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Alcohol, Nutrition, and Health Consequences

Humana Press

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Series Editor Page

The great success of the Nutrition and Health Series is the result of the consistent overriding mission of providing health professionals with texts that are essential because each includes: (1) a synthesis of the state of the science; (2) timely, in-depth reviews by the leading researchers in their respective fields; (3) extensive, up-to-date, and fully annotated reference lists; (4) a detailed index; (5) relevant tables and figures; (6) identification of paradigm shifts and the consequences; (7) virtually no overlap of information between chapters, but targeted, inter-chapter referrals; (8) suggestions of areas for future research; and (9) balanced, data-driven answers to patients' as well as health professionals' questions which are based upon the totality of evidence rather than the findings of any single study.

The series volumes are not the outcome of a symposium. Rather, each editor has the potential to examine a chosen area with a broad perspective, both in subject matter as well as in the choice of chapter authors. The editors, whose training is both research and practice oriented, have the opportunity to develop a primary objective for their book, define the scope and focus, and then invite the leading authorities to be part of their initiative. The authors were encouraged to provide an overview of the field, discuss their own research, and relate the research findings to potential human health consequences. Because each book is developed de novo, the chapters are coordinated so that the resulting volume imparts greater knowledge than the sum of the information contained in the individual chapters.

Alcohol, Nutrition and Health Consequences, edited by Dr. Ronald Ross Watson, Dr. Victor R. Preedy, and Dr. Sherma Zibadi is a very welcome addition to the Nutrition and Health Series. The 43 chapters in this comprehensive volume examine the clinical consequences of alcohol including the beneficial as well as detrimental effects. The book is logically organized into seven sections and begins with an overview section that includes informative chapters on the genetics of alcohol metabolism, laboratory models, and the very earliest effects of alcohol on the embryo and breast-fed neonate. The extensively referenced chapter on alcohol's effects during embryopathy contains excellent tables and figures that describe the consistent detrimental findings of ethanol-induced lipid peroxidation.

The second section contains six chapters that describe both the beneficial as well as the adverse effects of alcohol on the nutritional status of individuals and the nutritional value of certain foods. The chapters review these effects on overall metabolism. The chapter on specific effects on protein contains comprehensive figures and the chapters on lipids and the clinical consequences of alcohol-induced vitamin B12 deficiency contain important, relevant references. Additionally, there are chapters that examine at-risk, culturally specific populations including Native Americans.

The third section contains unique chapters that examine the potential for certain foods and food components to affect alcohol metabolism. Individual chapters review the effects of plant polyphenols, folic acid, zinc, tocotrienols, soy products, oats, and omega 3 fatty acids. Organ systems and disease conditions reviewed include mammary tissue, immune function, HIV infection, maternal to fetal nutrient transfer, gastrointestinal permeability and emptying, liver function including drug detoxification, alcoholic liver disease, cognitive function, and Alzheimer's disease.

Alcohol has been shown to interact with foods and food components to either enhance or depress the food's biological effects. Alcohol can also affect metabolism of foods and food components. Five chapters examine alcohol's interactions with dietary components. One example of the complex interactions involves the consumption of energy drinks especially among young adults who frequently use energy drinks as a mixer with alcohol. The most common active ingredients in energy drinks include caffeine, taurine, guarana, and ginseng. The combination of alcohol and energy drinks appears to increase alcohol absorption as well as the consumption of large volumes of alcohol. The combinations of caffeine and alcohol and cigarette smoking and alcohol are reviewed in the next two chapters that examine the potential benefits and risks of these combinations. The physiological rationale for the frequently seen co-use of cigarettes and alcohol may be due to their stimulation of specific brain areas, as reviewed in the next chapter. The final chapter in this section reviews the complex interactions between alcohol use and its effects on metabolism in individuals at risk for HIV and infected with HIV. The data suggest that there is no safe level of alcohol intake for HIV-infected individuals due to the interactions between alcohol, liver function, HIV drug detoxification, and other factors including the often malnourished state of the patient.

Alcohol consumption can affect the potential to develop certain chronic diseases as well as exacerbate already existing chronic conditions; however, moderate intake may reduce the risk of certain diseases. Section E, containing eight chapters, reviews the association of alcohol with chronic diseases. The chapter on cataracts reviews the role of lifestyle, type 2 diabetes, nutrient status, cigarette smoking, and other factors that are known to increase cataract risk and then examines the data suggesting that alcohol may be an independent risk factor for cataract development. The next chapter reviews the cross-sectional, longitudinal, and intervention trial data and finds consistent reporting of excessive consumption of alcohol and increases in both the level of blood pressure and the subsequent incidence of hypertension. Dyslipidemia is a disorder of lipoprotein metabolism, including lipoprotein overproduction or deficiency. Dyslipidemia may be manifested by elevated LDL cholesterol or elevated triglycerides or low HDL cholesterol. Excessive alcohol consumption is a major risk factor for dyslipidemia as outlined in the next chapter. Alcohol abuse is also associated with chronic pancreatitis, and symptoms may be reduced with antioxidant nutrient use as reviewed in the next chapter. Also included is an outline of the treatment algorithm. In contrast to the above chronic conditions, epidemiological studies have linked light to moderate alcohol consumption, i.e., 10–30 g alcohol per day, with about a 30 % decreased risk of type 2 diabetes compared to nondrinkers. There appears to be a U-shaped relationship between the amount and frequency of alcohol consumption and type 2 diabetes risk especially in women. The next chapter examines the association between alcohol consumption, adiposity, and obesity. Cross-sectional and prospective studies suggest that long-term, high alcohol intake (>3 drinks/day) is associated with increased abdominal adiposity and weight gain. In contrast to the obese patients, the next chapter describes the etiology of anorexia and it appears that alcohol may play a minor role in this condition whereas bulimics may have alcohol-related psychological dysfunctions. The next unique chapter reviews the influence of alcohol consumption on human cancers known to be caused by viral infections. This chapter includes comprehensive tables that outline those cancers that are associated with viral infections including, but not limited to, Epstein-Barr virus, hepatitis viruses, human papillomavirus, human lymphotrophic virus type 1, human herpesvirus 8, and human immunodeficiency virus (HIV).

