C4 levels drop significantly in the morbidly obese group over time and one year after bariatric surgery (Nestvold et al. 2015). Furthermore, deficiencies in the immune system of morbidly obese individuals include elevated levels of eosinophils, monocyte expressing CD14, and CD14+CD16+ monocyte subsets with the depression of monocyte and neutrophil expressing CD62L. Surgically-induced weight loss rapidly reverses these hematologic parameters. In this context, it is proposed that Roux-en-Y gastric bypass is not only a weight loss

operation but also appears to be an immune restorative procedure (Cottam et al. 2002).

Serum uric acid may contribute to the pathogenesis of metabolic comorbidities like hypertension, insulin-resistance and endothelial dysfunction in severely obese children. A significant decrease in serum uric acid, body mass index and HOMA-IR occurs 12 months after laparoscopic sleeve gastrectomy or laparoscopic Roux-en-Y-gastric bypass (Oberbach et al. 2014). Indeed, the prospective, controlled non-randomized Swedish Obese Subjects Study showed that in the 2- and 10-year follow-up period the incidence rates of hypertriglyceridemia, diabetes, and hyperuricemia are markedly lower in the surgically treated group. However, the incidence of hypertension and hypercholesterolemia did not improve over the 2- and 10-year (Sjöström et al. 2004). These results suggest that although bariatric surgery is a favorable option in the treatment of morbid obesity, not all obesity-associated risk factors are improved by sustained weight loss (Sjöström et al. 2004). Obese patients have approximately twofold higher serum phenylalanine levels. Both serum phenylalanine and serum alanine aminotransferase concentrations decrease 6 months after bariatric surgery (Swierczynski et al. 2009). While the amino acid profile shows increased concentrations of most amino acids at 3 months after Roux-en-Y gastric bypass, 6 months later; glutamic acid, serine, arginine, alanine, methionine, valine, phenylalanine, isoleucine, and tyrosine concentrations decrease. However, the total protein and albumin concentrations drop along the 12-month follow-up (Nicoletti et al. 2013).

Decreasing adiposity following Roux-en-Y gastric bypass correlate with longer-term HOMA-IR and peripheral insulin sensitivity values at 6 and 24 months, respectively. In patients exhibiting fasting hyperglycemia before surgery, β-cell function improves early following Roux-en-Y gastric bypass, due largely to increases in insulin secretion. For both normoglycemic and hyperglycemic subjects, further improvement or stabilization of β-cell function over the 2 years is due largely to improved peripheral insulin sensitivity associated with reduced adiposity (Lin et al. 2010).

3 Weight Regain After Bariatric Surgery

Several factors influence weight outcomes, including weight regain after bariatric surgery. Compared to malabsorptive procedures, restrictive procedures tend to have higher rates of weight loss failure and weight regain (Maggard et al. 2005). In this respect, successful weight loss is significantly greater in patients following duodenal switch in comparison to Roux-en-Y Gastric Bypass (Prachand et al. 2010). Although there is a lower food tolerance in bariatric surgery patients compared to nonsurgical obese patients (Suter et al. 2007), it is possible to regain weight as energy intake can exceed needs following surgery. On the other hand, low food tolerance related to the texture of some high protein foods may be a risk of protein malnutrition. Low intake of fruits and vegetables can contribute to low fibre intake and vitamin/mineral deficiency (Johnson Stoklossa and Atwal 2013). Roux-en-Y gastric bypass produces rapid weight loss even in super obese patients. But up to 20% cannot sustain their weight loss beyond 2–3 years after surgery (Guijarro et al. 2007; Meguid et al. 2008). The super-obese loses weight more rapidly and gains more rapidly after reaching the lowest weight at approximately 2 years than the morbidly obese patients. There is no difference in results between making the Roux-en-Y limb 100 cm long and to create a 40 cm Roux-en-Y limb. When the results are classified according to Biron et al. after 10 years, for morbidly obese patients the body mass index is less than 35 kg/m^2 and for super obese less than 40 kg/m^2 is accepted successfully. A significant increase in failures and decrease in excellent results are observed at 10 years. Furthermore, length of the gastric bypass limb length does not impact on the long-term weight loss (Biron et al. 2004; Christou et al. 2006). Roux-en-Y gastric bypass-induced weight loss and insulin sensitization in diet-induced obese mice could not be generated in leptin-deficient animals, which suffer a weight regain over the pre-surgery level. Thus, leptin is an important factor required for Roux-en-Y gastric bypass to prevent weight regain and diabetes

recurrence (Hao et al. 2015). Actually, Roux-en-Y gastric bypass reduces total body weight, fat and lean mass and causes a reduction in calorie intake in leptin-deficient mice. However, it fails to improve glucose tolerance, glucose-stimulated plasma insulin, insulin tolerance, and fasting plasma insulin. High fat diet eliminated the reduction in calorie intake that is observed after Roux-en-Y gastric bypass in leptin-deficient mice and promoted weight regain. Leptin is required for the effects of Roux-en-Y gastric bypass on glucose homeostasis but not body weight or composition (Mokadem et al. 2015).

Weight loss results in adaptive thermogenesis, and there is no indication for a change in adaptive thermogenesis up to one year, when weight loss is maintained (Camps et al. 2013). Less than expected weight loss with low-calorie diets can arise from an increase in fractional energy absorption, adaptations in energy expenditure, or incomplete patient diet adherence. Compliance with and the energy restriction itself play a role in the amount of weight loss and that the amount of weight loss determines the degree of adaptive thermogenesis (Heymsfield et al. 2007). In fact, limited therapeutic success may depend on adaptive thermogenesis, which represents the decrease in energy expenditure and this is accompanied by increases in adiposity. Decrease in energy expenditure is quantitatively sufficient to overcome the prescribed energy restriction. This suggests that adaptive thermogenesis plays an important role in unsuccessful weight loss interventions and reduced body weight maintenance (Major et al. 2007). Lower energy intake, increased energy expenditure and/or increased fatty acid oxidation are associated with less weight gain or greater weight loss over time (Stookey 2016). A lower resting metabolic rate may contribute to weight regain in patients who undergo Roux-en-Y gastric bypass. It is important to ensure ways to elevate energy expenditure in the patient, such as increasing the percentage of fat-free mass in the body (Faria et al. 2009). A sustained adaptive thermogenesis favors positive energy balance and may predispose to weight regain (Camps et al. 2013). Measurement of resting energy requirements would improve accuracy of nutrition prescriptions. Additionally, optimization of energy expenditure may be helpful to achieve energy balance to help the prevention of further weight regain (Johnson Stoklossa and Atwal 2013). Thus, one year after surgery, mitochondrial function is comparable to that of lean controls (Vijgen et al. 2013). Cellular and tissue mitochondrial respiration increases in morbidly obese patients after laparoscopic bariatric surgery. These changes are consistent in patients with postsurgical weight loss (Nijhawan et al. 2013).

Individuals who have intentionally lost at least 10% of their body weight and kept it off at least one year, are successful weight loss maintainers (Wing and Hill 2001). However, approximately 20–30% of patients do not achieve successful weight outcomes, and patients may experience a regain of 20–25% of their lost weight (Johnson Stoklossa and Atwal 2013). Bariatric surgery results in weight loss by reducing the amount of tolerable intake (restrictive) and reducing the amounts of nutrients absorbed by bypassing absorptive intestine (Concors et al. 2016). Weight regain after bariatric surgery may depend on energy intake due to enlargement of stoma and adaptive changes in the levels of gut and adipocyte hormones such as ghrelin and leptin, which regulate energy intake; decrease in physical activity; changes in energy expenditure. Additionally, Roux-en-Y gastric bypass surgery-associated weight regain may be related to iron, vitamin B12, folate, calcium, and vitamin D deficiencies (Shah et al. 2006). Morínigo et al. showed that morbidly obese patients underwent to Roux-en-Y-gastric bypass have a significant decrease in fasting and postprandial hunger and a significant increase in satiety after meal intake. It is likely that weight loss is associated with an improvement in the GLP-1 and total peptide YY (PYY) response to a liquid-meal intake after bariatric surgery (Morínigo et al. 2006). PYY suppresses appetite and exerts regulatory properties on body weight (Karra and Batterham 2010). Return to pre-surgical energy intake levels results in weight regain. Sustained weight loss is provided by producing adequate amount of PYY while suppressing leptin secretion. Weight regain is attributed to a failure to sustain elevated plasma

PYY concentrations and lower PYY/Leptin ratio. Combining Roux-en-Y gastric bypass with pharmacologic stimulation of PYY secretion in patients after Roux-en-Y gastric bypass who exhibit inadequate PYY concentration may increase long-term success of surgical weight reduction in morbidly obese adults (Meguid et al. 2008). The major source of GLP-1 in the body is the intestinal L cells. GLP-1 restores the glucose sensitivity of pancreatic beta-cells, with the mechanism involving the increased expression of GLUT2 and glucokinase. In bariatric surgery, the exclusion of duodenum and jejunum from food transit may also reduce gastrointestinal peptide (GIP) secretion of obese patient. Furthermore, the normalization of peripheral and beta-cell insulin sensitivity might play a relevant causative role in the down regulation of GIP secretion (Guidone et al. 2006). Recent bariatric procedures, Roux-en-Y gastric bypass and sleeve gastrectomy produce marked sustained weight reduction. However, there is a marked individual variability in this reduction. In these patients, post-operative weight loss follows a normal distribution with extremes of "good" and "poor" response. Profound anorexia and excessive weight loss post-sleeve gastrectomy may be associated with markedly elevated circulating fasting plasma PYY and post-meal plasma PYY and GLP-1 levels (Pucci et al. 2015). Laparoscopic sleeve gastrectomy decreases fasting and postprandial plasma acyl-ghrelin compared to presurgery and to laparoscopic Roux-en-Y gastric bypass. Nutrient-stimulated PYY3-36 and active GLP-1 concentrations increase post-operatively in both groups. However, laparoscopic Roux-en-Y gastric bypass induces greater, more sustained PYY3-36 and active GLP-1 increments compared to laparoscopic sleeve gastrectomy. Laparoscopic Roux-en-Y gastric bypass suppresses fasting hunger compared to laparoscopic sleeve gastrectomy. A similar increase in postprandial fullness is observed post-surgery following both procedures. Laparoscopic Roux-en-Y gastric bypass and laparoscopic sleeve gastrectomy produces comparable enhanced satiety and weight loss. However, laparoscopic sleeve gastrectomy and laparoscopic Roux-en-Y gastric bypass differentially alters gut hormone profiles (Yousseif et al. 2014). Forty-five primary articles show that there are alterations in GLP-1, peptide tyrosine-tyrosine, leptin, and ghrelin after Roux-en-Y gastric bypass procedure. GLP-1 and peptide tyrosine-tyrosine concentrations are usually found to be higher, whereas ghrelin levels are typically lower post-Roux-en-Y gastric bypass than in individuals with obesity, those who were overweight or of normal weight (Beckman et al. 2010). The secretion of gut hormones in patients with weight regain even after Roux-en-Y gastric bypass are the gold standard of bariatric operations. These hormone levels are different from that in patients with satisfactory weight outcome. After meal stimulation, reduced levels of glucose-dependent insulinotropic polypeptide and GLP-1 may indicate the influence of gut hormones in the process of weight regain (Santo et al. 2016). One of the most important factors associated with long-term weight regain after sleeve gastrectomy is residual gastric volume. Patients with higher weight regain have greater residual gastric volume. The remnant volume is significantly correlated with the weight gain percentage (Alvarez et al. 2016).

On the other hand, increased long chain fatty acid uptake in obese adipocytes is also a contributing factor in weight regain. Excess long chain fatty acid uptake reflects upregulation of a facilitated transport process and the permeability of adipocyte plasma membranes to long chain fatty acid. This process increases the cell surface area of obese adipocytes. Eventually, regulation of long chain fatty acid uptake by adipocytes is an important control point for body adiposity (Petrescu et al. 2005). In this respect, long chain fatty acid uptake by omental adipocytes also increases exponentially in obese and super-obese patients following bariatric surgery when compared to non-obese subjects. The increase of adipocyte long chain fatty acid uptake more than one year contributes to weight regain (Ge et al. 2016).

Microparticles are intact vesicles, which are derived from cell membranes; they vary in size from 0.2 to 2.0 μm (Piccin et al. 2015). Endothelial cells release qualitatively and quantitatively dis-

tinct endothelial microparticles during activation (Jimenez et al. 2003). There is a definitive relationship between adiposity and circulating microparticles in obesity. The percentages of reduction of all the microparticles are significantly correlated with the percentage of reduction of body mass index. In this context, the reductions of leukocyte-derived, tissue factor-bearing (TF+) and CD36+ microparticle are significantly correlated with the reduction of leptin and hs-CRP. In contrast, a trend of slight increase in all microparticle subtypes is detected 12 months after sleeve gastrectomy. This may indicate a possible underlying slow low-grade inflammatory state in adipose tissue before the potential overt weight gain (Campello et al. 2016).

The laparoscopic sleeve gastrectomy is emerging as an effective bariatric operation and is especially attractive in high-risk populations. Mean percent excess weight loss was 61% at an average of 22 months, with no significant difference between severely obese, morbidly obese, and super obese cohorts. Diabetes remission is seen in 56% of patients, hypertension remission in 51.6%, and obstructive sleep apnea remission in 46.4%, and gastroesophageal reflux disease improved or did not change in 83% (Eisenberg et al. 2013). Although a tendency for weight regain is noted after 5 years of follow-up evaluation, laparoscopic sleeve gastrectomy results in good to excellent health-related quality of life (D'Hondt et al. 2011). However, weight gain also is a common complication following Roux-en-Y gastric bypass surgery. Despite the percentage of weight loss over the first year, all patient groups regained on average between 21 and 29 % of lost weight. The mean weight regain for all patients is 23.4% of maximum weight loss. Excessive weight gain is approximately experienced by over one third of patients (Cooper et al. 2015). Weight regain due to gastric pouch dilatation after Roux-en-Y gastric bypass is seen more frequently during the long-term follow-up. The mean postoperative body mass index decreases to 32.8 ± 7.3 kg/m^2, and the median percentage excess weight loss is 29.1% by laparoscopic pouch resizing, but patients should be informed about possible protein-calorie malnutrition risks following resizing procedure (Al-Bader et al. 2015). In cases of weight regain or insufficient weight loss after laparoscopic sleeve gastrectomy, patients have a better weight loss with a secondary surgery, biliopancreatic diversion with duodenal switch; however, this procedure has the risk of complications, such as severe vitamin deficiencies (Carmeli et al. 2015; Homan et al. 2015). Twenty-four months after Roux-en-Y gastric bypass, 25.7% of the participants regained weight. In relation to the baseline, post-surgery reductions are found in vitamin C, beta-carotene, vitamin E, reduced glutathione, catalase, and ferric reducing antioxidant potential (Dadalt et al. 2013).

Analysis of 16 studies with a total of 4864 post-bariatric surgery patients indicated that weight regain appeared to be a multi-factorial and overlapping phenomena. In brief, weight regain following bariatric surgery varies according to duration of follow-up and the bariatric surgical procedure performed (Karmali et al. 2013). The indications and outcomes for reoperative bariatric surgery are procedure-specific but the current evidences are taken into account during the selection of additional treatment modalities for persistent obesity, co-morbid disease, and complications (Brethauer et al. 2014). As mentioned above, although laparoscopic Roux-en-Y gastric bypass is an effective treatment for morbid obesity, failure of weight loss has been reported in almost 10–30% of Roux-en-Y gastric bypass patients. Pericardial patch ring in addition to Roux-en-Y gastric bypass provides a mean percentage of excess weight loss of 57.4% at a mean follow-up of 11 months (Moon et al. 2013). Recently, a prospectively collected database showed that revision with pericardial patch ring after gastric bypass for weight regain or failure of weight loss is not a feasible, safe and effective procedure (Moon et al. 2014). On the other hand, despite the initial effectiveness of sleeve gastrectomy, some patients have inadequate weight loss or renewed weight gain. Conversion of sleeve gastrectomy to a mini gastric bypass by connecting the long narrow gastric tube to the jejunum at

a point 200cm downstream from the ligament of Treitz results in a loss of excess body mass index 26.8%, 37.2%, 48.6% and 51.6% at 3, 12, 18 and 24 months, respectively (Moszkowicz et al. 2013). Nevertheless, persistent satiety, obesity-related diseases, adaptive thermogenesis and gut hormones are important factors for the future of weight loss after bariatric surgery.