Two of the most serious diseases to affect chronic alcohol users are cancers, mainly of the digestive tract, and liver diseases. These two areas are reviewed in depth in the final 12 chapters of this comprehensive volume. Chronic alcohol users have an increased risk of many cancer types and alcohol use can affect the treatment of cancers not directly related to alcohol abuse. The effects of alcohol on the development and treatment of liver, colorectal, urinary tract, esophageal, and other digestive tract cancers are each reviewed in separate chapters. In contrast, chapters include the epidemiological findings that low or moderate intake of wine is associated with reduced risk of development of certain cancers. As indicated in previous chapters, the combination of alcohol use and cigarette smoking is

frequently seen. Their synergism in upper digestive system cancers is described in detail with excellent tables and figures and suggests that acetaldehyde, a human carcinogen derived from both alcohol and cigarettes, is a major factor.

The final section on alcohol and liver diseases contains eight comprehensive chapters. Topics reviewed include nonalcoholic fatty liver disease and nonalcoholic steatohepatitis (NASH); chronic viral infections in the liver; hepatic insulin resistance and other associations with effects of obesity and type 2 diabetes; cholesterol metabolism and its management; adverse effects of ceramide, a lipotoxin, and the use of ceramide-lowering drugs; dietary lipids and the potential for polyunsaturated fatty acids to reduce the chronic inflammation seen in many liver diseases; protein-calorie malnutrition and multiple micronutrient deficiencies associated with chronic liver diseases and the use of enteral and parenteral nutrition therapies; and the role of the liver in assuring adequate vitamin A delivery to the rest of the body once dietary vitamin A has been consumed. This final chapter reminds us of the liver's functions of storing and metabolizing vitamin A and synthesizing vitamin A binding proteins that permit the release of vitamin A from the liver to be distributed to all cells and tissues of the body.

The logical sequence of the sections as well as the chapters within each section enhance the understanding of the latest information on the current standards of practice with regard to chronic alcohol use and its consequences for clinicians, related health professionals including the dietician, nurse, pharmacist, physical therapist, behaviorist, psychologist, and others involved in the team effort required for successful treatment of alcoholism as well as liver diseases that may or may not be directly related to alcoholism. Other relevant diseases as well as conditions that adversely affect the liver's normal metabolic processes are also included. This comprehensive volume has great value for academicians involved in the education of graduate students and postdoctoral fellows, medical students, and allied health professionals who plan to interact with patients with relevant disorders.

The volume contains over 100 detailed tables and figures that assist the reader in comprehending the complexities of the metabolism as well as the potential benefits and risks of alcohol on human health. The over-riding goal of this volume is to provide the health professional with balanced documentation and awareness of the newest research and therapeutic approaches including an appreciation of the complexity of the effects alcohol can have on virtually every organ system within the body. Hallmarks of the 43 chapters include key words and bulleted key points at the beginning of each chapter, complete definitions of terms with the abbreviations fully defined for the reader, and consistent use of terms between chapters. There are over 3,400 up-to-date references; all chapters include a conclusion to highlight major findings. The volume also contains a highly annotated index.

This unique text provides practical, data-driven resources based upon the totality of the evidence to help the reader understand the basics, treatments, and preventive strategies that are involved in the understanding of how alcohol may affect healthy individuals as well as those with chronic alcohol use with or without relevant infectious diseases, obesity, diabetes, and/or neurocognitive declines. With equal importance, critical issues that involve patient concerns, such as malnourishment; potential effects on mental functions; and addiction and withdrawal are included in well-referenced, informative chapters. The overarching goal of the editors is to provide fully referenced information to health professionals so they may have a balanced perspective on the value of various preventive and treatment options that are available today as well as in the foreseeable future.

In conclusion, *Alcohol, Nutrition and Health Consequences*, edited by Ronald Ross Watson, Ph.D.; Victor R. Preedy, Ph.D., D.Sc., FRIPH, FRSH, FIBiol, FRCPath; and Sherma Zibadi, M.D., Ph.D., provides health professionals in many areas of research and practice with the most up-to-date, well-referenced, and comprehensive volume on the current state of the science and medical consequences of alcohol use. This volume will serve the reader as the most authoritative resource in the field to date and is a very welcome addition to the Nutrition and Health Series.

Adrianne Bendich, Ph.D., FACN, FASN
Series Editor

Preface

Humankind has had a complex relationship with alcohol from the beginning of recorded history. In most societies, some level of alcohol consumption is acceptable. In the United States, about 60% of high-school students illegally use alcohol. Alcohol-altered diet and nutrition directly affects ten million alcohol-abusing adults. It costs people in the United States more than $250 billion in health care, lost work, etc. Alcohol research is in a golden era. With more powerful tools for data collection and analysis and increased funding, the epidemiology of alcohol consumption, dietary consequences, role of nutrition in treatment of alcohol's pathology, and alcohol-related health issues are being better elucidated. Therefore, there is an overview section on nutrition and the effects of alcohol use on it to aid the reader. This includes genetics of alcohol metabolism and lessons learned from animal models.

Chronic alcohol use is associated with heart, liver, brain, and other organ pathology. Alcohol is a drug of abuse and a caloric food. It causes poorer intake and absorption of nutrients, thus playing a major role in many aspects of clinical consequences. Alcohol use lowers consumption of fruit and vegetables, lowers tissue nutrients, and, in some cases, requires nutritional therapy by clinicians. Thus the next section deals with diverse chapters relating to oxidation, body weight, health inequalities, specific problems to Native Americans, and biology. Clearly, metabolites of ethanol such as acetaldehyde are important modifiers of nutrients and metabolism of protein which are reviewed. In addition, the effects of alcohol abuse on nutrients' actions including vitamin E, vitamin B12, and zinc in the body's biology are assessed. Alcohol modifies use and metabolism of diverse foods with oats, fish oil, and soy being examples that are reviewed.

Infectious diseases, particularly viral ones including HIV/AIDS and viral infections promoting cancer can be changed by alcohol abuse which is defined in this book. More importantly chronic diseases are susceptible to chronic alcohol abuse. These include a wide range of nutritional diseases such as cataracts, high blood pressure, dyslipidemia, diabetes, obesity, and bulimia. This book helps to define the causes and types of nutritional changes due to alcohol use and how nutrition can be used to ameliorate its consequences. The role of antioxidant nutrients and foods as partial therapies is carefully defined.

Chapters deal with application of current nutritional knowledge by physicians and dietitians in understanding alcohol and cancer promotion. Reviews describe alcohol use in liver, colorectal, urinary, and digestive systems. Of course, toxic metabolites, acetaldehyde plays an important role in digestive tract cancer described in a chapter. An intimate, detailed knowledge of the effects of alcohol on the biochemical reactions and nutritional changes is critical in preventing or treating biomedical consequences.

Specific areas involving alcohol-related damage due to alcohol-combined effects with foods are reviewed, specifically the interaction with caffeine in foods, tobacco smoke and nicotine, and energy drinks. Because of alcohol's effects on the liver with a diverse range of diseases, they become a major section. Therefore the roles of nutrients as therapies for alcoholic liver diseases are defined including the actions of dietary fats, vitamin A, and native plant foods in reducing and exacerbating them.

The book will become a desk reference for alcohol therapists and researchers as well as primary care physicians and dietitians. These professionals frequently need information on the nutritional effects of alcohol as well as the role of nutritional supplementation and diet in the therapy of alcohol pathology. Research progress encourages us to summarize and evaluate in detail advances in understanding changes in nutritional biochemistry and physiology caused by ethanol (alcoholic beverages). It will assist the clinician, student, and dietitian to comprehend the complex changes caused by direct and indirect effects of ethanol at the cellular level via its nutritional modification. This book will stimulate research while educating health-oriented laypersons as well as scientists and health-care professionals.

Tucson, AZ, USA — Ronald Ross Watson
London, UK — Victor R. Preedy
Tucson, AZ, USA — Sherma Zibadi

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Chapter 1
Alcohol and Nutrition: An Overview

Francisco Santolaria and Emilio González-Reimers

Key Points

- Excessive ethanol intake may cause both overweight and malnutrition. Malnutrition develops mainly in heavy drinkers and is not related to dependence but to marginality with loneliness and to liver cirrhosis with ascites.
- Alcoholics frequently have social and family problems which disrupt social links and lead to an irregular lifestyle. Meals of lonely male alcoholics are often irregular. As alcoholics increase ethanol intake, they change their feeding habits; some meals are missed, and the quality of the diet consumed is poor.
- Body mass index (BMI) is a misleading method to detect nutritional changes in cirrhotics. Both fluid retention and obese-type malnutrition (decreased lean mass with increased fat mass) are common in cirrhotics, emphasizing the importance of nutritional assessment by compartments. Moreover, decreased albumin, prealbumin, transferrin, and IGF-1 are unreliable nutritional markers in alcoholics, since they may depend more on liver function, infection, or injury than on nutritional impairment.
- Regarding prognosis, the protein compartment, especially muscle protein, is more important than body fat stores.
- Malnutrition in alcoholics is a chronic process, which ensues over years, and is related to heavy and prolonged consumption. In most studies dealing with this problem, alcohol intake was higher than 200 g/day and lasted for 20 years or more. Probably, all these factors had been in play for a long time before protein and calorie malnutrition becomes evident as a clinical problem.

Keywords Alcoholism • Malnutrition • Caloric wastage • Irregular feeding • Liver cirrhosis • Prognosis

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Introduction

Although alcohol consumption is very frequent in Western countries, nutritional disorders due to alcohol are relatively uncommon, and they are mainly restricted to heavy consumers. However, malnutrition is one of most relevant medical problems of alcoholic patients, since it is related to advanced alcoholism and to survival.

Some years ago, we reviewed general pathogenetic and clinical aspects of alcohol-related malnutrition [1]. Despite intensive research in trace elements and specific nutrients, relatively few new data related to general clinical aspects of alcohol-related malnutrition have appeared in the medical literature. They will be commented in this chapter.

Ethanol is a highly energetic (7.1 kcal/g), readily oxidizable compound, often present in the Western diet. It accounts for 5.6% of the total energy intake of the average American diet, despite the fact that about one-third of the population is teetotaler [2]. Ethanol accounts for up to 10% of the total energy intake among social drinkers, this proportion reaching more than 50% in heavy alcoholics. Due to its high caloric content, ethanol consumption has been considered a risk factor for weight gain and obesity. However, weight loss is common among heavy drinkers [3]. But it is noteworthy that alcohol dependence per se is not a main cause of malnutrition. The alcoholic patient who becomes malnourished is that one with social and familial problems, socially marginated, who loses meals, and finally spent most of money and time in drinking. Another way of malnutrition is the development of organic pathology such as liver cirrhosis with ascites.

Mechanisms of Malnutrition in Alcoholics

Primary Malnutrition

Shift of Nutrients

Moderate ethanol consumption increases rather than decreases dietary intake. Indeed, Westerterp-Plantenga et al. (1999) showed that 24-h energy intake was higher on days in which a drink was consumed as an aperitif [4]. In contrast, heavy alcoholism leads to a substantial reduction of dietary intake, so consumption of other nutrients progressively decreases as ethanol intake increases [5, 6]. Moreover, since heavy alcoholics underreport the amount of ethanol consumed and overreport their nonalcoholic energy intake, this effect is probably even more important [7, 8].