4 Conclusion

Surgical therapy is the most effective option in terms of extent and duration of weight reduction with acceptable operative risks. The proportion of newly developed sleeve gastrectomy technique sharply increased during the period of 2008–2014. However, malabsorptive approaches is more effective than their restrictive alternatives in morbidly obese patients. In this context, compared to malabsorptive procedures, restrictive procedures tend to have higher rates of weight loss failure and weight regain. The super-obese loses weight more rapidly and gains more rapidly after reaching the lowest weight at approximately 2 years than the morbidly obese patients. A significant increase in failures and decrease in excellent results are observed at 10 years. A sustained adaptive thermogenesis favors positive energy balance and may predispose to weight regain. Individuals who have intentionally lost at least 10% of their body weight and kept it off at least one year, are successful weight loss maintainers.

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Obesity-associated Breast Cancer: Analysis of risk factors

25

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Abstract

Several studies show that a significantly stronger association is obvious between increased body mass index (BMI) and higher breast cancer incidence. Furthermore, obese women are at higher risk of all-cause and breast cancer specific mortality when compared to non-obese women with breast cancer. In this context, increased levels of estrogens due to excessive aromatization activity of the adipose tissue, overexpression of pro-inflammatory cytokines, insulin resistance, hyperactivation of insulin-like growth factors (IGFs) pathways, adipocyte-derived adipokines, hypercholesterolemia and excessive oxidative stress contribute to the development of breast cancer in obese women. While higher breast cancer risk with hormone replacement therapy is particularly evident among lean women, in postmenopausal women who are not taking exogenous hormones, general obesity is a significant predictor for breast cancer. Moreover, increased plasma cholesterol leads to accelerated tumor formation and exacerbates their aggressiveness. In contrast to postmenopausal women, premenopausal women with high BMI are inversely associated with breast cancer risk. Nevertheless, life-style of women for breast cancer risk is regulated by avoiding the overweight and a high-fat diet. Estrogen-plus-progestin hormone therapy users for more than 5 years have elevated risks of both invasive ductal and lobular breast cancer. Additionally, these cases are more commonly node-positive and have a higher cancer-related mortality. Collectively, in this chapter, the impacts of obesity-related estrogen, cholesterol, saturated fatty acid, leptin and adiponectin concentrations, aromatase activity, leptin and insulin resistance on breast cancer patients are evaluated. Obesity-related prognostic factors of breast cancer also are discussed at molecular basis.

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1 Introduction

High body mass index (BMI) is associated with increased risk of some cancer types. In 1402 of 108,812 individuals from the general population developed breast cancer during a median of 4.7-years follow-up. In this series of patients, corresponding risk of breast cancer was 20% higher in postmenopausal women with high BMI (Benn et al. 2016). However, a significantly stronger association between increased BMI and higher breast cancer incidence is observed in the Asia-Pacific group than in European-Australian or North-American group (Wang et al. 2016). In the Asia-Pacific region, every additional 5 kg/m^2 increase in BMI corresponds to a 31% increase in postmenopausal breast cancer risk (Renehan et al. 2008). Although overweight is related to a higher risk of mortality, the number of obese female breast cancer survivors is growing as a consequence of advances in treatment strategies and early diagnosis. In this respect, the number of breast cancer survivors is increasing by approximately 3% each year (Maddams et al. 2009). Evaluation of 79 publications from 82 follow-up studies clearly indicate that, amongst the breast cancer survivors, higher BMI is consistently associated with lower overall and breast cancer survival. Analysis of 23,182 deaths of 213,075 breast cancer survivors confirmed that the current guideline for breast cancer survivors is to remain as lean as possible within the normal range of body weight (Chan et al. 2014). Another meta-analysis includes results from 43 studies show that obese women are at higher risk of all-cause and breast cancer specific mortality when compared to non-obese women with breast cancer (Demark-Wahnefried et al. 2012). In this respect, overweight women should avoid weight gain during treatment and obese women should lose weight immediately after treatment (Rock et al. 2012). Of 53,816 women treated for early-stage breast cancer, 18,967 patients with complete follow-up have been evaluated up to 10 years and up to 30 years for the loco-regional recurrences or distant metastases and for death, respectively. The risk of developing distant metastases after 10 years was significantly increased which was by 46%, and the risk of dying as a result of breast cancer after 30 years was significantly increased which was by 38% for patients with a BMI of 30 kg/m^2 or more. However, BMI had no influence on the risk of loco-regional recurrences (Ewertz et al. 2011). Indeed, high BMI is associated with worse long-term outcomes among obese women, who have breast cancer (Kamineni et al. 2013). In addition, increase in post-diagnostic body weight is common in women with breast cancer. In this respect, particularly chemotherapy treatment has been considered as a significant contributory factor through reduced metabolism (Demark-Wahnefried et al. 2012). Inter-linked molecular mechanisms between obesity and breast cancer might be involved in the pathogenesis in postmenopausal women. In this context, increased levels of estrogens due to excessive aromatization activity of the adipose tissue, overexpression of inflammatory cytokines, insulin resistance, hyperactivation of insulin-like growth factors (IGFs) pathways, adipocyte-derived adipokines, and excessive oxidative

stress contribute to the development of breast cancer in obese women. These factors also interfere with intracellular signaling in the mitogen-activated protein kinase (MAPK) and phosphatydilinositol-3-phosphate (PI3P)/mammalian target of rapamycin (mTOR) pathway (Simone et al. 2016).

The interaction between human-derived adipocytes and primary breast cancer cells increase the secretion of pro-inflammatory cytokines. Furthermore, contact with immature adipocytes increase the abundance of cancer cells with tumor-forming and metastatic potential in vivo (Picon-Ruiz et al. 2016). The expression level of the fat mass-obesity associated genes in breast cancer tissues is significantly higher than that in the adjacent healthy breast tissues. However, there is no correlation between fat mass-obesity associated gene expression and age, tumor stage, lymph node status, TNM stage, Ki67, and BMI in breast cancer. Despite all these, fat mass-obesity associated gene expression has very important role in human epidermal growth factor receptor 2 (HER2)-overexpressed breast cancer (Tan et al. 2015). In the women undergoing mastectomy for breast cancer risk reduction or for breast cancer treatment, white adipose tissue inflammation in breast specimens is detected in 52% and 41% of patients, respectively. In these patients, white adipose tissue inflammation is defined by the presence of dead adipocytes surrounded by macrophages forming crown-like structures in the breast adipose tissue (Iyengar et al. 2016).

During the 4.7 years-follow-up of 73,542 premenopausal and 103,344 postmenopausal women from nine European countries, 1879 incidental invasive breast cancers have been diagnosed. While excess breast cancer risk with hormone replacement therapy is particularly evident among lean women, in postmenopausal women who are not taking exogenous hormones, general obesity is a significant predictor of breast cancer. In contrast to postmenopausal women, among premenopausal women, high BMI is inversely, but not significantly, associated with breast cancer risk. This meta-analysis indicates a 2% reduction in risk per unit of increase in BMI (1 kg/m^2) among premenopausal women, whereas a 3% increase in risk of breast cancer among postmenopausal non-hormone replacement therapy users (Lahmann et al. 2004). Considering the relationship between breast cancer and obesity; the postulated mechanisms for the increased risk of breast cancer in obese women, such as elevated estrogen levels, insulin resistance and the influences of IGF, fatty acids, leptin and adiponectin will be discussed in this chapter.

2 Estrogens as a Cause of Breast Cancer in Obesity

There is abundance of data linking obesity-related breast cancer to estrogen receptor signaling. As comorbidities of obesity; excessive local production of estrogens in adipose tissue, the influence of adipokines and inflammatory cytokines, finally hypercholesterolemia have also been established as independent risk factors for breast cancer in postmenopausal women (Boyd and McGuire 1990; McDonnell et al. 2014a). Despite accumulated observational evidences, risks and benefits of estrogen use are evaluated with the increase in absolute excess risk for occurrence of invasive breast cancers. On May 31, 2002, after a mean of 5.2 years of follow-up, the data and safety monitoring board recommended stopping the trial of estrogen-plus-progestin versus placebo, because the test statistic for invasive breast cancer exceeded the average incidence of this adverse effect (Rossouw et al. 2002). Over an average follow-up of 6.8 years, the use of 0.625 mg/day of conjugated equine estrogen increases the risk of stroke, decreases the risk of hip fracture, and does not affect coronary heart disease incidence in postmenopausal women with prior hysterectomy. Finally, in February 2004, the National Institutes of Health decided to end the intervention phase of the trial early by taking into consideration of these data (Anderson et al. 2004). In a total of 16,608 women without hysterectomy and randomized to the estrogen-plus-progestin trial, the higher breast cancer risk seen during intervention that is followed by a substantial drop in the risk of the early post-intervention phase, but the

higher breast cancer risk remains during the late post-intervention phase of follow-up period. The 10,739 women with prior hysterectomy are randomized according to the estrogen alone trial. Despite the lower breast cancer risk that is observed during the early post-intervention phase, during the late post-intervention period a higher breast cancer risk is determined (Chlebowski et al. 2015). Collectively, the results of these data indicate that in estrogen-plus-progestin group, breast cancer incidence is higher, and the cases are more commonly node-positive. Breast cancer mortality also appears to be increased with combined use of estrogen-plus-progestin (Chlebowski et al. 2010). Breast cancer incidence rate decreases with body mass among premenopausal women in high-risk countries, but this ratio rises with the increase in body mass in all other groups of women (Pathak and Whittemore 1992). More detailed analysis revealed that decreased risk of breast cancer associates with increased body size among premenopausal women, however this event is limited to the youngest age group only (Peacock et al. 1999). Furthermore, obesity may influence the levels of endogenous sex-steroid and IGF-related hormones in the circulation, especially after menopause. In the post-menopausal group, increases in estrogens, testosterone and androstenedione are correlated with increasing BMI. In contrast, sex hormone-binding globulin decreases with increasing BMI (Lukanova et al. 2004; McTiernan et al. 2006). Obese women have 35% higher concentrations of estrone and 130% higher concentrations of estradiol compared with normal-weight women. Testosterone concentrations also increase with increasing levels of adiposity. Concentrations of free estradiol and free testosterone are two to three times greater in overweight and obese women compared with normal-weight women (McTiernan et al. 2003). Obese never users of hormone therapy have 1.7- to 2.3-fold elevated risks of ductal and estrogen receptor (ER) positive-progesteron receptor (positive-PR) positive breast cancer, respectively, compared to thinner women. Estrogen-plus-progestin hormone therapy users for more than 5 years have 2.1 to 9.6-fold elevated risks of lobular and ER positive-PR positive tumors compared to never users of hormone therapy regardless of BMI. Current estrogen-plus-progestin hormone therapy users for more than 5 years with a BMI less than 24.9 kg/m^2 also have a 2.6-fold elevated risk of ductal breast cancer (Li et al. 2006). In eleven original articles, the case-case comparison showed a significant association between triple negative breast cancer and obesity. When the patients are stratified based on menopausal status, significant results are observed only in the premenopausal group and triple negative tumors with ER negative-PR negative/HER2-found to have positive associations with obesity but only among premenopausal women (Pierobon and Frankenfeld 2013). In a prospective cohort of 67,754 postmenopausal women, 1821 cases of invasive ductal breast cancer and 471 cases of invasive lobular or mixed lobular cancer occurred during 13 years of follow-up. Use of exogenous estrogen plus progesterone is associated with an increased risk of both ductal and lobular breast cancer. Risk increased within the first 2–3 years of use and attenuated 2 years after cessation. In contrast, use of estrogen-only is not associated with an overall increased risk of invasive ductal cancer. Estrogen-only use is associated with a 50% increased risk of invasive lobular cancer after more than 10 years of use (Calle et al. 2009). In this case, estrogens are converted to metabolites, particularly the catechol estrogen-3,4-quinones (CE-3,4-Q), that can react with DNA to form depurinating adducts. These adducts are released from DNA to generate apurinic sites. Depurinating adducts 4-hydroxyestrone (estradiol), 4-OHE1(E2)-1-N3Ade and 4-OHE1(E2)-1-N7Gua constitute more than 99% of the total DNA adducts formed. Increased levels of these quinones and their reaction with DNA occur when estrogen metabolism is unbalanced (Cavalieri et al. 2006). Risk for ER negative-PR negative tumors among postmenopausal women slightly increases, but this is significantly different from risk for ER positive-PR positive tumors. The association between adult weight gain and postmenopausal breast cancer risk is heterogeneous according to ER/PR status and stronger for

ER positive-PR positive than for ER negative-PR negative tumors (Vrieling et al. 2010).

3 Cholesterol and Breast Cancer in Obesity

In association with oncogenic stimuli, increased plasma cholesterol leads to accelerated tumor formation and increases tumor burden. In mammary tumors increased plasma cholesterol levels are associated with increased expression of cyclin D1, a marker associated with tumor formation, and decreased expression of markers associated with protection. Both high density lipoproteins and scavenger receptor type BI proteins are elevated in animals that are fed a cholesterol-rich diet. An increase in plasma cholesterol levels accelerates the development of tumors and exacerbates their aggressiveness (Llaverias et al. 2011). The increased availability of high density lipoproteins may increase the estradiol access to the cancer tissue. While lipoproteins may regulate endothelial function in the tumor, they may also affect the transport of estrogen. In addition, scavenger receptor type BI may promote estradiol uptake (Brodeur et al. 2008). The binding of high density lipoprotein (HDL) to scavenger receptor type BI activates Ras in a protein kinase C (PKC)-independent manner with subsequent induction of the MAPK signaling cascade (Grewal et al. 2003). Many metabolic pathways, which have critical roles in progression of breast cancer may induce MAPK signaling cascade. In this respect, fatty acid binding protein 4 (FABP4) enhances the proliferation of breast cancer cells by inducing the Akt and MAPK signaling cascades (Guaita-Esteruelas et al. 2016). Adiponectin receptor 1 (AdipoR1), adaptor protein APPL1, ER-alpha, Insulin like growth factor 1 receptor (IGF-R1), and c-Src that is also responsible for MAPK signaling activation in ER-delta positive breast cancer cells (Mauro et al. 2014). Furthermore, circulating factors in the serum of obese postmenopausal women stimulate ER-alpha positive breast cancer cell viability and growth by facilitating non-genomic ER-alpha crosstalk with the PI3K/Akt and MAPK signaling pathways (Bowers et al. 2013). Indeed, increased phosphatidylinositol 3-kinase (PI3K) activity and Akt phosphorylation is a favorable setting for mammary tumor growth and metastasis in hypercholesterolemic animals (Alikhani et al. 2013) (Fig. 25.1).

Actually, the oxysterol 27-hydroxycholesterol (27HC) is synthesized from cholesterol by CYP27A1 which is a rate limiting enzyme. Hence in humans, the circulating levels of 27HC closely reflects those of cholesterol and hypercholesterolemia (Karuna et al. 2011) 27HC is a liver X receptor (LXR) agonist and as such serves to limit cholesterol accumulation in cells (Fu et al. 2001) LXRs, as antiproliferative factors suppress growth of both normal and breast cancer cells (Vigushin et al. 2004). Interestingly, LXRs might have a dual role in this respect. On the one hand, LXRs are antiproliferative in the breast cancer cells. On the other hand, LXR-induced expression of lipogenic genes peaked at lower 27HC concentration are needed for the antiproliferative effect of LXR (Vedin et al. 2009). Nevertheless, genetic or pharmacological activation of LXR results in estrogen deactivation, which in turn inhibits breast cancer growth (Gong et al. 2007). In contrast, 27HC is an estrogen receptor agonist in breast cancer cells and it stimulates the growth and metastasis of tumors in breast cancer of obese women (McDonnell et al. 2014b). In human breast cancer specimens, tumor grade is correlated with CYP27A1 expression levels. In high-grade tumors, both tumor cells and tumor-associated macrophages display high expression levels of this enzyme. Thus, 27HC actually promotes the proliferation of ER-positive, but not ER-negative breast cancer cell lines (Nelson et al. 2013). In ER-positive breast cancer patients, 27HC content in normal breast tissue is increased compared to the cancer-free controls, and the tumor 27HC abundance is further elevated. Cancer 27HC content is not influenced by circulating levels of 27HC or its precursors. Increases in tumor 27HC are directly related to diminished expression of the 27HC metabolizing enzyme CYP7B1. Actually, 27HC is a locally-modulated, non-aromatized ER ligand that promotes ER positive breast cancer growth (Wu et al.