Despite the fact that alcoholic beverages may account for up to 5% of the total energy intake, they should not be considered as a food, or, in the best of the cases, only as a poor-quality food, since they provide only one nutrient, lacking proteins, essential lipids, minerals, and the majority of trace elements and vitamins. Therefore, although the diet of a heavy drinker matches or even surpasses the caloric requirements, it may be inadequate in terms of protein, essential lipids, and other nutrients.

Caloric Wastage

Pirola and Lieber (1972), in classic studies, found a weight loss of about 1 kg after consumption for 14 days of a diet in which 50% of calories were substituted by ethanol. Moreover, no significant weight gain was observed when 2,000 kcal – in the form of ethanol – were added to the diet, whereas subjects experienced a weight gain of nearly 3 kg when the same amount of calories was consumed in the form of chocolate. These findings were attributed to the metabolism of ethanol by energy-wasting pathways in chronic alcoholics [9, 10].

Ethanol is a xenobiotic product, which cannot be stored in the body but becomes rapidly oxidized, displacing other fuels. Two main mechanisms are involved in ethanol metabolism: the alcohol dehydrogenase (ADH) pathway and the microsomal ethanol-oxidizing system (MEOS). The ADH pathway requires reduction of NAD to NADH + H, but MEOS requires oxidation of NADPH to NADP, a process that consumes ATP and dissipates heat. Therefore, the ADH pathway yields 16 mol ATP/mol of ethanol oxidized, whereas MEOS, only 10. MEOS pathway scarcely works in occasional ethanol consumers but is induced in chronic alcoholics [11, 12].

In healthy volunteers, short-term ethanol administered as 25% of the total energy requirements, either added to the diet or given instead of other food, increases 24-h energy expenditure [13, 14]. Since this experiment was carried out in healthy nondrinkers, ethanol should have been mainly metabolized by the ADH system and not by the MEOS. Therefore, mechanisms other than MEOS must be involved in the alcohol-mediated increase in energy expenditure, such as acetaldehyde-induced catecholamine secretion. When moderate amounts of ethanol, 5–10% of total daily calories, were added to the diet (as occurs with social drinkers), no change was observed in resting energy expenditure (REE) [15, 16]. However, Addolorato et al. (1998) report an increase in REE in long-term heavy drinkers (mean consumption of 195 g ethanol/day) when compared with social drinkers; chronic alcoholics show a significantly lower weight due to lower fat mass and increased fat oxidation [17, 18]. Levine et al. (2000) also showed an increased fat oxidation and an increased REE, which is related to ethanol ingestion, since both decrease 4 days after withdrawal [19]. Thus, it seems that ethanol increases REE by an increased catecholamine secretion and uncoupled oxidative phosphorylation due to mitochondrial damage [20, 21].

Effect of Ethanol on Fat Synthesis and Oxidation

Ethanol may inhibit fat mobilization due to the antilipolytic effect of acetate [22]. In addition, an increased NADH/NAD ratio may enhance liver fatty acid and triglyceride synthesis. These data theoretically favor lipid accumulation and weight gain. However, epidemiologic studies support the conclusion that even moderate ethanol consumers (less than 50 g/day), despite an increase in the total energy intake, show weight loss [23, 24]. So, studies dealing with changes in body composition in chronic heavy drinkers describe fat loss. Addolorato et al. (1998), in chronic heavy drinkers (mean ethanol intake of 195 g/day) without liver cirrhosis or malabsorption, found a lower body weight due to fat mass reduction (the triceps skinfold was reduced but not the midarm muscle circumference) and a preferential use of lipids as fuel when compared with social drinkers [17, 18].

Effects of Ethanol on Protein Metabolism

Ethanol increases urinary nitrogen excretion [25, 26]. Reinus et al. (1989) studied eight alcoholic patients continuously fed by nasogastric tube. When ethanol accounted for 30% of the total caloric intake (about 100 g/day), an amount which does not surpass the hepatic clearance rate, negligible ethanol concentrations were detected in blood, and no increase in urea nitrogen excretion was observed. However, when the amount of ethanol was increased to 40–60% of the total calories (about 180 g), blood ethanol concentration ranged from 250 to 300 mg/dl, urinary urea nitrogen and 3 methylhistidine increased – pointing to muscle wastage – and weight loss ensued [27].

Ethanol administered to rats leads to reduced protein synthesis and type II muscle fiber atrophy, an effect more dependent on acetaldehyde than on ethanol itself. Moreover, type IIb fiber atrophy is more intense when a low protein diet is added to ethanol [28]. The association between ethanol, malnutrition, and muscle atrophy is complex. It has been clearly shown that ethanol leads to muscle atrophy and cardiomyopathy in the absence of nutritional impairment [29]. However, malnutrition is frequently associated to alcoholic myopathy [30]. Histologically assessed muscle atrophy was found

in one-third of 64 heavy alcoholics, drinkers of 217 g ethanol/day. Patients with muscle atrophy consistently showed an impaired nutritional status, affecting not only muscle mass but also subcutaneous fat [31]. Fernandez-Sola et al. (1995) reported that protein-calorie malnutrition is an independent predictive factor of type II fiber atrophy [32, 33]. However, muscle atrophy implies a reduction in total body protein burden, and is, thus in itself, a criterion of malnutrition. In any case, as Fernandez-Sola et al. (2000) show, alcoholic myopathy only appears with heavy ethanol consumption at levels at which malnutrition is frequent. Interestingly, it may recover without total abstinence, only by lowering the dose of ethanol consumption [34].

In addition to muscle protein, ethanol and acetaldehyde may alter protein synthesis in every body tissue. They decrease protein synthesis in the majority of the tissues, such as bone, decreasing collagen; liver, decreasing albumin, prealbumin, IGF-1, its binding protein IGF1BP3, and osteocalcin; and whole-body nitrogen balance. But they also increase liver collagen synthesis [35].