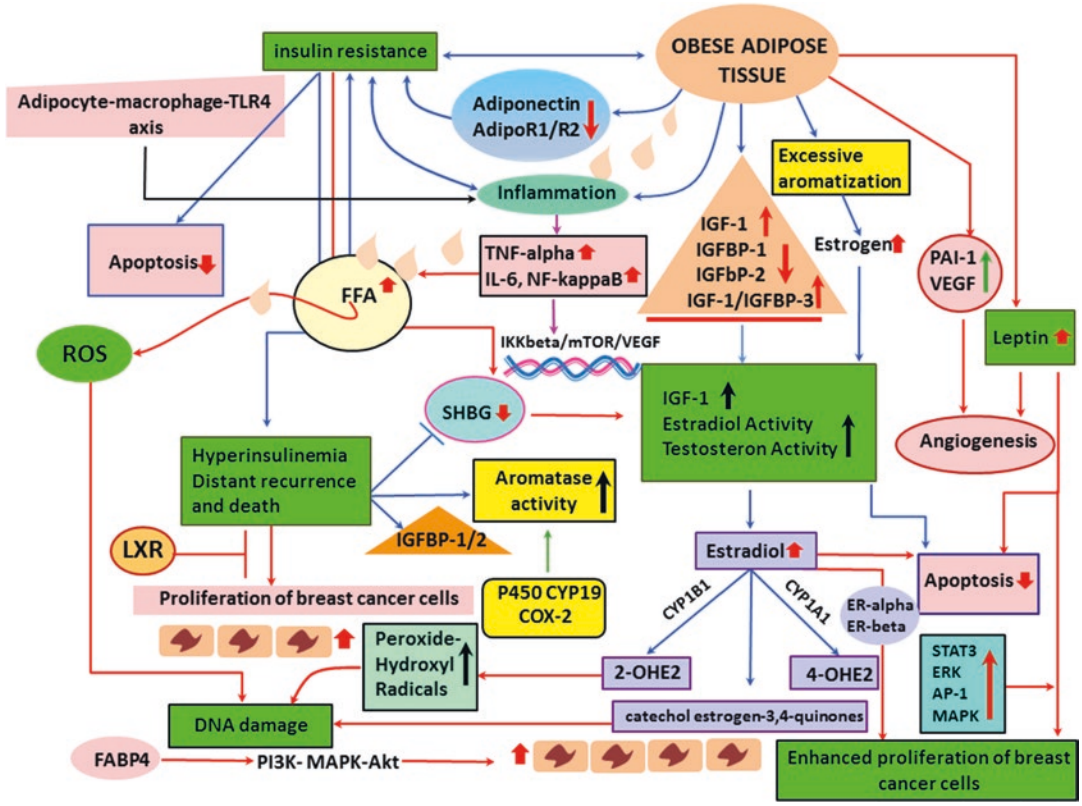

Fig. 25.1 The postulated mechanisms for the increased risk of breast cancer in obese women; elevated estrogen levels, insulin resistance and the influences of IGF, fatty acids, leptin and adiponectin (*ROS* reactive oxygen radicals, *PI3K* phosphatidylinositol 3-kinase, *MAPK* mitogen-activated protein-kinase, *Akt* protein kinase B, *FFA* free fatty acid, *SHBG* sex hormone-binding globulin, *TNF-alpha* tumor necrosis factor-alpha, *IL-6* interleukin-6, *NF-kappaB* nuclear factor-kappaB, *IKK* inhibitor kappa B kinase, *mTOR* mammalian target of rapamycin, *VEGF* vascular endothelial growth factor, *PAI-1* plasminogen activator inhibitor-1, *IGF-1* insulin-like growth factor-1, *IGFBP* insulin-like growth factor binding protein, *CYP19* aromatase cytochrome P450, product of the CYP19 gene, *CYP1B1* cytochrome P-450 1B1, *CYP1A1* cytochrome P-450 1A1, *2-OHE2* 2-hydroxy estradiol, *4-OHE2* 4-hydroxyestradiol, *ER-alpha* estrogen receptor-alpha, *ER-beta* estrogen receptor-beta, *STAT3* signal transducers and activators of transcription-3, *ERK* extracellular signal-regulated kinase, *AP-1* activating protein-1, *MAPK* mitogen-activated protein-kinase, *TLR4* Toll like receptor-4, *COX-2* cyclooxygenase-2, *LXR* liver X receptor, *Adipo R1/R2* adiponectin receptors, *FABP4* fatty acid binding protein 4)

2013). Interestingly, tumor infiltration by macrophages is associated with their ability to produce 27HC within tumors and induce ER-alpha activity (McDonnell et al. 2014a) (Fig. 25.1).

4 Obesity-Related Aromatase Activity in Breast Cancer

While the ovaries are the primary source of estrogens in premenopausal women, estrogen synthesis also occurs in the adipose tissue. Estrogens may play a role in the pathogenesis of breast cancer that operates independent of their well-documented function in binding the ER molecules expressed by breast cancer cells. In this context, despite the lack of clinical responses of ER-negative breast cancers to tamoxifen therapy, aromatase inhibitor is able to block tumor growth. Actually, estrogen also enhances the growth of ER-negative tumors by increasing the systemic capacity for angiogenesis and the recruitment of bone marrow-derived stromal cells (Gupta et al. 2007). Breast cancer patients

have increased exposure to unbound circulating estradiol. An increased percentage of estradiol bound to albumin may influence the availability of estradiol, considering its low binding affinity to albumin (Ota et al. 1986). Weight loss as a result of caloric restriction and exercise significantly decreases estrogens and free testosterone, and increases sex hormone-binding globulin (SHBG) (Campbell et al. 2012). In contrast, increasing obesity correlates with a progressive fall in SHBG level. Thus increasing upper body fat localization is inversely proportional to levels of SHBG in breast cancer patients and healthy individuals. Premenopausal breast cancer patients are found to have significantly lower levels of SHBG compared with age-matched and weight-matched individuals. Lower levels of SHBG also result in increased levels of circulating unbound androgens which may result in tumor progression by themselves and further conversion to estrogens by adipose tissue (Schapira et al. 1991). Thus, the levels of estradiol that are detected in breast cancers are 50 to 100-fold higher than the concentrations of this hormone that are found in the circulation of postmenopausal women. The high estradiol concentrations found in breast cancers could arise either due to uptake from the circulation or from endogenous synthesis. Since similar concentrations of estradiol are present in tumors with or without estrogen receptors, endogenous synthesis is considered to be the major pathway by which tumor estrogens originate. Three main enzyme complexes are involved in the synthesis of estrogens in peripheral tissues. Firstly, aromatase is responsible for the aromatisation of androstenedione to estrone. Secondly, estrone sulfatase catalyses the formation of estrone from estrone sulfate, and finally estradiol-17β-hydroxysteroid dehydrogenase Type 1 is responsible for the reduction of estrone to the biologically active estrogen and estradiol (Purohit et al. 2002). The aromatase enzyme, estrogen synthase (CYP19 p450), belongs to a family of p450 enzymes (Simpson et al. 1981). In postmenopausal women, it is located mainly in adipose tissue but is also present in normal and malignant breast tissues. Immunocytochemical studies indicate that aromatase settles in the stromal part of breast tumors (Sasano and Harada 1998), as well as in epithelial location. There is a significant, but inverse, correlation between the aromatase activity and the estrogen receptor status, indicating the likelihood of negative estrogen receptors if substantial aromatase activity is present (Esteban et al. 1992). Three-fold higher human aromatase mRNA levels are found in the mammary gland of female mice that are fed with a high fat diet compared with their littermates on normal chow. Because overweight females have twice the mammary gland mass as lean controls, thereupon, the overweight mice could have up to six-fold higher human aromatase mRNA in their mammary gland compared with lean counterparts. Hence, weight loss may reduce breast cancer risk via reduction in local aromatase production of the breast in premenopausal obese women (Chen et al. 2012). As mentioned above, epithelial cells are the primary site of estrogen synthesis in the breast and breast cancers. Aromatase expression is detected in the cytoplasm of tumor epithelial cells and the surrounding stromal cells of over 50% of tumors. Although intra-tumoral aromatase activity does not correlate with steroid receptors, ER-positive tumors express aromatase. These data indicate that some breast cancers synthesize sufficient estrogen to stimulate their own proliferation (Brodie et al. 1997). Assays of aromatase levels in adipose tissue from the different quadrants of mastectomy specimens from patients with breast cancer indicate that activity is always higher in breast quadrants associated with tumor as compared with non-involved quadrants. These results emphasize the importance of local estrogen synthesis within the breast (Miller and O'Neill 1987). Loss of the ovarian function to supply estrogen and progesterone after menopause can cause deregulation of the body's metabolism and the availability of systemic estradiol dramatically decreases (Boonyaratanakornkit and Pateetin 2015). Elevated in situ estrogen concentrations in postmenopausal human breast cancer patients are derived from intratumoral aromatization. Furthermore, in patients with estrogen-dependent breast carcinoma, intratumoral estrogens function as an autocrine growth and a mitogenic fac-

tor and could impart a growth advantage to these cancer cells, regardless of serum concentration of estrogens (Sasano et al. 2006). Furthermore, in postmenopausal breast cancer patients, up to 50% of deaths have been attributed to obesity (van Kruijsdijk et al. 2009). As mentioned above, aromatase activity is highest in adipose tissue of the breast quadrant containing the tumor. In these breast tissues, a significant correlation is also found between interleukin-6 (IL-6) production and aromatase activity (Reed et al. 1993). In accordance with declining systemic estradiol at menopause, the incidence of ER-alpha-positive breast cancer dramatically increases (Pfeilschifter et al. 2002; Sasser et al. 2007). BMI, leptin, IL-6 and reactive oxygen species (ROS) is higher in ER positive compared with ER negative patients. Among ER positive patients, BMI, leptin, IL-6 and ROS correlate with tumor size and metastasis. Additionally, leptin, IL-6 and ROS positively correlate also with lymph node metastasis. Eventually, weight gain, inflammation and oxidative stress are involved in post-menopausal estrogen-dependent breast cancer prognosis (Madeddu et al. 2014). Evidently, obesity is associated with a worse breast cancer prognosis, particularly in ER-alpha-positive, postmenopausal patients. Obesity-associated systemic IL-6 indirectly enhances preadipocyte aromatase expression via increased breast cancer cell prostaglandin E2 (PGE2) production (Bowers et al. 2015). Increased nuclear factor-kappaB (NF-kappaB) binding activity and elevated aromatase expression and activity are found due to chronic inflammation in breast tissue of overweight and obese women. The severity of breast inflammation of obese women is defined as the crown-like structures of the breast index and this severity status is correlated with both BMI and adipocyte size. The obesity-inflammation-aromatase axis is an indicator of the increased risk of hormone receptor-positive breast cancer and is a sign of poor prognosis in breast cancer of overweight and obese women (Morris et al. 2011; Subbaramaiah et al. 2012). Indeed, increased secretion of prostaglandins via constitutive cyclooxygenase (COX)-1 and inducible COX-2 isozymes in the breast cancer tissue microenvironment can result in increased aromatase activity and subsequently increased estrogen biosynthesis through autocrine mechanisms in breast epithelial cells and through paracrine mechanisms in breast stromal cells (Richards et al. 2002). Furthermore, COX-2-derived PGE2 stimulates the cyclic adenosine monophosphate (cAMP)-phosphokinase A (PKA) signal transduction pathway that activates CYP19 transcription. Consequently, higher aromatase expression and increased progesterone receptor levels are observed in breast tissues of overweight and obese women (Subbaramaiah et al. 2012). Actually COX-2 directly regulates gene expression of specific aromatase promoter regions of the p450 Cyp19 enzyme, aromatase and its product, estradiol. Inhibition of COX-2 or blocking the biological effects of PGE2 may be useful in significantly limiting aromatase activity and proliferation of human breast tumor cells despite the presence of COX-2 protein (Prosperi and Robertson 2006).

Estrogen or other selective estrogen receptor modulators (SERMs) bind to the ER, a ligand-activated transcription factor that regulates transcription of target genes in the nucleus by binding to estrogen response element (ERE). The ER exists in two main forms, ER-alpha and ER-beta, which have distinct tissue expression patterns (Mueller and Korach 2001). ERs are expressed in white adipose tissue, and play an important role in regulating white adipose tissue in females. Absence of ER-alpha causes adipocyte hyperplasia and hypertrophy in white adipose tissue and is accompanied by insulin resistance and glucose intolerance in both males and females (Cooke et al. 2001). Obese patients have 35% higher levels of estrone and 130% higher concentrations of estradiol. Concentrations of free estradiol and free testosterone are two to three times greater in overweight and obese women compared with lean-matched controls (McTiernan et al. 2003). A traditional hypothesis for how obesity affects breast cancer recurrence risk is based on the mitogenic effect of excess estrogen produced in fat cells via the enzymatic conversion of adrenal steroids to estrogen (Dignam et al. 2003) (Fig. 25.1). In addition, cytokines secreted by the adipocytes can upregulate the aromatase enzyme

to further increase the estrogen production, which may stimulate breast cancer cell growth (Cleary and Grossmann 2009). Indeed, obesity is associated with increased adipose tissue mass and aromatase activity, thereby aromatase inhibitors are less effective in women who are overweight or obese (Goodwin 2013). For women with lymph node-negative, ER-positive breast cancer, tamoxifen might be offered as the best approach for the breast cancer risk reduction among obese women, because obesity is associated with increased risks of contralateral breast cancer, other primary cancers, and overall mortality (Dignam et al. 2003). However, in postmenopausal women with early-stage breast cancer and high BMI, after a median of 8.7 years of follow-up, obesity shown to be an independent adverse prognostic factor for death from breast cancer. Aromatase inhibitors are more effective than tamoxifen in reducing overall deaths, breast cancer recurrences, and distant metastases (Ewertz et al. 2012). In any way, risk factors associated with breast cancer reflect cumulative exposure of the breast epithelium to estrogen (Henderson and Feigelson 2000). Two current hypotheses exist to explain this relationship (Yue et al. 2005). In the first hypothesis; according to genomic mechanism, estrogens diffuse into the cell and bind to the estrogen receptor, which is located in the nucleus. This nuclear estrogen-ER complex binds to ERE sequences directly or indirectly through protein-protein interactions with activator protein 1 (AP1) in the promoter region of estrogen-responsive genes (Deroo and Korach 2006). Thereby, estrogen modulates this mechanism by two separate processes. One involves the binding of estradiol to ER-alpha with stimulation of cell proliferation. Consequently, errors in DNA occurring during replication result in fixed mutations when not repaired. The other process results from the formation of genotoxic metabolites of estradiol, which can bind to DNA, cause depurination, and result in mutations (Santen et al. 2009). In brief, estrogens increase the rate of cell proliferation by stimulating ER-mediated transcription. Thus increasing number of errors occur during DNA replication (Yue et al. 2005). At the same time, estrogen can act more quickly via non-genomic mechanisms, either through the estrogen receptor, which is located in or adjacent to the plasma membrane, or through other non-estrogen receptor, plasma membrane-associated estrogen-binding proteins. Eventually, estrogen-mediated cellular responses are increased levels of Ca^{2+} or nitric oxide (NO), and activation of kinases (Deroo and Korach 2006). In the second process, estradiol is metabolized to quinone derivatives, which directly remove base pairs from DNA through depurination. The third pathway involving estrogen metabolism is mediated by cytochrome p450. Reactive electrophilic estrogen o-quinones and ROS are generated through redox cycling of o-quinones (Bolton and Thatcher 2008). All these data indicate that both ER-dependent and genotoxic ER-independent effects of estradiol mediate breast cancer development (Santen et al. 2009). The hormonal changes across the perimenopause and increased risk of obesity at menopause may be caused by the decrease in circulating estrogen, and, for fat distribution shifts, the relative increase in the androgen-estrogen ratio (Davis et al. 2012; Lovejoy 2003).

5 Breast Cancer Recurrence in Obesity

Obesity has been repeatedly shown to be a risk factor for breast cancer recurrence and poor survival. In a meta-analysis of 43 studies, compared with non-obese, obese women with breast cancer showed to have poorer survival which is similar for overall and breast cancer specific survival. This association does not differ by menopausal status (Protani et al. 2010). Adjusting for clinical factors and potential confounders, 10% weight gain and obesity are associated with increased risk of late recurrence (Nechuta et al. 2016).

Compared to normal-weight women, obese women have experience of increased risk of recurrence and risk of breast cancer death, 12.2% and 6.9%, respectively within 10 years of diagnosis. Although, there is no association between BMI and all-cause mortality, obese women have significantly faster growing tumors (Kamineni et al. 2013). The cohort consisted of

3385 women enrolled in National Surgical Adjuvant Breast and Bowel Project (NSABP) protocol B-14 showed that obesity is not associated with an increase in recurrence risk in lymph node-negative, ER-positive breast cancer cases. However, obesity is associated with increased risks of contralateral breast cancer and of overall mortality (Dignam et al. 2003). In 153 case-control pairs of perimenopausal and postmenopausal women, total estradiol, bioavailable estradiol and free estradiol concentrations are significantly associated with risk for recurrence. Recurred women have an average total estradiol concentration that is double than that of non-recurred women (Rock et al. 2008). Serum levels of soluble IL-6 receptor are lower in patients with ER-positive cancer. However, higher serum levels of soluble IL-6 receptor at diagnosis are associated with significantly shorter relapse-free survival in patients with ER-positive breast cancer (Won et al. 2013). Direct application of IL-6 on breast cancer cells inhibits proliferation in ER-positive cells, while high circulating IL-6 levels are correlated with a poor prognosis in breast cancer patients (Dethlefsen et al. 2013). Interleukins could stimulate many signaling pathways and thus regulate the transcription of target genes that are involved in tumor growth, invasion and metastatic potential (Gelaleti et al. 2012). Firstly, estradiol antagonizes IL-6 function by repressing both IL-6 and its signaling receptor, gp130 (Pfeilschifter et al. 2002). Five-year follow-up of 1199 women with hormone receptor positive and human HER2-negative invasive breast cancer are evaluated for the impact of obesity on time to either local or distant recurrence or new breast cancer, or death due to breast cancer. Moderate to severe obesity is associated with a poorer invasive breast cancer prognosis; this is also true for women with Stage 1 disease, and is independent of age and treatment (Robinson et al. 2014). Weight gain after diagnosis of breast cancer is associated with higher all-cause mortality rates compared with maintaining body weight. Adverse effects are greater for weight gains of 10% or higher (Playdon et al. 2015).

Actually, high BMI is significantly associated with larger tumor size both in pre and postmenopausal women. Obese premenopausal women show worse histopathologic features including more metastatic axillary lymph nodes, and presence of vascular invasion, compared to under or normal weight group. Postmenopausal patients with BMI less than 25 develop more frequently estrogen and progesterone receptor positive cancers, while no association is found in premenopausal women (Biglia et al. 2013). There are no differences in estradiol levels between lean and obese women under aromatase inhibitors. The known impact of obesity on recurrence risk in women under aromatase inhibitors treatment may not be due to incomplete aromatase inhibition (Diorio et al. 2012). Aromatase inhibitors are less efficient at suppressing estradiol serum levels in obese when compared with non-obese women (Pfeiler et al. 2013). The higher levels of estrogens in overweight postmenopausal breast cancer patients before and during aromatase inhibition may be due to the effects of BMI on estrogen metabolism rather than aromatization (Lønning et al. 2014). Actually, weight gain is a significant determinant of recurrences. In multivariate analyses, BMI variation more than 5.71% is associated with higher rates of recurrences in early-stage breast cancer patients (Fedele et al. 2014). BMI and weight gain is even more strongly associated with fatal postmenopausal breast cancer. In these cases, the percentage of postmenopausal breast cancer accounted for by weight gain alone is approximately 16% and by hormone replacement therapy alone was 5%. But when the interaction between these variables is taken into account, their impact is about one third of postmenopausal breast cancers (Huang et al. 1997). However, the effect on outcomes is not only limited to excess weight at presentation, as weight gain after chemotherapy for breast cancer negatively impacts disease-free survival, local recurrence, and death as well. Adjuvant chemotherapy is associated with greater weight gain in node-positive, postmenopausal breast cancer patients. The amount of weight gain appears greater for premenopausal than postmenopausal women. It is clear that excessive weight gain in premeno-

pausal women may be associated with an increase in the relapse risk of breast cancer and cancer-related deaths (Camoriano et al. 1990). Thus, patients included 5204 Nurses' Health Study participants diagnosed with incident, invasive, non-metastatic breast cancer between 1976 and 2000; 860 total deaths, 533 breast cancer deaths, and 681 recurrences have been recorded during median follow-up of 9 years. Weight at diagnosis and weight gain is positively associated with increased rates of breast cancer recurrence and mortality (Kroenke et al. 2005). Furthermore, during breast cancer chemotherapy, 31% of patients present a notable weight variation which is greater than 5% of their initial weight. In multivariate analyses, weight change more than 5% is positively associated with an increased risk of both recurrence and death (Thivat et al. 2010).

6 Fatty Acids and Breast Cancer in Obesity

Obesity is strongly associated with changes in the physiological function of adipose tissue, leading to insulin resistance, chronic inflammation, and altered secretion of adipokines (van Kruijsdijk et al. 2009). The increase in circulatory non-esterified fatty acids, adipose tissue dysfunction and the activation of toll-like receptors (TLR) induce signal transduction pathways that lead to the activation of transcription factors. Adipocyte-macrophage-TLR4 axis might be involved in the chronic low-grade inflammatory process occurring in obesity. Indeed, the obesity is associated with a marked infiltration of macrophages within the adipose tissue (Wolowczuk et al. 2008). Actually, saturated fatty acids plus TLR4 are mainly responsible for the amplification of this inflammatory process. In this vicious cycle, increased amount of saturated fatty acids which are provided either by high-fat feeding or adipocyte lipolysis could serve as ligands for TLR4. Finally, the activated adipocytes and macrophages produce more pro-inflammatory products (Wolowczuk et al. 2008) (Fig. 25.1). During obesity, the enhanced production of CCL2 and IL-1beta by the breast adipose tissue increases recruitment of macrophages and the formation of crown-like structures. The recruited macrophages are subsequently stimulated by CCL2 and IL-1beta to secrete CXCL12. In this case, induction of angiogenesis supports the expansion of adipose tissue. Macrophages promote tumor progression from ductal carcinoma in-situ to aggressive breast cancers. Finally, inflammation within the obese mammary gland contributes to angiogenesis through a novel CCL2/IL-1beta/CXCL12 pathway that bypasses the vascular endothelial growth factor/vascular endothelial growth factor receptor (VEGF/VEGFR) pathway (Bowers et al. 2015). Both the systemic and local consequences of chronic adipose inflammation provide key potential mechanistic links between obesity and breast cancer (Iyengar et al. 2013). Adipose tissue macrophages can comprise up to 40% of the cells in adipose tissue of obese humans and represent a rich source of cytokines which are key mediators of the increased risk of insulin resistance associated with obesity (Uysal et al. 1997; Weisberg et al. 2003). Furthermore, TLR4 constitutes a molecular link between nutrition, lipids, and inflammation and that the innate immune system participates in the regulation of energy balance and insulin resistance (Shi et al. 2006). In this respect, saturated fatty acids can activate TLR4 and its downstream signaling pathways involving myeloid differentiation primary response 88/IL-1 receptor-associated kinase/TNF receptor associated factor6 (MyD88/IRAK/TRAF6) and PI3K/Akt. Constitutively, active Akt is sufficient to induce NFkappaB activation and COX-2 expression (Lee et al. 2003). In brief, obesity-inflammation axis is important for the development and progression of breast cancer. Macrophages forming crown-like structures in the breast adipose tissue correlate with both BMI and adipocyte size (Morris et al. 2011). Necrotic adipocytes surrounded by macrophages form crown-like structures in both mammary glands and visceral fat. Saturated fatty acids, which have been linked to obesity-related inflammation and crown-like structures are associated with activation of NF-kappaB and increased levels of tumor necrosis factor-alpha (TNF-alpha), IL-1beta and COX-2. Each of these

cytokines contributes to the induction of aromatase in preadipocytes (Subbaramaiah et al. 2011). Seventy-five per cent of obese and 70% of overweight breast cancer patients have crown-like structures, while among normal-weight breast cancer patients only 8.3% have crown-like structures. This corresponds to an approximately nine-fold change in crown-like structures comparing obese patients to normal-weight women (Morris et al. 2011).

Actually, TNF-alpha is the major mediator of cancer related inflammation. Besides increasing total estrogen metabolites, TNF-alpha significantly decreases the Estrogen1 (E1)/Estrogen2 (E2) ratio due to a decrease in the level of E1 with a concomitant increase in E2 concentration. TNF-alpha increases the expression levels of CYP1A1 and CYP1B1. Estradiol is metabolized into 2-hydroxyestradiol (2-OHE2) and 4-hydroxyestradiol (4-OHE2) by CYP1A1 and CYP1B1, respectively. These catechols undergo further oxidation into semiquinones and quinones that react with DNA to form depurinating adducts leading to mutations, which are associated with breast cancer (Kamel et al. 2012). Additionally, breast inflammation, as determined by crown-like structures of the breast, is paralleled by increased NF-kappaB binding activity and elevated levels of aromatase mRNA and aromatase activity (Subbaramaiah et al. 2012) (Fig. 25.1). NF-kappaB activation underlies many aspects of breast cancer cell proliferation, invasion and metastasis (Rose and Vona-Davis 2014). In this context, seventeen cytokines; IL-1beta, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12 [p70], IL-13, IL-17, granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage (GM-CSF), interferon-gamma (IFN-gamma), monocyte chemoattractant protein (MCP)-1, macrophage inflammatory protein (MIP)-1beta and TNF-alpha are expressed in breast carcinoma, whereas only nine of these could be detected in normal breast. Most cytokines are more abundant in breast carcinoma than in normal breast, with IL-6, IL-8, G-CSF, IFN-gamma, MCP-1 and MIP-1beta being very abundant. IL-2, IL-6, IL-8, IL-10, IFN-gamma, MCP-1, MIP-1beta and TNF-alpha, and to a lesser extent IL-1beta and IL-13 exhibit levels of expression that are inversely correlated to ER and PR status. However, IL-1beta, IL-6, IL-8, IL-10, IL-12, MCP-1 and MIP-1beta are more abundant in high-grade tumors than in low-grade tumors. In addition, IL-8 and MIP-1beta are expressed to a greater degree in HER2-positive patients compared to HER2-negative ones (Chavey et al. 2007). Obese adipose tissue-derived inflammatory mediator production could exacerbate inflammation-associated tumorigenic effects. These mediators and adipokines such as leptin and inflammatory cytokines with a concomitant reduction in adiponectin are produced in both visceral adipose tissue depots and also within mammary adipose tissue depots. All these depots contribute to the development of a more severe breast cancer phenotype via stimulating breast cancer growth, invasion and metastasis (Dalamaga et al. 2012). n-6 polyunsaturated fatty acid (n-6 PUFA)-derived eicosanoids are known as pro-inflammatory and pro-carcinogenic lipid mediators. In contrast, n-3 PUFA-derived lipid mediators functionally oppose the synthesis and activity n-6 PUFA-derived eicosanoids. Thereby, several of the known risk factors of these eicosanoids for breast cancer may be modified by dietary omega-3 fatty acid supplementation (Rose and Connolly 1999). Indeed, n-3 PUFA have been shown to concurrently target in the multiple aspects of the obese breast cancer phenotype. In this respect, reduction of macrophage adipose tissue infiltration, crown like structure formation and down-regulation of critical adipokine production are the major goals. Collectively, increased n-3 PUFA intake could attenuate obesity-associated breast cancer (Monk et al. 2014). Eventually, breast cancer risk has been found to be negatively associated with specific n-3 PUFAs (eicosapentaenoic acid and docosahexaenoic acid) and positively associated with n-6 PUFAs (linoleic acid and arachidonic acid) in breast adipose tissues, which are collected from 73 breast cancer patients and 74 healthy subjects (Bagga et al. 2002). Excessive dietary intake of n-6 PUFA versus n-3 PUFA results in a significantly greater proportion of eicosanoids generation from n-6 PUFA (Calder 2012). Even

short-term dietary intervention can lead to statistically significant increases in omega-3/omega-6 polyunsaturated fatty acid ratios in plasma and breast adipose tissue of women with high-risk localized breast cancer (Bagga et al. 1997). Actually, inflammatory process leads to the induction of COX-2 expression and a consequent elevation in prostaglandin (PG) production. Together with other pro-inflammatory cytokines, the eicosanoids promote further development and growth of breast cancers by the induction of aromatase, particularly in estrogen positive breast cancers. Meanwhile, the more aggressive, estrogen-independent tumors may develop by direct stimulatory effect of PGE2 and lipoxygenase (LOX) products (Vona-Davis and Rose 2013).

The high energy consumption is indispensable for both primary tumor growth and secondary tumor cell metastasis (Phoenix et al. 2010). Thereby, most breast cancer cell types are addicted to fatty acids, which they require for membrane phospholipid synthesis, signaling processes, and energy production. Expression of the enzymes required for fatty acid synthesis is closely linked to each of the major classes of signaling molecules that stimulate breast cancer cell proliferation (Kinlaw et al. 2016). Obesity, which is characterized by hyperlipidemia and an elevation of circulating free fatty acids, is also associated with enhanced cancer risk (Soto-Guzman et al. 2010). Thus, diet-induced obesity has been shown to promote the incidence of breast cancer development (Cleary et al. 2004a). Additionally, fatty acid synthase is strongly associated with the postmenopausal breast cancer. BMI and pathological stage of tumor is also related to fatty acid synthase expression of breast cancer cells (Porta et al. 2014). By analyzing mastectomy specimen, the amounts of glandular and fat tissue are quantified in the resected breasts tissue. Adipose tissue is a major component, comprising up to 56% of the total breast volume (Vandeweyer and Hertens 2002).

As mentioned above, linoleic acid is a dietary n-6 PUFA that is known to induce proliferation and invasion in breast cancer cells. It promotes focal adhesion kinase and Src activation, as well as cell migration in breast cancer cells (Serna-Marquez et al. 2013). At first, oleic acid mediates the production of arachidonic acid (AA), and then AA metabolites mediate focal adhesion kinase phosphorylation and cell migration in breast cancer cells. In contrast, Gi/Go proteins, phospholipase C, LOXs and Src inhibitor prevents focal adhesion kinase phosphorylation and cell migration (Navarro-Tito et al. 2010). Insulin, leptin, and adiponectin has no effect on oleic acid, AA, and eicosapentaenoic acid uptake by breast cancer cells. However, preferential uptake of AA in breast cancer cells, and the fatty acid uptake activity of these cells is influenced by TNF-alpha (Kaur et al. 2009). In breast cancer, the free oleic acid induces tumor invasion through an epidermal growth factor receptor (EGFR), guanosine triphosphate (GTP)-binding proteins; Gi/Go proteins, matrix metalloproteinases, PKC and Src-dependent pathway, but it is not able to promote invasion in non-invasive breast cancer cells (Soto-Guzman et al. 2010). The phospholipase C, mitogenic-extracellular signal-regulated kinase 1/2 (MEK 1/2), Src, and PI3K/Akt signaling pathways are involved in the proliferative signal induced by oleate. This effect is mediated at least in part via the G protein-coupled receptor (GPR) 40. Eventually, fatty acids control breast cancer cell growth via GPR40, and may provide a link between fat and cancer (Hardy et al. 2005). Consequently, G-protein-coupled cell surface receptor for long-chain fatty acids is involved in human breast cancer cell proliferation (Yonezawa et al. 2004). Saturated fatty acids released from adipocytes have been linked to obesity-related white adipose tissue inflammation. In this respect, saturated fatty acid stimulates Akt-dependent activation of NF-kappaB. Later on, TNF-alpha, IL-1beta and COX-2 levels are increased in macrophages. Release of these cytokines strongly provoke aromatase activity (Subbaramaiah et al. 2013).