Socioeconomic Status, Social and Family Problems, and Irregular Feeding

Malnutrition has been more frequently reported among skid row and low class alcoholics than in middle class ones [36–38]. In this sense, Goldsmith et al. (1983) found that only 8% of alcoholics of middle and high socioeconomic status were malnourished, in contrast with 32% of those belonging to a low social class [39]. Alcoholics frequently have social and family problems which disrupt social links and lead to an irregular lifestyle. Meals of lonely male alcoholics are often irregular. As alcoholics increase ethanol intake, they change their feeding habits; some meals are missed, and the quality of the diet consumed is poor [6].

In a study performed on drug addicts – mainly heroin consumers – admitted for detoxification, we found that disruption of social and family links were related to anorexia and poor food intake and also to a more intense drug addiction [40]. In our culture, regular meals and adequate food intake are related to family life, and family rupture leads to progressive marginalization and poverty. These factors, together with the anorexigenic effect of alcohol and the lack of interest for everything besides ethanol consumption, may lead to progressive malnutrition. In this line, we studied 181 alcoholic patients, consumers of about 180 g of ethanol daily. The heaviest drinkers showed the most irregular feeding habits and were severely underweighted. The worst situation was suffered by the skid row alcoholics, all of them unemployed, homeless, and without family support. Most of these patients (73%) showed a BMI below 20 kg/m^2, a finding which was observed only in 11% of non-skid row alcoholics and in none of the controls. Skid row alcoholics also showed an intensely decreased lean and fat mass assessed by midarm anthropometry and double-energy X-ray absorptiometry (DEXA), and, subsequently, decreased handgrip strength. However, skid row alcoholics did not show more somatic complications [41].

Alcoholics eat frequently in bars or taverns instead of at home. They miss meals, meals are scanty, and portions are small and deficient in protein. Alcoholics who confessed irregular feeding habits had more social and family problems, drank more ethanol, and suffered a more intense malnutrition with decreased fat, lean, and bone mass (pointing to a relationship between malnutrition and osteopenia); low serum albumin, prealbumin and transferrin, cholesterol and triglyceride, and also serum folate and magnesium; and a decreased handgrip strength when compared with the remaining alcoholics. Thus, loneliness and irregular feeding may be the link between social and family problems and malnutrition [41, 42].

Recently, a Japanese study supports this hypothesis. It included 467 patients with a daily ethanol consumption of 119±65 g; 50.5% of the subjects consumed three meals a day; 32.8%, two meals; 12.2%, one meal; and 4.5% scarcely ate. The meals mainly consisted of carbohydrates and protein, with few vegetables. Daily alcohol consumption was inversely related to the frequency of meals. The subjects who

lived with their family (72.8%) consumed more meals than the subjects living alone. BMI of excessive drinkers directly depends on ethanol consumption and inversely on the number of lost meals. The group with the lowest BMI values (<18.5) accounted for 19.3% of the subjects, and those with the highest BMI values (> or = 25) accounted for 11.5% [43]. So, excessive ethanol intake may cause both overweight and malnutrition. Malnutrition develops mainly in heavy drinkers and is not related to dependence but to marginality and loneliness. Alcoholics with social and familial disturbs are those who lose meals and become malnourished. Menari AP et al. (2003) did not find differences in the degree of malnutrition between the harmful drinkers (mild dependency) and heavily dependent alcoholics. Although the whole population of the study showed one or more deficiencies in macro- or micronutrients intake, one-third were below normal body weights, but one-quarter showed overweight [44].

Serum folate levels are reduced in alcoholics [41, 45–48]. In a study on 103 male alcoholics, drinkers of a mean of 205 g/day, we found decreased serum folate and B6 levels but increased B12. Thirty percent of our alcoholics showed serum folate levels below 3 ng/l. The decrease in serum folate was not related to liver function impairment or to ethanol intake; instead, it was related with nutritional data and especially, again, with irregular feeding habits (only one meal per day and one dish per meal) and poor consumption of one or more of the main food groups. Decreased B6 levels were also related to malnutrition [48]. As serum folate and B6 levels were inversely related to homocysteinemia, ethanol abuse may lead to hyperhomocysteinemia [46–48].

Early start in alcohol abuse. Alcohol intake in teenagers may impair growth. The height of alcoholic patients was 4 cm less than that of the controls. Height of the alcoholics was related to age at the onset of drinking, which was before 15 years in nearly half the cases. Alcoholics who drank before 15 years of age were 3 cm shorter than the remaining alcoholics who did not drink at this age and also showed a higher current ethanol intake [41, 49]. Alcohol intake was related to decreased serum IGF-1 and osteocalcin levels, even among those alcoholics without liver disease [41, 42, 45]. Two studies performed on Harris lines, which may be related to growth arrest due to metabolic stress, showed a relation with ethanol intake during growth [49, 50].

Secondary Malnutrition

Many alcohol-related diseases may lead to malnutrition, mainly by interfering with intake or absorption of nutrients. Chronic alcoholic gastritis, with anorexia and vomiting, and chronic diarrhea are common complications of alcohol consumption. However, chronic pancreatitis and liver disease are the two main causes of secondary malnutrition in alcoholics. Moreover, alcoholics frequently suffer episodes of infection and injuries, leading to superimposed stress malnutrition. Nicolas et al. (1993), in a study performed on 250 male chronic alcoholics, who drank a mean of 235 g ethanol per day, with stable social status and familial support, who entered a treatment program for alcoholism, found that impaired nutritional status was mainly due to organic complications but not to alcohol itself or dependence. Indeed, nutritional status of alcoholics without organic complications was similar to that of the controls [51]. Alcohol dependence does not seem to play an important role in alcoholic malnutrition, provided that social and familial links are not disturbed. Alcoholics with major withdrawal symptoms either at admission or during hospital stay showed a nutritional status similar to those without withdrawal symptoms [41].

Compensated liver cirrhosis may be associated with a normal or only slightly impaired nutritional status, even with overweight. In cirrhotics, interpretation of decreased serum albumin, transferrin, and prealbumin levels may be difficult, since they may be secondary to liver failure rather than to malnutrition or may be even related to infection or injury [52]. Serum IGF-1 and IGFBP3 levels show a better correlation with liver function than with nutritional status [45, 53].

Alcoholics with liver disease show some metabolic disturbances which may clearly influence nutritional status. A hypermetabolic state with increased thermogenesis has been observed in these patients, especially in those with superimposed alcoholic hepatitis [54–56]. However, these changes are not specific of alcoholic liver disease, since they are also observed in other forms of liver disease as postviral cirrhosis [57]. Furthermore, not all cirrhotics are hypermetabolic. In fact, Muller et al. (1992) report hypermetabolism in 18% and hypometabolism in 31% of their cirrhotics. Those who were hypermetabolic showed a reduced muscle mass, whereas those who were hypometabolic, an increased fat mass [58]. Hypermetabolism has been related to increased serum levels of pro- and anti-inflammatory cytokines [59].

In contrast to cirrhotics with ascites, compensated cirrhotics show a better nutritional status, even with overweight in half of cases. This overweight is related to an excess of fat, as lean mass was shown to be reduced both by creatinine excretion and by DEXA. Indeed, arm lean mass and handgrip strength were both decreased to a similar degree in compensated cirrhotics and noncirrhotic alcoholics [41, 42, 45, 60]. Other studies have also shown an excess of fat in cirrhosis. Overweight was reported in 18% of the 883 male cirrhotics who entered the Italian Multicentre Study (1994), and Bunout et al. (1983) found higher values of body weight (110% of ideal weight) and midarm fat area (113% of the standard) in alcoholics with cirrhosis or alcoholic hepatitis [61, 62]. Therefore, obesity is not an uncommon finding in cirrhotics. However, the increased fat mass often coexists with a decreased lean mass, which is a criterion of malnutrition: obese-type malnutrition [63].

Nutritional status of decompensated cirrhotics (mainly by ascites or alcoholic hepatitis) is worse than that of noncirrhotic alcoholics [41, 42, 60, 64, 65]. Cirrhotics with ascites showed reduced lean and fat mass. Ascites causes anorexia and early satiety due to gastric compression and abdominal distension but not to altered gastric emptying: large-volume paracentesis improves satiety and dietary intake but has no effect on gastric emptying [66]. Ascites drainage by peritoneovenous shunting improves fat and muscle mass, serum albumin and transferrin, and lymphocyte count [67, 68]. Transjugular intrahepatic portosystemic shunt (TIPS), as therapy for refractory ascites, decreases portal hypertension and improves intestinal absorption. Allard et al. (2001) studied ten cirrhotics with refractory ascites who underwent TIPS. Total body nitrogen, body fat, REE, caloric intake, and muscle strength were all reduced at baseline and showed a marked improvement 12 months later [69].

Thus, body weight is a misleading method to detect nutritional changes in cirrhotics. Both fluid retention and obese-type malnutrition (decreased lean mass with increased fat mass) are common in cirrhotics, emphasizing the importance of nutritional assessment by compartments. Moreover, decreased albumin, prealbumin, transferrin, and IGF-1 are unreliable nutritional markers in alcoholics, since they may depend more on liver function, infection, and injury than on nutritional impairment.

Nutritional assessment by body compartments may be performed either by anthropometry, bioelectrical impedance, or absorptiometry. DEXA is the most accurate of these procedures and allows a separate evaluation of fat, lean, and bone mass, although it has the drawback that retained water – as ascites or edema – is counted as lean mass [70]. However, since fluid retention is habitually less pronounced, or absent, in arms, compartmental analysis of the upper limbs allows an accurate assessment of lean mass [41].

Complications of Alcohol Abuse Closely Related to Malnutrition

Some complications of alcoholism are more frequent among severely malnourished alcoholics. Some of them are the logical consequence of vitamin and trace element deficiencies. Diverse studies such as the Italian Multicentre (1994), Leo and Lieber (1999), and Bergheim et al. (2003) have shown vitamin and trace element deficiencies in alcoholics with and without liver disease, with decreased serum levels of vitamin C, retinol, carotene, selenium, and zinc [61, 71, 72]. Manari et al. (2003)

report in UK alcohol abusers' low intakes of vitamin E and folate, selenium and vitamin D, calcium and zinc, and vitamins A, B1, B2, B6, and C below UK recommended standards [44]. Wernicke encephalopathy (vitamin B1 deficiency), pellagra (niacin), xerophthalmia (vitamin A), scurvy (vitamin C), and folate and B12 deficiencies are only seen in severely malnourished alcoholics [73–76]. Interestingly, consequences of B12 deficiency, such as megaloblastic anemia, are sometimes observed among alcoholics with normal cobalamin serum levels (Fragaso A 2010), pointing out to the existence of nonfunctional forms of cobalamin [77].

Other alcohol complications, such as cerebral and cerebellar shrinkage, hypophosphatemic rhabdomyolysis, chronic alcoholic myopathy, bone disease with decreased bone mineral density, and paralysis associated with hypokalemia and hypomagnesemia, have not a direct relation with vitamin deficiency but globally with malnutrition. In all of them, a close relationship with malnutrition has been reported but also a remarkable improvement after abstinence [78–83].

Alcohol Abuse, Malnutrition, and Survival

Malnutrition, irrespective of its etiology, is related to a poor prognosis, since it depresses immunity and favors infection. Therefore, mortality of malnourished alcoholic inpatients is increased to a similar degree to that of similarly undernourished nonalcoholics [83].