Actually, lipid desaturation is an essential process for cancer cell survival. Stearoyl-CoA desaturase mRNA and protein expression is elevated in human breast cancers and predicts poor survival. Therefore, inhibition of stearoyl-CoA desaturase activity could efficiently control synthesis of unsaturated fatty acids and limit breast

tumor growth (Peck et al. 2016). Lipogenesis is regulated by the enzyme fatty acid synthase (FASN); and breakdown of fatty acids is regulated by carnitine palmitoyltransferase-1 (CPT-I). FASN is highly expressed in breast cancers (Puig et al. 2008). Basal expression of rate-limiting enzymes FASN, stearoyl-CoA desaturase and lipolytic phospholipase A2 group IVB, as well as lipogenesis transcription factors peroxisome proliferator activated receptors alpha (PPARalpha), sterol regulatory element binding factor 2 (SREBF2) and cAMP responsive element modulator (CREM) are higher in breast cancer cells. Over-expression of lectin-like oxidized-low density lipoprotein (OX-LDL) receptor-1 in breast cancer cells also enhances cell migration, without affecting their adherence to TNF-alpha-activated endothelium or transendothelial migration. Additionally, lectin-like OX-LDL receptor-1 inhibits apoptosis and stimulates cancer cell proliferation through NF-kappaB signaling pathway (Khaidakov et al. 2011). Triple negative breast cancer cell lines show overexpression of FASN (Giró-Perafita et al. 2016). Through a PI3K-dependent pathway, HER2 stimulates the FASN promoter and ultimately mediates increased fatty acid synthesis. Interestingly, pharmacological inhibition of FASN preferentially induces apoptosis of HER2-overexpressing breast epithelial cells relative to matched control cells (Kumar-Sinha et al. 2003). By contrast, blocking the FASN leads to apoptosis of HER2-positive breast carcinoma cells (Blancafort et al. 2015).

A high-fat diet with estrogen deprivation leads to development of insulin resistance, which may accelerate mammary tumor growth through the insulin receptor-mediated Akt pathway and inactivation of AMP-activated protein kinase (AMPK) in vivo. High circulating insulin in combination with increased expression of insulin receptor in tumor tissues may result in stimulation of Akt/mTOR signaling leading to the acceleration of breast tumor growth (Kim et al. 2015). Janus Kinases (JAKs) are a unique class of tyrosine kinases that associate with cytokine receptors. Upon ligand binding, IL-6, IL-4 and G-CSF activate members of the Signal Transducers and Activators of Transcription (STAT) family through phosphorylation on a single tyrosine. Activated STATs form dimers, translocate to the nucleus, bind to specific response elements in promotors of target genes, and transcriptionally activate these genes (Heim 1999). After the activation of JAK 1 tyrosine kinase and STAT1 and STAT3 transcription factors by IL-6, expression of dominant negative STAT3 in the cells strongly reduces IL-6-mediated growth inhibition but does not prevent IL-6-induced cell migration. IL-6 treatment leads to activation of the MAPK and the PI3K pathways. Inhibition of MAPK or PI3K activity reverses IL-6- stimulated migration (Badache and Hynes 2001). The cancer cell death signaling c-Jun N-terminal kinase (JNK) pathway is inhibited by IL-6. Consequently, IL-6 effectively protects cancer cells from the apoptosis and allows for the proliferation of malignant cells (Lin et al. 2001). Additionally, IL-6 and TNF-alpha acts as a regulator of estrogen synthesis and aromatase activity in normal and malignant breast tissues (Purohit et al. 1996). Thereby, IL-6 and IL-1 beta regulates proliferation of breast cancer cells through estrogen production by steroid-catalyzing enzymes in the breast tissue (Honma et al. 2002) In this respect, serum IL-6 concentration is significantly higher in patients with breast cancer compared to healthy controls. Thus, median IL-6 serum levels are nearly ten times higher in patients with metastatic breast cancer compared to those with loco-regional disease. Therefore, serum IL-6 level is the most discriminative factor separating healthy controls and the loco-regional and metastatic breast cancer patient groups (Benoy et al. 2002). Moreover, the patients with high IL-6 levels show significantly poorer survival than patients with low IL-6 levels (Zhang and Adachi 1999). Indeed, higher production of IL-6 is associated with worse outcomes in breast cancer patients at high risk of relapse, however these effects are limited to those patients with ER-positive tumors. IL-6 may exert its effect on breast cancer cells through hormonal pathways (DeMichele et al. 2009). Although both IL-6 and TNF-alpha are expressed by adipose tissue, there are important differences in their systemic release. In contrast to TNF-alpha, IL-6 is released from the subcutaneous adi-

pose tissue depot and thereby able to signal systemically (Mohamed-Ali et al. 1997). Consequently, there is a positive relationship between IL-6 concentration and percentage of body fat (Vozarova et al. 2001). IL-6 genotypes may influence breast cancer risk in conjunction with central adiposity in postmenopausal women (Slattery et al. 2008). Interestingly, the breast tumors of larger size and/or with lymph nodes involvement exhibit higher levels of IL-6 in tumor surrounding adipocytes. Adipocytes participate in a vicious cycle, which is controlled by cancer cells. Thus, invasive cancer cells dramatically affect surrounding cancer-associated adipocytes. Furthermore, cancer-associated adipocytes modify the cancer cell phenotypes leading to a more aggressive behavior (Dirat et al. 2011). Deleterious function of cancer-associated adipocytes is dependent on their crosstalk with invasive cancer cells. Indeed, this event leads to dramatic phenotypic and/or functional modifications of both cell types. These adipocytes exhibit delipidation and acquire a fibroblast-like shape. A high number of cancer-associated adipocytes might be predicted to be detrimental in obesity (Tan et al. 2011). Therefore, IL-6 seems to play a key role in the acquired proinvasive feature of breast cancer cells. Furthermore, IL-6 induces cell migration through the activation of the MAPK pathway, acts as an anti-apoptotic factor, promotes the osteoclasts formation, and inhibits the differentiation of dendritic cells. In this wise, IL-6 facilitates the metastases of breast cancer (Macciò and Madeddu 2011).

Furthermore, tumor-associated IL-1alpha and IL-1beta are present in the tumor microenvironment and may play a pivotal role in regulating invasive cancer and ductal carcinoma in-situ growth and metastasis (Kurtzman et al. 1999). The presence and distribution of IL-1 cytokines and its receptors (IL-1R) in human breast cancer suggests that the local expression of IL-1 results in the activation of a population of cells within the human breast cancer microenvironment. This activation of the IL-1/IL-1R cytokine family via autocrine and/or paracrine mechanisms leads to a cascade of secondary pro-tumorigenic cytokines (Pantschenko et al. 2003) (Fig. 25.1).

Adipocyte fatty acid binding protein 4 (FABP4) is a key adipokine for fatty acid transport. Exogenous FABP4 enhances the proliferation of breast cancer cells and induces the Akt and MAPK signaling cascades. The inhibition of these pathways reduces the exogenous FBAP4-mediated cell proliferation (Guaita-Esteruelas et al. 2016). Although serum adipocyte-FABP (A-FABP) levels are found to be significantly higher in obese than in non-obese women, independent of obesity, the serum A-FABP levels are significantly higher in breast cancer patients than in healthy controls. Furthermore, A-FABP is positively correlated with tumor size and nodal-status (Hancke et al. 2010). In fact, FABP4, adiponectin and retinol binding protein 4 (RBP4) are most down regulated genes in breast cancer. These genes also display strong connections with the other molecules of lipid metabolism pathway in breast cancer (Merdad et al. 2015).

7 Adiponectin and Breast Cancer

Adiponectin is synthesized and secreted almost exclusively by the white adipose tissue. AdipoR1 and AdipoR2 serve as receptors for globular and full-length adiponectin. These receptors mediate the increased AMPK, PPAR-alpha ligand activities, and glucose uptake and fatty-acid oxidation by adiponectin. Obesity decreases expression levels of AdipoR1/R2. Hence, adiponectin sensitivity is reduced in obesity (Kadowaki and Yamauchi 2005). Additionally, reduced expressions of AdipoR1/R2 in obese animals are significantly correlated with decreased binding of adiponectin to membrane fractions. This situation is defined as adiponectin resistance. Increase in adiponectin resistance in turn may play a role in worsening insulin resistance in obesity (Tsuchida et al. 2004). In women with low-circulating adiponectin levels, breast tumors may present a more aggressive phenotype, by large tumor size, high-histological grade, estrogen receptor negativity, and increased

angiogenesis and metastasis (Mantzoros et al. 2004). Adiponectin may act on tumor cells directly by binding and activating adiponectin receptors and downstream signaling pathways (Barb et al. 2006). In fact, breast cancer cells can express both AdipoR1/R2 but not adiponectin. In contrast, women with the highest adiponectin levels have a 65% reduced risk of breast cancer (Körner et al. 2007). The analysis of 885 cases of breast cancer revealed that adiponectin levels are not related to the risk of breast cancer in premenopausal women, however, lower adiponectin levels are associated with a higher risk of breast cancer in postmenopausal women (Ye et al. 2014). Thus, an inverse association is found between adiponectin and postmenopausal breast cancer risk in 1477 incident breast cancer cases. However, a modest correlation is estimated with ductal type of breast cancer, but not lobular tumors. Adiponectin may only influence breast cancer cell proliferation in a low estrogen environment (Tworoger et al. 2007). Nevertheless, the frequency of lymph node metastasis of tumor is significantly increased in the patients with low plasma adiponectin levels. Furthermore, the frequency of tumors with negative estrogen receptor is significantly increased in the patients who have less than the median adiponectin level (Kang et al. 2007) (Fig. 25.1).

The highest high molecular-weight adiponectin levels and lower BMI have shown a significantly reduced risk of breast cancer in both pre and postmenopausal women in a case-control study, including 66 sets of Japanese female breast cancer cases and age and menopausal status matched controls (Minatoya et al. 2014). Low total or high molecular-weight adiponectin associates with risk of breast cancer particularly among premenopausal and obese women. In contrast to cancer tissue, adiponectin expression is elevated in adjacent non-tumor adipose tissues of breast, compared with controls. In both tumor tissue samples and breast cancer cell lines, AdipoR1 expression is two to four times higher than that of AdipoR2 (Körner et al. 2007). Adiponectin decreases breast cancer cell proliferation by inhibiting the entry into S-phase without inducing apoptosis, and this inhibitory effect is mediated through AdipoR1 (Nakayama et al. 2008). In contrast, adiponectin also triggers cellular apoptosis in breast cancer cells in the presence of 17-beta estradiol. A cross-talk between adiponectin and estrogen receptor signaling exists in breast cancer cells (Pfeiler et al. 2008). Higher circulating levels of leptin found in obese subjects could be a growth-enhancing factor, whereas low adiponectin levels in obese women may be permissive for growth-promoting effect of leptin. In any case, estrogen and its receptors have a definite impact on the response of human breast cancer cell lines to leptin and adiponectin (Grossmann et al. 2010). Obesity leads to altered expression profiles of various adipokines and cytokines including leptin, adiponectin, IL-6, TNF-alpha and IL-1beta. The increased levels of leptin and decreased adiponectin secretion are directly associated with breast cancer development (Khan et al. 2013). The leptin/adiponectin ratio provides significant adjunctive information to the risk of metabolic syndrome beyond homeostatic model assessment-insulin resistance (HOMA-IR)) alone (Yoon et al. 2011). In patients with breast cancer, extracellular leptin is higher and adiponectin is lower in tumors than in normal adjacent breast tissue. While estrogen exposure increases leptin secretion in postmenopausal group, tamoxifen treatment increases adiponectin and decreases leptin and the leptin/adiponectin ratio (Morad et al. 2014). A significant reduction in tumor volume is observed in human ER-alpha-negative breast cancer cells, which are pretreated with adiponectin, whereas an increased tumor growth is evident in the human ER-alpha-positive breast cancer cells. Cyclin D1 mRNA and protein levels are also up-regulated in ER-alpha-positive cells by adiponectin (Mauro et al. 2015). Actually, exogenous adiponectin may induce the inhibition of cell proliferation and promotion of apoptosis in breast cancer cells (Chen and Wang 2011). Conversely, breast cancer arising in women with the low serum adiponectin levels are more likely to show a biologically aggressive phenotype (Miyoshi et al. 2003). Since, adiponectin acts directly on breast cancer cells by inhibiting proliferation and angiogenesis or by stimulating apoptosis, it is proposed that

replacement of adiponectin may be of major importance in the prevention and the treatment of breast cancer in obese patients (Delort et al. 2012). Consequently, serum adiponectin levels in ER-negative/PR-negative breast cancer show an inverse relationship with the risk of recurrence (Oh et al. 2011).

8 Insulin Resistance and Insulin-Like Growth Factor in Breast Cancer

The association of insulin with breast cancer outcomes is nonlinear. Insulin resistance is significantly correlated with obesity, which is associated with distant recurrence and death (Goodwin et al. 2002). This connection between insulin resistance and obesity is strongly valid for only postmenopausal patients with high proliferative luminal B/HER-2-negative type of breast cancer (Nam et al. 2016). Evaluation of 22 studies with 7478 cases indicated that fasting insulin and non-fasting or fasting C-peptide levels are not different between women with and without breast cancer, whereas HOMA-IR levels in breast cancer patients are significantly higher than in women without breast cancer (Hernandez et al. 2014). In this respect, hyperinsulinemia is a consequence of insulin resistance or the impaired responsiveness of cells to insulin. This metabolic imbalance is more common in obese women than in normal-weight women (Lazarus et al. 1998). Evaluation of 816 randomly chosen postmenopausal breast cancer cases revealed 2.4-fold increase in breast cancer incidence in women with the highest quartile of fasting insulin concentrations. Ultimately, hyperinsulinemia and insulin or insulin like growth factor-1 (IGF-1)-signaling are independent risk factors for breast cancer and may have a substantial role in explaining the obesity-breast cancer relationship (Gunter et al. 2009). Actually, IGFs are mitogenic and anti-apoptotic peptides that influence the proliferative behavior of many cell types, including normal and transformed breast epithelial cells. IGF binding proteins (IGFBPs), and IGFBP proteases regulate circulating IGF-I levels (Pollak 1998). Circulating insulin levels and plasma levels of IGFBP-3 are simultaneously elevated in women with premenopausal breast cancer (Del Giudice et al. 1998). Approximately 1% of circulating IGF-1 remains unbound, while the rest is mainly bound to IGFBP-3 (Pollak 2008). IGFBP-binding to cell surface results in the release of more IGF to the receptors (McCusker et al. 1990). Secretion of IGFBP-1 and IGFBP-2 is further suppressed by insulin and is diminished with increasing obesity (Hoeflich and Russo 2015). The insulin-cancer hypothesis postulates that chronic hyperinsulinemia is associated with decreased concentrations of IGFBP-1 and IGFBP-2, leads to increased availability of IGF-1 and concomitant changes in the cellular environment that favor tumor formation (Renehan et al. 2006). The IGF-1 and IGFBP-2 are amongst the most potent mitogens for mammary epithelial cells and there are accumulating evidences that they interact with the estradiol-axis to regulate mitogenesis, apoptosis, adhesion, migration and differentiation of mammary epithelial cells. Such interactions are bi-directional and estradiol has been shown to regulate the expression and activity of IGF-axis genes with the general effect of sensitizing breast epithelial cells to the actions of IGFs and insulin (Hawsawi et al. 2013). IGF-1 can specifically target its receptors on human breast epithelial cells to induce mitogenic and anti-apoptotic pathways. Both the PI3K and MAPK pathways are important for IGF-1-stimulated proliferation of human breast cancer cells in vitro. Insulin-receptor substrate (IRS)-1, but not IRS-2, is the predominant signaling molecule activated by IGF-I and insulin (Jackson et al. 1998). For overall survival of breast cancer patients, only histological grade and IGF-1R mRNA emerges as significant predictors. Increased IGF-1R mRNA implies poorer prognosis among the different subtypes of breast cancer, and that may be associated with the lack of responsiveness to tamoxifen in cases with a positive hormone receptor status (Peiró et al. 2011). In contrast, IGF-1R correlates with good prognostic markers among patients with early breast cancer and is differentially expressed with vari-

able prognostic impact among breast cancer subtypes (Yerushalmi et al. 2012). However, IGF-2 is expressed both in the epithelial tumor cells and in stromal cells, in 84% and 50% of breast cancer cases, respectively. IGF-2 expression in cancer cells is related to a non-metastatic disease at diagnosis or to low tumor grade (Toropainen et al. 1995). IGF-1R may be overexpressed in all breast cancer subtypes, regardless of the hormone receptor or HER2 status and its expression rates range from 43.8 to 87% by Allred score (Shimizu et al. 2004; Yerushalmi et al. 2012). There is a significant correlation between IGF-1R and ER status, but not between IGF-1R and PR status. Patients with IGF-1R-positive and ER-negative breast cancers are in a worse situation than IGFR-negative ER-negative cancer patients (Railo et al. 1994). Within 4 years of initial breast tumor diagnosis, elevated levels of IGF-1R are strongly associated with ipsilateral breast tumor recurrence. IGF-1R predisposes to early relapses after radiation therapy, which is thought to be true for the recurrences. After more than 4 years, recurring tumors are more likely to represent de novo primary breast cancers, therefore IGF-1R expression would not be expected as a marker of radioresistance (Turner et al. 1997). On the other hand, reduction of serum IGF-1 levels results in significantly decreased burden of tumors, despite modestly elevated levels of circulating insulin and leptin (Lashinger et al. 2011). In this context, IGFBP-3 induces apoptosis in an IGF-IGFR axis-independent manner through the activation of caspases involved in a death receptor-mediated pathway in human breast cancer cells. Consequently, IGFBP-3 functions as a negative regulator of breast cancer cell growth (Kim et al. 2004) (Fig. 25.1). The characteristics and phenotypic behavior of malignant breast ductal epithelial cells associate with the synergistic activity of adipocyte-derived factors. Adipocyte-secreted substances can affect tumorigenesis by increasing the stabilization of pro-oncogenic factors (Iyengar et al. 2003). Adipocytes from subcutaneous adipose tissue specimens of obese individuals are capable of producing larger amounts of IGF compared with lean women. IGF-1 is a pivotal factor in adipocyte regulation of breast cancer cell growth and it is upregulated at early period of adipocyte differentiation (D'Esposito et al. 2012). Indeed, obesity is associated with significant changes in the growth hormone (GH)/IGF system. In non-diabetic obese subjects, adipocytes produce large amount of free IGF-1 and IGF-2, total IGF-2, IGFBP-3 and growth hormone-binding protein (GHBP), reduced IGFBP-1 and IGFBP-2 when compared to lean subjects. Conversely, in obese Type 2 diabetics, free IGF-I is insignificantly reduced, when compared to non-diabetic obese subjects (Frystyk et al. 1999). Adipocytes-derived IGF-1 release is regulated by glucose and fatty acids and may contribute to the control of breast cancer cell growth in obese individuals (D'Esposito et al. 2012). Actually, IGF-1 and IGF-2 stimulate both normal growth development and breast cancer cell proliferation. IGF-2 induction of the aryl hydrocarbon receptor (AHR) promotes the expression of Cyclin D1 and the proliferation of breast cancer cells (Tomblin and Salisbury 2014). The AHR also regulates cell cycle in part by binding with Cyclin D1 and cyclin dependent kinase 4 (CDK4) in human breast cancer cells (Barhoover et al. 2010). Adipocytes secrete IGF-2 at levels that stimulate the proliferation of human ER-positive breast cancer cells. By contrast, specific exogenous AHR ligands inhibit the proliferative effects of mitogenic adipokines, including IGF-2 in human ER expressing breast cancer cells (Salisbury et al. 2013). In the absence of an exogenous AHR agonist, the AHR is located in the cytoplasm bound to chaperon proteins. In the classical mechanism of AHR action, upon activation by an agonist, the AHR dissociates from chaperon proteins, translocates into the nucleus, and stimulates transcription by binding to AHR response elements (AHRE) (Denison et al. 2011). AHR-dependent gene transcription is terminated by dissociation of the liganded AHR-AHR nuclear translocator (ARNT) complex from the dioxin-responsive element (DRE), subsequent nuclear export of the AHR into the cytosol is mediated by its N-terminal nuclear export sequence followed by ubiquitin-mediated AHR degradation (Pollenz 2002). Although the ER

signaling pathway has many mechanistic similarities to that of the AHR, there are some differences. ER isoforms are localized primarily in the nucleus in their unliganded state and are found complexed with two chaperone proteins (Heldring et al. 2007). However, AHR-ER crosstalk is multifactorial in nature, effecting both ERalpha and ERbeta, and involving a combination of both classical and non-classical AHR-dependent mechanisms that results in inhibition of estrogen response or responsiveness. Eventually, induction of gene expression by the classical AHR-ARNT-DRE signaling pathway can repress ER-dependent signaling (Denison et al. 2011).

Considering breast cancers overall, the relationship between obesity and cancer risk is complex. Obesity is associated with an increased risk of postmenopausal breast cancer. Contrarily, in premenopausal women, obesity has no or even reducing effect on breast cancer risk. However, epidemiologic data have demonstrated that obesity is strongly associated with an increased risk of triple-negative breast cancer in both pre- and postmenopausal women (Millikan et al. 2008). Actually, triple-negative breast cancers are aggressive tumors, which are typically ER-negative, PR-negative and human EGFR-2 negative tumors and targeted therapies for triple-negative breast cancers are almost currently unavailable (Toft and Cryns 2011).

Insulin may influence prognosis to the greatest extent during the first 5 years after diagnosis, whereas obesity-related factors, particularly leptin continue to be important with longer follow-up (Goodwin et al. 2012). IGF-I activates downstream signaling molecules within the leptin receptor and IGF-1R pathways. In contrast to IGFI, leptin does not induce phosphorylation of IGF-1R, indicating that receptor cross-signaling is unidirectional and signal transmission occurs from IGF-1R to leptin receptor in human breast cancer cells (Ozbay and Nahta 2008). Elevated HOMA-IR scores and low levels of adiponectin are both associated with obesity and increased breast cancer mortality in obese breast cancer cases. Hyperinsulinemia is an independent risk factor for poor prognosis for breast cancer and is associated with low levels of adiponectin and shorter breast cancer survival (Duggan et al. 2011). Actually, adiponectin and HOMA-IR have also prognostic significance in breast cancer recurrence. Treatments related to these factors may protect against recurrence in ER-negative/PR-negative patients. Similar results could not be achieved in the case of ER-positive/PR-positive patients (Oh et al. 2011). Interestingly, an inverse, significant association between insulin resistance and cancer recurrence is observed in the ER-positive/PR-positive breast cancer patients. Furthermore, ER-positive/PR-positive patients with hyperglycemia showed decreased risk of recurrence (Oh et al. 2011).

9 Adipose Tissue Hypoxia in Breast Cancer

Increased adipose tissue hypoxia, accompanies obesity. Obesity-induced hypoxia and oxidative stress affect the production of many adipocyte-derived proteins involved in angiogenesis, inflammation and extracellular matrix remodeling. All these alterations can establish a pro-malignant environment for breast tissues (Yao-Borengasser et al. 2015). Cancer-associated adipocytes (CAAs) are essential for breast tumor development and progression. Furthermore, CAAs modify the cancer cell characteristics and phenotype as leading to a more aggressive tumor behavior (Dirat et al. 2011). Adipocytes can modify ER gene expression and promote epithelial-mesenchymal transition in breast cancer cells through upregulation of hypoxia-inducible factor 1 alpha (HIF-1alpha). Obesity plays an important role in the development of an aggressive breast cancer (Yao-Borengasser et al. 2015). Decreased oxygen availability stimulates cells to consume glucose and produce lactate. This response is coordinated by the HIF-1, which is expressed under the control of signaling of the PI3K/Akt/mTOR pathway in tumor cells (DeBerardinis et al. 2008). A significant correlation is demonstrated between impaired glucose tolerance and disease progression in postmenopausal women receiving treatment of breast can-

cer. In particular lactate, are predictive of reduced response to chemotherapy and is prognostic for weight gain during early breast cancer chemotherapy (Stebbing et al. 2012). Hyperinsulinemia could induce breast cancer progression through leptin-dependent mechanisms. Thus, insulin stimulated leptin expression is associated with increased activation of the leptin gene promoter. Excess insulin increases nuclear accumulation of transcription factors HIF-1alpha and their loading on the leptin promoter (Bartella et al. 2008). Furthermore, serum lactate inhibits lipolysis and triglyceride breakdown in adipocytes through a direct activation of the orphan G-protein-coupled receptor, GPR81(Liu et al. 2009). Breast cancer cells can switch to a form that can utilize lactate as a primary source of energy, allowing them to survive in case of glucose deprivation and this activity confers resistance to PI3K/mTOR inhibitors. Estrogen-related receptor alpha (ERR-alpha), is shown to regulate the expression of genes required for lactate utilization (Park et al. 2016). COX-2 directly regulates gene expression of specific aromatase promoter regions and regulates aromatase enzyme activity in breast cancer cells (Prosperi and Robertson 2006). PGE2 also increases HIF-1alpha transcript and protein expression, nuclear localization and binding to aromatase promoter-II in human breast adipose stromal cells. Actually, HIF-1alpha as a modulator of aromatase promoter II-driven aromatase expression in human breast tumor-associated stroma and provides a special mechanism for estrogen regulation in obesity-related, post-menopausal breast cancer (Samarajeewa et al. 2013).

10 Leptin and Breast Cancer

Adipocytes account for more than 90% of human breast volume and secrete adipocytokines, including leptin. Breast adipose tissue-derived leptin promotes the growth of developing breast cancer (Guo et al. 2012; Khandekar et al. 2011). Indeed, the breast cancer cells are surrounded by an adipocyte microenvironment, which is more extensive in obese people. Thus, adipocyte-derived leptin is strongly involved in mammary carcinogenesis by contributing to the local pro-inflammatory mechanisms. Therefore, obese patients with breast cancer have increased metastatic potential and greater risk of mortality (Delort et al. 2015). Serum leptin levels and leptin/BMI ratio are significantly increased in patients with breast cancer (Romero-Figueroa et al. 2013). In the primary breast cancer cases, expressions of leptin and leptin receptor are found in 85% and 75%, respectively. In these cases, the expression of leptin is significantly correlated with leptin receptor. Additionally, leptin receptor expression in primary breast cancer is positively correlated with estrogen receptor expression and tumor size but not with Ki67 or progesterone receptor expressions (Jardé et al. 2008). Both normal epithelial cells and carcinoma cells express leptin. However, overexpression of leptin is observed in 92% of invasive ductal carcinomas but in no normal epithelium. Furthermore, normal mammary epithelial cells do not express a significant level of leptin receptor, whereas 83% of breast cancer cases have leptin receptors. Accordingly, distant metastasis is detected in 34% of all leptin receptor-positive tumors with leptin overexpression, but none of the patients with leptin receptor-negative and weak leptin expressing tumors are associated with distant metastasis (Ishikawa et al. 2004). Higher circulating levels of leptin found in obese individuals and act as growth-enhancing factor. In contrast, low adiponectin levels in obese women may be permissive for leptin's growth-promoting effects (Grossmann et al. 2010) (Fig. 25.1). Leptin/adiponectin ratio are increased significantly in the breast cancer patients, in comparison to controls. BMI is negatively and positively correlated to serum adiponectin and leptin levels, respectively. Low serum adiponectin levels and high serum leptin levels are associated with an increased risk for breast cancer. The blood levels of estradiol increase in parallel to those of leptin. The increased serum ratio of leptin/adiponectin may indicate the presence of aggressive breast cancers (Chen et al. 2006). Furthermore, high leptin levels in obese and overweight individuals are also positively corre-

lated with body fat and adipocyte size. In terms of obesity, leptin is unable to regulate appetite and size of fat deposits leading to a "leptin resistance status" (Guo et al. 2012).

Almost all of the breast cancer samples co-express leptin and its two main isoforms of receptors, leptin receptor-L (long form) OBR-L and OBR-S (short form) at the mRNA and protein levels. OBR-L or OBR-S is found to be positively correlated with the lack of progesterone receptor. Elevated OBR-S expression has longer relapse-free survival, whereas high OBR-L/OBR-S is associated with a shorter relapse-free survival in breast cancer patients. This inverse ratio between OBR-L and OBR-S could be a marker of tumor aggressiveness (Révillion et al. 2006).

Actually, circulating leptin levels are proportional to the body fat mass, thus serve as an adiposity signal of the total body energy stores. However, despite increased leptin levels, animals fed a high-fat diet without decreasing their caloric intake, make them become obese. This suggests that a high content of dietary fat changes the "set point" for body weight, at least in part by limiting the action of leptin (Frederich et al. 1995). Therefore, regulating leptin sensitivity is extremely important to control body weight. In this respect, leptin exerts its biological action through binding to and activating OBR-L (Elmquist et al. 1998; Scott et al. 2009). In parallel with its activation of AMPK, leptin suppresses the activity of acetyl coenzyme A carboxylase, subsequently stimulates the oxidation of fatty acids in muscle. (Minokoshi et al. 2002). OBR-L is capable of signaling to IRS-1 and MAPK via JAK, in addition to activating STAT pathways (Bjørbaek et al. 1997). The short isoform (OBR-S) is inactive in both proliferation and JAK activation. Thereby, resistance to leptin occurs despite the presence of the OBR-S isoforms (Ghilardi and Skoda 1997). Binding of the SH2 domain of SH2B1 to phospho-Tyr813 in JAK2 enhances the leptin induction of JAK2. Actually, SH2B1 acts as signal transducer and signaling adaptor in obesity and its deletion is associated with severe obesity. Overexpression of SH2B1 enhances JAK2-mediated tyrosine phosphorylation of IRS-1 in response to leptin (Li et al. 2007). SH2B1 directly binds to both IRS-1 and IRS-2 and mediates the formation of a JAK2/SH2B/IRS-1 or IRS-2 protein complexes. Consequently, SH2B dramatically enhances leptin-stimulated tyrosine phosphorylation of IRS-1 and IRS-2 and subsequent activation of the PI3K pathway (Duan et al. 2004). mRNA expression of leptin and leptin receptors in adipose tissue and matched tumor samples, respectively, are associated with obesity status in breast cancer. Increasing insulin resistance is a predominant feature of this higher leptin and leptin receptors expression (Carroll et al. 2011). In response to leptin, STAT3 binds to phospho-Tyr1138, allowing JAK2 to phosphorylate and activate STAT3. The JAK2-dependent and -independent pathways act coordinately and synergistically to promote STAT3 activation (Morris and Rui 2009). Indeed, chronic leptin signaling causes significant phosphorylation of JAK2 and STAT3 together with the higher breast cancer cell proliferation rate. In addition to the high ERalpha/ERbeta ratio, estrogen-dependent transcriptional activity, E2-dependent cell growth and resistance to antiestrogen agents is enhanced (Valle et al. 2011). Activated STAT3 and G9a histone methyltransferase in epigenetic silencing of miR-200c promotes the formation of breast cancer stem-like cells. These cells elevate the cell surface levels of the leptin receptors. In breast cancer subjects with diet-induced obesity, STAT3 blockade suppresses the leptin receptor expression and inhibits tumor progression (Chang et al. 2015). Most of obese humans are characterized by leptin resistance (Maffei et al. 1995). Leptin specifically induces expression of SOCS-3 mRNA (Bjørbaek et al. 1998). Thus, increased levels of SOCS-3 in peripheral tissues may therefore result in leptin resistance at these sites. SOCS-3 binds to JAK2 in a leptin-dependent manner and suggest that SOCS-3 attenuates leptin receptor signaling by inhibiting JAK-induced tyrosine phosphorylation of the receptor and of JAK itself (Bjørbaek et al. 1999). Collectively, the deterioration of the leptin signals results in obesity. Leptin resistance in obesity may occur with various mechanisms such as; reduction in leptin transport into the brain, defects in OBR-L trafficking, as well as in OBR-L

expression, increase in SOCS3 expression, chronic endoplasmic reticulum stress by activation of various unfolded protein response-signaling pathways and impairment of melanocortin-4 or brain-derived neurotrophic factor receptors (Morris and Rui 2009).