The prognostic value of malnutrition in alcoholics has been extensively analyzed in those affected by liver disease: acute alcoholic hepatitis and liver cirrhosis. The prognosis of decompensated liver cirrhosis is very poor, with a 2–5-year mortality of 50% [84, 85]. The Child system, a widely used prognostic score of liver disease, included in its first version (Child and Turcotte classification 1964) a subjective nutritional assessment. However, this parameter was later substituted by prothrombin in the Child-Pugh score (1973) [86, 87]. Therefore, in the current version of the Child-Pugh score, no nutritional parameter is included.

The question is, therefore, whether nutritional data – other than liver-synthesized proteins and BMI in cases of fluid retention – may improve the prognostic value of the Child-Pugh score regarding survival. In this line, Abad et al. (1993) showed that midarm circumference (MAC) improves the prognostic capacity of the Child-Pugh score, a result also obtained by Alberino et al. (2001) with midarm muscle circumference (MAMC) and triceps skinfold (TSF), with MAMC yielding a closer prognostic value than TSF [84, 88]. Merli et al. (1996) found that a MAMC below the fifth percentile is associated with an increased mortality in Child A and B patients but not in class C ones, whereas a decrease in adipose tissue did not worsen the prognosis in any of the Child groups [85]. Mendenhall et al. (1995), in patients with acute alcoholic hepatitis, report that creatinine excretion and handgrip strength – both related to muscle mass – are better indicators of survival than other nutritional parameters [89].

Our group (2008) reported that lean arm mass assessed by DEXA yields a long-term survival value after a follow-up period of 88 months [90, 91]. Moreover, loss of lean mass after a 6-month period is related to impaired prognosis. One hundred and five alcoholic patients (including 66 of those who underwent two DEXA assessments) were followed up for a median of 18 months. During this period, 33 died (including 20 of those who had undergone a second DEXA assessment).

Forty-two of the patients had abstained from alcohol. Of these, 69.04% gained lean mass, compared with only 35.71% of those who had continued drinking ($p=0.006$). However, no associations were found between alcohol abstinence and changes in fat parameters. Analysis by means of Kaplan-Meier curves showed that loss of total lean mass and loss of total fat mass were all significantly associated with reduced survival. However, within 30 months of the second evaluation, significant associations were observed between changes related to lean mass and mortality, but no association between changes in fat parameters and mortality [92]. Taken together, these observations suggest that the protein compartment, especially muscle protein, is clinically more important than body fat stores

in patients with alcoholic malnutrition. In this way, searching for those nutritional data best related to prognosis, Alvares-da-Silva et al. (2005) compared handgrip strength, subjective global assessment, and a prognostic nutritional index to predict clinical outcome in cirrhotic outpatients and found that handgrip was the only technique that predicted a significant incidence of major complications within 1 year in undernourished cirrhotic patients [93].

Malnutrition in Alcoholics Is Multifactorial

As mentioned, many factors such as the amount of ethanol intake, the disruption of social and family links, the irregularity of meals, and the development of organic complications predispose to malnutrition in alcoholics. All these factors may be related to each other. Therefore, in order to discern which of them yield an independent value in the development of malnutrition, as well as their hierarchical importance, we performed a multivariate analysis, defining malnutrition as a DEXA-assessed reduction in lean mass in the upper limbs. Irregularity of food habits was the parameter most closely related to malnutrition, and liver cirrhosis with ascites also showed a predictive value. In turn, the irregularity of feeding habits was dependent on disruption of social and family links with loneliness and a heavy ethanol intake [41].

Malnutrition in alcoholics is a chronic process, which ensues over years, and is related to heavy and prolonged consumption. In most studies dealing with this problem, alcohol intake was higher than 200 g/day and lasted for 20 years or more. Probably, all these factors had been in play for a long time before protein and calorie malnutrition becomes evident as a clinical problem. Finally, superimposed organic complications, such as chronic pancreatitis, decompensated liver cirrhosis, acute alcoholic hepatitis, acute or chronic infections, and injury, may further impair nutritional status making recovery unlikely.

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Chapter 2
Genetics of Alcohol Metabolism

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Key Points

- Alcohol metabolism occurs mainly via hepatic oxidation and is governed by the catalytic properties of the alcohol-metabolizing enzymes, alcohol dehydrogenase (ADH), and aldehyde dehydrogenase (ALDH2).
- Genetic polymorphisms in *ADH1B* and *ALDH2*, and ethnic differences in the prevalence of these polymorphisms, result in increased variation in alcohol metabolism among individuals.
- Polymorphisms in *ADH1B* result in variants that code for isozymes that tend to show a faster rate of alcohol metabolism, while the *ALDH2*2* polymorphism results in a "deficient" form of *ALDH2* that causes an accumulation of acetaldehyde and its associated physiological effects.
- *ADH* and *ALDH* polymorphisms are also associated with a protective effect on the development of alcoholism. The allele frequencies of *ADH1B*2*, *ADH1B*3*, and *ALDH2*2* are significantly lower in individuals diagnosed with alcohol dependence compared to controls.
- Further evaluation of the factors, both genetic and environmental, regulating the rates of alcohol and acetaldehyde metabolism, will help improve our understanding of the metabolic basis and consequences of alcohol's effects, including the risk and consequences of alcohol-related organ damage, developmental problems, as well as alcohol dependence.

Keywords Alcohol metabolism • Alcohol dehydrogenase (ADH) • Aldehyde dehydrogenase (ALDH) • Genetic polymorphism • Ethnic differences • Cytochrome P450 • Catalase • Pharmacogenetics

Introduction

Ethanol (also referred to as alcohol in this chapter) is probably the most widely investigated drug in the world, not only because of its ubiquitous use and its widespread abuse but also because of its unique pharmacological properties. Following administration, systemic concentrations of alcohol are a consequence of the absorption, distribution, and metabolism of alcohol, which display very unique characteristics and demonstrate substantial interindividual variability [1]. As the pharmacological

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effects of alcohol depend on its systemic concentrations, variability in the pharmacokinetics of alcohol can have a significant impact on its pharmacodynamic effects.