On the other hand, in approximately 30% of population, leptin is able to stimulate aromatase activity in adipose stromal cells at high concentrations. The elevated levels of aromatase activity may contribute to increase in the circulating estrogen levels in obese women. In postmenopausal obese women, adipose tissue is the only place of estrogen production by aromatization of C19 steroid androstenedione. Hence, the total pool of estrogens is increased by the aromatization process in postmenopausal obese women (Magoffin et al. 1999). Plasma leptin levels correlate to plasma levels of estradiol and estrone sulfate in breast cancer patients as well as in healthy postmenopausal females. In addition, plasma leptin levels also correlate to total body aromatization rate in breast cancer patients with obesity (Geisler et al. 2007). Factors that increase endogenous estrogen production or reduce the binding of estradiol to sex hormone-binding globulin may increase a woman's risk of developing breast cancer later in life (Toniolo et al. 1995). On the other hand, PPAR-gamma ligands show an inhibitory effect on the growth of breast cancer cells. PPAR-gamma is a member of the nuclear receptor family of ligand-dependent transcription factors (Elstner et al. 1998). In this respect, activation of PPAR-gamma also decreases leptin receptors, inhibits their transductional pathways, and negatively interferes with estrogen signaling through the down-regulation of aromatase gene expression and the inhibition of ER-alpha transactivation (Catalano et al. 2011).

Twenty-four per cent of 1352 total patients with breast cancer, are classified as obese. When the obese patients are compared with the normal weight cancer patients, obesity is found to be associated with non-palpable tumors, larger tumors, a higher incidence of lymph node metastasis, lower incidence of HER2 positivity, lower incidence of multifocality (Haakinson et al. 2012). Actually, mRNA levels of the progesterone receptor isoforms positively correlated with protein levels of estradiol and progesterone receptors. The PR isoforms' mRNA levels are inversely correlated with clinical-pathological markers of tumor aggressiveness. Furthermore, the progesterone receptor isoforms are positively correlated with the mRNA levels of HER/ErbB receptors and ligands which are associated with more differentiated phenotypes of breast cancer (Lindet et al. 2012). An interaction between leptin signaling and the transmembrane tyrosine kinase receptor HER2 has been shown in breast cancer. Leptin receptor and HER2 are co-expressed in these tumors and leptin/OBR system contributes to the enhanced HER2 activity and reduced sensitivity to anti-HER2 treatments (Fiorio et al. 2008). Overexpression of HER2 in a series of breast carcinoma cells increases the aldehyde dehydrogenase-expressing cancer stem cell population which displays increased invasion and increased tumorigenesis (Korkaya et al. 2008). Furthermore, in different human breast cancer cell lines, leptin enhances the expression of a chaperone protein heat shock protein 90 (Hsp90). Eventually, HER2 protein levels are increased. In contrast, silencing of Hsp90 gene expression by RNA interference inhibits leptin-mediated HER2 up-regulation. The adipocyte-secreted leptin modulates Hsp90/HER2 expressions in breast cancer cells. This mechanism links the obesity to breast cancer growth and progression. However, long-term leptin exposure reduces sensitivity of breast cancer cells to the anti-estrogen agents (Giordano et al. 2013). Synergy between the leptin/leptin receptor/STAT3 signaling pathway and the HER2 receptor protects tamoxifen-treated HER2 overexpressing cells from the inhibitory effect of tamoxifen through differential regulation of apoptosis-related genes (Papanikolaou et al. 2015). Leptin enhances cyclin D1 gene transcription by inducing the binding of ER-alpha to the promoter of cyclin D1 gene. In contrast, leptin receptor deficiency significantly enhances the inhibitory effects of tamoxifen on tamoxifen-resistance-breast cancer cell proliferation and survival. However, long-term endocrine therapy facilitates leptin and leptin receptor overexpression in breast

cancer cells, which attenuates the inhibitory effect of tamoxifen by activating both the ERK1/2 and STAT3 signaling pathways and upregulating cyclin D1 gene expression (Qian et al. 2015).

Collectively, growth of malignant cells could be regulated by various leptin-induced second messengers like STAT3, AP1, MAPK and extracellular signal-regulated kinases (ERKs). They are all involved in aromatase expression, generation of estrogens and activation of ER-alpha in malignant breast epithelium (Sulkowska et al. 2006). In this respect, leptin switches on transcription of aromatase, and activates this enzyme with engagement of AP1 promoter, STAT3 and ERK2 by promoting estradiol synthesis (Catalano et al. 2003). Leptin also activates ER-alpha via the MAPK pathway (Catalano et al. 2004). ER-alpha36 is mainly expressed on the cell surface and mediates membrane-initiated or non-genomic estrogen signaling (Wang et al. 2005). Hence, in ER-negative breast cancer cells Src acts as a switch in ER-alpha36-mediated biphasic estrogen signaling through the Src/EGFR/STAT5 pathway. Similar to ER-positive breast cancer cells, ER-alpha36 mediates mitogenic signaling of low-concentration estrogen through the EGFR/Src/STAT5 pathway in ER-negative breast cancer cells (Zhang et al. 2012). After exposure to leptin, ER-alpha-positive breast cancer cells undergo proliferation. This process is mediated by active STAT3 and ERK1/2. However, elevated serum levels of leptin maintain resistance to anti-estrogen drugs during hormonal therapy of breast cancer (Garofalo et al. 2004). Despite 12 to 20-fold higher leptin levels of obese mice than that of lean mice, malfunction leptin receptors effectively protect against mammary tumor incidence (Cleary et al. 2004b).

Actually, there is a crosstalk between Notch, IL-1 and leptin in breast cancer. Leptin induction of proliferation/migration and upregulation of VEGF/VEGFR-2 in breast cancer cells are related to an intact Notch signaling axis (Guo and Gonzalez-Perez 2011). However, leptin upregulation of VEGF/VEGFR2 is impaired by IL-1 signaling blockade (Zhou et al. 2011). The notch ligands are single-pass transmembrane proteins and are members of the Delta/Serrate/LAG-2 family of proteins. Translocation of the Notch intracellular domain into the nucleus induces the transcriptional activation of Notch target genes. The carcinogenesis process is reinforced by Notch crosstalk with many oncogenic signaling pathways suggest that Notch signaling may be a critical drug target for breast cancer (Guo et al. 2011).

11 Conclusion

Obese women have elevated risks of ductal and ER-positive-PR-positive breast cancer compared to lean subjects. Estrogen-plus-progestin therapy for more than 5 years significantly increases risks of lobular and ER positive-PR positive breast cancer compared to never users of hormone therapy regardless of BMI. In addition, elevated estrogen concentrations in post-menopausal breast cancer patients are largely derived from obese adipose tissue aromatization. Most breast cancer cell types are addicted to fatty acids. Hence, elevation of circulating free fatty acids in obesity is associated with enhanced cancer risk. The obesity-inflammation-aromatase axis is an indicator of the increased risk of hormone receptor-positive breast cancer and is a sign of poor prognosis. Increase in risk of recurrence and death in breast cancer is significantly higher within 10 years of diagnosis in obese women. Low-circulating adiponectin levels in breast cancer patients are associated with the increased rate of more aggressive, high-histological grade, estrogen receptor negative, and metastatic tumors. Hyperinsulinemia is an independent risk factor for poor prognosis in breast cancer, and is associated with low levels of adiponectin and shorter breast cancer survival in obesity. Serum leptin levels, leptin-BMI and leptin-adiponectin ratios are significantly higher in obese patients with breast cancer.

Body weight control and avoiding estrogen-plus-progestin therapy is an effective strategy for primary prevention of breast cancer among post-menopausal women. Because of these,

recommendations should be taken into account also for young adult pre-menopausal women, obesity has impacts on the responsiveness to the endocrine therapy with aromatase inhibitors of pre-menopausal breast cancer patients. Excessive weight gain in premenopausal women is associated with the increase in relapse risk and higher mortality rate in breast cancer.

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Lipotoxicity in Obesity: Benefit of Olive Oil

26

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Abstract

The clinical implication of Lipotoxicity in obesity derives primarily from its potential to progress to insulin resistance, endothelial dysfunction and atherosclerosis. Olive oil rich diet decrease accumulation of triglyceride in the liver, improved postprandial triglyceride levels, improve glucose and GLP-1 response in insulin resistant subjects, and up regulate GLUT-2 expression in the liver. The exact molecular mechanism is unknown but, decreasing NFkB activation, decreasing LDL oxidation and improving insulin resistance by less production of inflammatory cytokines (TNF-a, IL-6) and improvement of kinases JNK-mediated phosphorylation of IRS-1 are the principle mechanisms. The beneficial effect of the Mediterranean diet derived from monounsaturated fatty acids (MUFA), mainly from olive oil. In this review we document lipotoxicity in obesity and the benefit of olive oil.

Keywords

Lipotoxicity • Insulin resistance • Inflammation • Endothelial dysfunction • Atherosclerosis • Obesity • Steatohepatitis • Fatty liver • Olive oil

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1 Introduction

Obesity reduce life expectancy of up to 7 years compared with normal weight individuals and predict the future development of metabolic syndrome, Diabetes (T2DM), and cardiovascular disease (Freedman 2011). Excess adiposity and poor cardiorespiratory fitness drive the epidemic of T2DM and early cardio vascular disease (Peters et al. 2003).

Cardiovascular complications are the main cause of morbidity and mortality in obese, insulin resistance and type 2 diabetes mellitus (T2DM). Complications can be divided in micro vascular (retinopathy, neuropathy, nephropathy) and macrovascular (atherosclerosis, CAD, PVD, and Stroke). Multiple mechanisms contribute to these clinical outcomes including hyperglycemia, hyperinsulinemia, insulin résistance, inflammation, change in circulating adipokines, and alteration in intracellular signaling pathway. Increased circulating concentration of lipids and altered tissue metabolism of lipids is consistent feature of this prevalent condition and contribute importantly to cardiovascular complications.

2 Lipotoxicity

Lipotoxicity is the diverse effect of fatty acid accumulation in non-adipose tissue, the liver, muscle, pancreatic beta-cell, cardiac and arteries are the main targets. The term was coined by Unger to describe the deleterious effects of tissue fat accumulation on glucose metabolism; however the term has assumed added significance (Kashyap et al. 2003).

Mechanism of Toxicity: Spectroscopic studies by MRI have demonstrated that intra myocellular and intrahepatic fat accumulations are closely associated with organ specific insulin resistance by impairing insulin signaling and multiple intracellular steps of glucose metabolism (Mayerson et al. 2002; Belfort et al. 2006; Miyazaki et al. 2002; Bajaj et al. 2003). The deleterious effect of increase intracellular fat on insulin sensitivity is supported by the work of Kim et al. who over expressed lipoprotein lipase in muscle and or liver in mice (Kim et al. 2001). Plasma NEFA elevation by lipid infusion also increased intramyocellular diacylglycerol, a potent activator of protein kinase C (PKC) isoforms, which inhibits insulin signaling through serine phosphorylation of IRS-1 (Heydrick et al. 1991), were demonstrated in animals and humans (Yu et al. 2002; Itani et al. 2002). Ceramide levels are also increased in muscle and plasma in obese and T2DM individuals, correlating with severity of insulin resistance (Adams et al. 2004; Haus et al. 2009; DeFronzo 2010).

Adiposities have a great ability to adapt to over feeding by means of hypertrophy and hyperplasia. Within this context, adipose tissue must be viewed primary as a protective tissue that store and prevent excessive exposure of other organs to fatty acid (Lefterova and Lazar 2009; Lumeng and Saltiel 2011). Protection from chronic energy supply and access triglyceride accumulation in tissue (liver, pancreatic beta-cell, muscle) require an extraordinary adaptation by adiposities that involves activation of several inflammatory pathway but at the cost of insulin resistance, the most relevant of this pathway in obesity are the inhibitor kB Kinase/ nuclear factor kB pathway in which FFA active Toll-like-receptor (TLR-4) in macrophage and adiposities. Pharmacologically decreasing plasma FFA levels restores hepatic insulin sensitivity (Lefterova and Lazar 2009; Lumeng and Saltiel 2011; Holland et al. 2011).

2.1 Role of Lipotoxicity in Liver, Muscle, Beta-Cells, Cardiac and Vessel Disease

In Skeletal muscle: Increase plasma FFA concentration impairs insulin signaling and cause skeletal muscle insulin resistance, and increase in intra myocellular lipids (Cusi et al. 2007). Intra myocellular diacylglycerols concentration have been reported to be markedly high, this metabolites impair insulin signaling and activate inflammatory pathway, including certain protein kinas C isoforms and IkB/nuclear factor kB. A decrease

in plasma FFA improves rapidly insulin sensitivity (Cusi et al. 2007).

In Pancreatic beta-cell: One-third of obese adults aged >60 have "pre-diabetics" in the USA. Sustained elevation of plasma FFA levels impairs insulin secretion in lean subjects, and in non-diabetic subjects genetically predisposed to develop T2DM (Unger et al. 1801). FFA induced pancreatic bet-cell dysfunction and can be rapidly reversed in these subjects by decreasing the release of FFAs (Cusi et al. 2007).

Liver: Two third of fatty acid delivery are supplies by the adipocytes. In lean subjects insulin resistance can be induced rapidly by a lipid infusion (Barrows and Parks 2006; Belfort et al. 2005). The presence of diabetes is associated with advanced liver disease, cirrhosis, and hepatocellular carcinoma (Harrison 2006; Cusi 2009; Beymer et al. 2003; Porepa et al. 2010; Starley et al. 2010; Okanoue et al. 2011). There is close relationship between obesity, T2DM and non alcoholic fatty liver disease (NAFLD) (Ortiz-Lopez et al. 2012). The prevalence of steatohepatitis (NASH) among patients with NAFLD range from 15 to 40% and increases in the presence metabolic syndrome (Beymer et al. 2003; Porepa et al. 2010; Starley et al. 2010; Okanoue et al. 2011; Ortiz-Lopez et al. 2012; Musso et al. 2010; Chitturi et al. 2011). Disease progression from bland steatosis to NASH is associated with mitochondrial dysfunction, endoplasmic reticulum stress, reactive oxygen species formation, and active inflammatory pathway by toxic lipids metabolites such as diacylglycerols, ceramides and others (Choi and Diehl 2008; Gentile and Pagliassotti 2008). Development of severe advanced fibrosis is believed to occur in 10–20% of patients with NASH (Gentile and Pagliassotti 2008; Vernon et al. 2011; Wong et al. 2012). Recent metabolic studies in patients with NASH suggests that liver fibrosis correlates closely with sever adipose tissue insulin resistance (Ratziu et al. 2010), rendering further support to the link between obesity and fibrosis and making adipose tissue a potential target for the prevention of disease progression.

Vessel: Endothelial dysfunction is a hallmark in obesity and diabetes-related vascular dysfunction. Central aspect of endothelial dysfunction is reduced nitric oxide (NO) bioactivity. Multiple alterations such as hyperglycemia, oxidative stress, activation of rennin-angiotensin system and increased pro-inflammatory cytokines contribute independently and synergistically to endothelial dysfunction. Vascular insulin resistance correlates with endothelial dysfunction (Kim et al. 2006; Wende et al. 2012a). This relationship is driven by a pathway-selective inhibition of insulin mediated activation of NO synthase (eNOS) by PI3K and AKT, whereas MAPK signaling to endothelin 1 (ET-1) is intact or even augmented (Jiang et al. 1999). The resulting endothelial cell dysfunction renders the vascular wall more susceptible to atherosclerosis and less responsive to agonist-induced vasodilatation (Rask-Madsen et al. 2010; Maeno et al. 2012).

3 Lipotoxicity, Insulin resistance and Atherosclerosis (Fig. 26.1)

Increase FFA impair endothelial cell insulin signaling and NO production through the activation of the inhibitor β/nuclear factor kB pathway, and experimentally induced plasma FFA elevation in humans alter endothelial function (Boden 2008; Boden et al. 2005). In vivo studies provide that insulin promote atherogenesis (Cruz et al. 1961; Duff and McMillan 1949; Stamler et al. 1960). Non diabetic chickens fed high-cholesterol diet develops sever atherosclerosis, which regress when switched to low-cholesterol diet (Stamler et al. 1960). Insulin administration prevented regression of coronary atherosclerosis (Duff and McMillan 1949). In other experiment; dogs receiving a low dose insulin infusion into hind limb develop sever atherosclerosis, whereas all other arteries remained free of atherosclerosis plaque (Cruz et al. 1961). Other effect of FFA is inducing inflammation. Palmitic, Oleic, and Linoleic acid comprise 70% of the total circulating FFA (Knopp et al. 2000). Palmitate signals

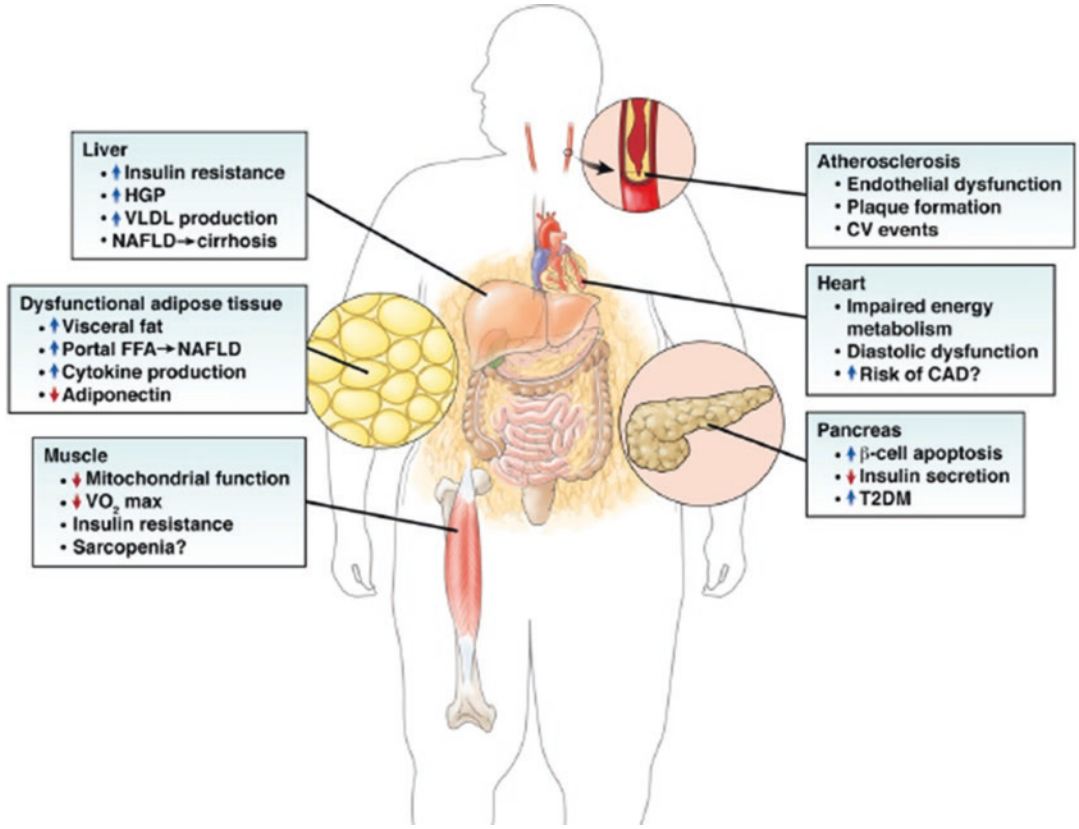

Fig. 26.1 Link between lipotoxicity, insulin resistance, inflammation and atherosclerosis: increased plasma NEFA/intramyocellular levels of toxic lipids metabolites (long-chain fatty acyl CoAs, diacylglycerol, ceramides) play a role in the pathogenesis of muscle, liver and other tissue insulin resistance

via TLR4, a pattern recognition receptor that is essential for initiating inflammatory responses associated with innate immunity (Schwartz and Reaven 2006; Schwartz et al. 2010). When palmitate signal via TLR4, allowing nuclear translocation of NF kβ-kinase (IKK), a transcriptional activator of the expression of many gens involved in inflammation (Schwartz and Reaven 2006).

4 Myocardial Steatosis and Cardiac Function

Recently, proposed mechanisms involved in cardiac lipid utilization and the development of lipotoxicity in the heart that lead to contractile dysfunction. The mechanism includes altered AMPK signaling, ceramide accumulation, endoplasmic reticulum (ER)-stress, ROS, and mitochondrial dysfunction (Wende and Abel 1801). Studies via MRI (O'Connor et al. 2011; Labbé et al. 2011) have increased the understanding of myocardial lipid accumulation and metabolic fat of circulating fatty acid. Analysis of cardiac tissue has provided impair cardiac function in subjects with obesity and T2DM, with or without heart failure (Anowski et al. 2010; Marfella et al. 2009). Change in cardiac lipids content can also be induced in otherwise healthy individual subjected to short-term hyperinsulinemia and hyperglycemia (Winhofer et al. 2012), temporally increasing circulating

FFAs in healthy individuals exercising the fasted state. Reducing lipid accumulation in obese individual leading to improved cardiac function (Schrauwen-Hinderling et al. 2010). Heart failure is associated with increased lipid accumulation in cardiac tissue (García-Rúa et al. 2012), lipid accumulation in the failing heart may be reversed by mechanical unloading, which partially alleviate mitochondrial dysfunction and insulin resistance (Chokshi et al. 2012). However reducing circulating FFAs during heart failure is not sufficient to restore cardiac function (Halbirk et al. 2010).This accumulation of FFAs reflect complex interaction between the metabolic and neurohumoral milieu and change in mechanisms governing cardiac lipid uptake and metabolism. However the functional implication of myocardial steatosis is context-dependent, where it may be maladaptive, or could represent adaptation that might be cardio protective. Where triglycerides per se are not likely to be toxic, but may be a biomarker for the accumulation of more toxic and reactive lipids. Paradoxically, increase dietary fat, may lead to cardio protection in heart that are already undergoing left ventricular remodeling (Wende et al. 2012a).

5 Benefit of Olive Oil

Diet and nutrition, in particular the amount and type of fat intake, has been linked to insulin resistance, an increased risk of developing type 2 diabetes and impaired postprandial lipid metabolism (Hu et al. 2001; Thomsen et al. 1999). "Mediterranean diet" has been associated with higher survival and for lower all-cause mortality. The main characteristics of the Mediterranean diet include an abundance of plant food (fruits, vegetables, whole-grain cereals, nuts, and legumes); olive oil as the principal source of fat; fish and poultry consumed in low- to moderate amounts; relatively low consumption of red meat; and moderate consumption of wine (Trichopoulou et al. 2003; Knoops et al. 2004).

5.1 Composition of Olive Oil

Each 100 g of olive oil contains the following fatty acids: MUFA 73.7 g (n-9 oleic acid 18:1); saturated fatty acids (SFA) 13.5 g (16:0 palmitic acid); polyunsaturated fatty acids (PUFA) 7.9 g (n-6 linoleic acid 18:2, and n-3 alpha linoleic acid 18:3) (DeFronzo 2010). MUFAs include palmitic (C16:1), oleic (C18:1), elaidic (C18:1) and vacentic acids (C18:1). The most abundant MUFA in the diet is oleic acid (C18:1 n-9) (Lefterova and Lazar 2009). In Mediterranean countries, the main source of MUFA is olive oil (74 g/100 g). Other oil sources of MUFAs are canola (59 g/100 g), peanut (46 g/100 g), sunflower (32 g/100 g), corn (29 g/100 g), soybean (24 g/100 g) and safflower oils (14 g/100 g) (Lumeng and Saltiel 2011). Additionally, new oil variants, rich in oleic acid have been developed including high-oleic acid sunflower oil (84 g/100 g) and high oleic acid safflower oil (74 g/100 g) (Holland et al. 2011). In addition to a high MUFA content, virgin (unrefined) olive oil contains a significant amount of antioxidants and α-tocopherol and phytochemicals. However, when refined or heated, olive oil loses these natural compounds (Ros 2003). Olive oil is graded according to its acidity. Extra virgin olive oil, the first pressed oil, having maximum free acidity, contains an abundance of squalene and phenolic antioxidants including simple phenols (hydroxytyrosol, tyrosol), aldehyde secoiridoids, flavonoids and lignans (acetoxypinoresnol, pinoresinol). Interestingly, it contains significantly higher concentrations of phenolic antioxidants and squalene than refined virgin and seed oils. In addition, seed oils, which contain very low amounts of squalene, have none of the phenolic antioxidants that are present in virgin and refined olive oils (Owen et al. 2000). The exact composition of olive oil depends not only on the growth conditions in the year preceding the harvest, but also on the degree of ripeness of the fruit and the technical processing (cold pressing, refining) (Ros 2003).

Oleic Acid: Olive oil is approximately 72% oleic acid, a monounsaturated fatty acid. Olive oil is unique with respect to the high oleic acid content because the majority of seed oils are

composed primarily of polyunsaturated fatty acids, including the essential omega-6 fatty acid, linoleic acid. Compared to polyunsaturated fatty acids, oleic acid is monounsaturated, meaning it has one double bond, making it much less susceptible to oxidation and contributing to the antioxidant action, high stability, and long shelf life of olive oil.

Mechanism action of oleic acid and other minor compounds of olive oil (Table 26.1): the main mechanism by which the components of olive oil influence endothelial activation involves inhibition and/or scavenging of ROS. Oleic acid and h-sitosterol may reduce intracellular ROS by creating a less-oxidant environment through inhibition of intracellular ROS production. H-sitosterol may also enhance SOD activity, hence decreasing O_2^- levels. This reduction has also observed for the terpenoid oleanolic acid, although the mechanism not known, tocopherols and phenolic compounds are potent antioxidant that may help lipid peroxidation and scavenge intracellular free ROS and free NO, reduction of $OONO^-$. ROS can active the NF-κB, which is then translocated into the nucleus, where it binds to recognition sequences in DNA to induce gene expression. This mobilization of NF n B is blocked by α-tocopherol succinate but not by a-tocopherol. In contest, phenolic compounds have been proposed to act blocking the formation of NF-κB/DNA binding complexes. NF-κB modulation the expression of cytokines. LOX and COX, thereby affecting the levels of adhesion molecules and eicosanoids. However, some of the minor compounds of olive oil may act directly on this enzymes and cytokines. LOX and COX activities are inhibited at different points by phenolic and triterpenoids whereas IL-1 expression is inhibited by phenolic and tocopherols, contributing to protect the endothelium against vasoconstriction, platelet aggregation and monocyte adhesion. Vasodilatation is also suggested to be enhanced by oleuropein and oleanolic acid through an increase in the production of NO (Wende et al. 2012b) (Table 26.1).

Table 26.1 Mechanisms of action of olive oil on lipotoxicity

Mechanism	Component involved
Anti-inflammatory and immuno modulatory effects	Oleic acid
	Phenolic compounds
Anti-oxidants	Oleic acid
Decrease lipid peroxidation	Phenolic compounds: hydroxytyrosol, oleuropein, caffeic acid, o-coumaric acid, vanillic acid, and dihydroxyphenylethanol
Decrease oxidative DNA damage	
Modulation of transduction pathways	Oleic acid
Decrease arachidonic acid	Phenolic compounds: protocatechuic acid
Inhibit lipoxygenase	Hydroxytyrosol
Inhibit HMG-CoA reductase	Squalene
Decrease in RAS activation	Squalene
Regulation of gene expression in liver regeneration (Oleic acid inhibit ð6-desaturase which decrease PGE2 and inhibit liver regeneration)	Oleic acid
	Minor compounds
Change in membranes fluidity and membrane Peroxidation (estrogen modulator, regulate G protein)	Oleic acid
	Lignans

6 The Effect of Olive Oil on Specific condition (Gill et al. 2005)

Coronary Heart Disease : Oxidation of LDL cholesterol has been identified as one of the first steps in the development of atherosclerotic lesions by promoting injury to the arterial wall through several mechanisms, including growth factor and chemotactic protein expression, inflammation, and increased local macrophages. Macrophages bind to

and engulf oxidized LDL—an innate immune response to tissue damage. This engulfment produces a fatty foam cell, which, when combined with other cells, produces a fatty streak in the blood vessel (Ebaid et al. 2010). Oxidized LDL can also be taken up directly by endothelial and smooth muscles cells, leading to formation of fatty streaks, which is the first sign of atherosclerosis. The lesions forming atherosclerotic plaques are made up of lipids, endothelial and smooth muscle cells, and extracellular matrix. The plaque environment is proinflammatory. Inflammation occurring prior to the formation of fatty streaks and atherosclerotic lesions causes alterations to the endothelial cell wall, which increases the adhesion of leukocytes, LDL cholesterol, and platelets. This contributes to the development of atherosclerosis and cardiovascular disease (Ebaid et al. 2010).

The Giovanna study (Perona et al. 2006) have demonstrate for the first time that olive oil enhanced fat oxidation and regulated myocardial metabolic enzymes, optimizing cardiac energy metabolism in obesity conditions. Olive oil and its minor phenolic compounds, oleuropein and caffeic acid had myocardial antioxidant activity in standard-fed conditions.

Hypertension: As with other aspects of cardiovascular diseases, there is a reduced incidence of hypertension in populations that consume the Mediterranean diet (Ruiz-Gutierrez et al. 1997; Psaltopoulou et al. 2004), and adherence to the Mediterranean diet is inversely related to systolic and diastolic blood pressure (Gilani et al. 2005). Several studies have demonstrated the antihypertensive properties of olive oil (Keys et al. 1986; Ferrara et al. 2000a; Alonso et al. 2006). Epidemiological data from studies in three Mediterranean countries—Italy, Greece, and Spain—as well as non-Mediterranean countries, suggest a protective effect for monounsaturated fatty acids or olive oil, while non Mediterranean countries show little or no positive effects (Ferrara et al. 2000).

Ferrara et al. compared a diet rich in polyunsaturated fatty acids (from sunflower oil) with a diet high in monounsaturated fatty acids (from olive oil) in patients taking antihypertensive medications (Herrera et al. 2001) and found individuals who consumed an olive oil-rich diet were able to reduce the dosage of antihypertensive medication. Either tan the mechanism of action for blood pressure reduction is unknown, although several theories have been proposed. Giuliani et al. concluded that olive oil is a calcium channel antagonist; closely mimicking the effects of the calcium channel blocker drug verapamil.1 another suggested mechanism is via improved endothelial function (Ruiz-Gutierrez et al. 1997; Psaltopoulou et al. 2004; Gilani et al. 2005; Keys et al. 1986; Ferrara et al. 2000; Alonso et al. 2006; Herrera et al. 2001; Newmark 1997). Phenols and oleic acid may contribute to improved endothelial function by reducing ROS. Other potential mechanisms have been suggested, including decreasing vascular tone and changes to the fatty acid and phospholipid composition of the aorta (Herrera et al. 2001).

Effect on low density lipoprotein (LDL): Animal studies have shown Squalene added to the diet of rats resulted in an 80% increase in serum squalene levels and inhibition of the hepatic enzyme HMG-CoA reductase the enzyme inhibition may be due to squalene or its metabolites. HMG-CoA reductase (Relas et al. 2000), the rate-limiting enzyme in the biosynthesis of cholesterol, results in decreased production of cholesterol and the intermediates formed during its biosynthesis (Relas et al. 2000). Following acute administration of squalene, the rate of cholesterol synthesis increased 9–24 h post-administration. This apparent conflicting data may be a result of the single dose of squalene used (Strandberg et al. 1990).

These observed differences may be due to the dose of squalene. Short-term studies have shown increased dietary squalene, while increasing serum squalene levels, does not cause an increase in serum cholesterol or atherosclerosis (Strandberg et al. 1990; Fraser et al. 2008).

Liver: Decreasing total fat consumption and shifting to MUFAs found in olive oil (20–40% of total energy) or n-3 PUFAs found in fish oil (2 g/d) could lead to a decrease in postprandial lipidemia and steatosis. In one study (Esposito et al. 2004) a modified Mediterranean diet

(42% carbohydrates) was associated with lower alanine aminotransferase (ALT) levels at both 6 and 12 months compared with both the 2003 American Diabetes Association (ADA) diet and a low-glycemic-index diet, independent of weight changes. People allocated to a Mediterranean diet have lower circulating levels of triglycerides and less abdominal obesity, as compared with control diets (Rodríguez-Rejón et al. 2014; Fitó et al. 2014; Paniagua et al. 2007).

7 Conclusion

Dietary fat content modified fat accumulation in obese subjects. Obese patients with NAFLD have a higher postprandial TG response and an increased production of VLDL suggesting that the metabolism of dietary fat is impaired in these individuals. Decreasing total fat consumption and shifting to monounsaturated fat found in olive oil could lead to a decrease in postprandial lipidemia and in Lipotoxicity. Further studies in humans are needed to ascertain whether the consumption of olive oil may be helpful in obese individuals with lipotoxicity and NAFLD.

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