Following oral ingestion, alcohol is absorbed by passive diffusion, primarily from the small intestine [2, 3]. The rate of absorption depends on several factors, both genetic and environmental, and is highly variable. Some of these factors include the volume, concentration, and nature of the alcoholic beverage [2, 4, 5]; the rate of drinking [4]; the fed or fasted state [6]; the nature and composition of food [6, 7]; the rate of gastric emptying [8, 9]; the gender differences in first-pass metabolism [10, 11]; and other drugs including histamine (H1) receptor antagonists like cimetidine and ranitidine [12, 13]. Ethanol is a small polar molecule and its volume of distribution is comparable to total body water [3]. No plasma protein binding has been reported for alcohol. Elimination of alcohol occurs primarily through metabolism with small fractions of the administered dose being excreted in the breath (0.7%), sweat (0.1%), and urine (0.3%) [3]. Alcohol metabolism occurs mainly via hepatic oxidation and is governed by the catalytic properties of the alcohol-metabolizing enzymes, alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH). The cytochrome P450 enzymes (CYP2E1) and catalase also contribute to alcohol metabolism and alcohol-related cytotoxicity under specific circumstances [14].

Alcohol metabolic rates show a considerable degree of interindividual and ethnic variability, in part due to allelic variants of the genes encoding ADH and ALDH producing functionally different isozymes [15–17]. Functional polymorphisms of the *ADH1B* and *ALDH2* genes have been shown to increase the variance in alcohol metabolism among individuals. Additionally, a multitude of environmental factors can influence the metabolic regulation of alcohol metabolism, which results in a large three- to four-fold variance in the alcohol elimination rate in humans [16, 18]. Factors that have been shown to be important determinants of alcohol metabolism include age [19, 20], gender [21, 22], ethnicity and genetics [21, 23–26], body mass and liver size [22], as well as environmental factors such as food intake [27].

This chapter will focus on genetic variation in the alcohol-metabolizing enzymes and its impact on the metabolism of alcohol.

Alcohol-Metabolizing Enzymes and Genetic Aspects

Alcohol Dehydrogenase

The genes for the human *ADH* family cluster in a region of chromosome 4q21 spanning ~370 kb [28]. The alcohol dehydrogenase (*ADH*) gene family encodes oxidative enzymes that metabolize a wide variety of alcohols including ethanol, retinol, other aliphatic alcohols, hydroxysteroids, and lipid peroxidation products [15, 17]. Currently, seven human *ADH* genes have been identified and organized into five classes based on amino acid sequence alignments, catalytic properties, and patterns of tissue-specific expression [29]. Human ADH enzyme is a dimeric molecule, arising from the association of different subunits expressed by the seven genes. Thus, there are over 20 ADH isozymes that vary greatly with regard to the types of alcohols they preferentially metabolize and the maximal rate at which they oxidize ethanol [15]. The five classes of ADH are divided according to their subunit and isozyme composition (Table 2.1).

The class I isozymes are found in liver and consist of homo- and heterodimeric forms of the three subunits (i.e., αα, αβ, ββ, βγ, γγ, etc.). Classes II, III, and IV enzymes are homodimeric forms of the π, χ, and σ subunits, respectively. All the class I ADHs metabolize ethanol and are inhibited by pyrazole derivatives [17]. The *ADH1* subunits share about 94% sequence identity. The relative order of catalytic efficiency (kcat/Km) for ethanol oxidation at ethanol concentrations of about 100 mg% and saturating coenzyme NAD+concentration (0.5 mM) is $\beta2 > \beta1 > \gamma1 > \gamma2 \approx \sigma >> \beta3 > \alpha >> \pi$. However, the relative order of kcat at saturating concentrations of both ethanol and NAD+is $\sigma > \beta3 \approx \beta2 > \gamma1 > \gamma2 \approx \pi > \beta1$. Thus, the relative contributions of each of the ADH isozymes to ethanol oxidation change with the hepatic concentration of alcohol [16, 17, 30].

Table 2.1 Nomenclature for alcohol dehydrogenase genes

ADH class	Official gene nomenclature	Former gene nomenclature	Enzyme subunit nomenclature	Km for ethanol [mM]
I	*ADH1A*	*ADH1*	α	4.0
I	*ADH1B*1*	*ADH2*1*	β1	0.05
I	*ADH1B*2*	*ADH2*2*	β2	0.9
I	*ADH1B*3*	*ADH2*3*	β3	40
I	*ADH1C*1*	*ADH3*1*	γ1	1.0
I	*ADH1C*2*	*ADH3*2*	γ2	6.0
II	*ADH4*	*ADH4*	π	30
III	*ADH5*	*ADH5*	χ	>1,000
IV	*ADH7*	*ADH7*	σ	30
V	*ADH6*	*ADH6*	Not identified	?

For official gene nomenclature, go to: http://www.genenames.org/genefamilies/ADH (HUGO Gene Nomenclature Committee at the European Bioinformatics Institute)

The human *ADH* genes are differentially expressed in different tissues, and this is a fundamental determinant of the physiological consequences of alcohol metabolism in specific cells and tissues [31]. The liver contains a large amount of ADH (about 3% of soluble protein) and expresses the widest number of different isozymes. ADH4 (π-ADH) is solely expressed in liver. Only ADH7 (σ-ADH) is not highly expressed in liver. ADH5 (χ-ADH) is ubiquitously expressed in human tissues. *ADH1C*, *ADH4*, *ADH5*, and *ADH7* are expressed in gastrointestinal tissues. The expression of ADH6 in humans and its role in ethanol metabolism remains to be elucidated. The expression of ADH in other tissues such as skeletal muscle, and the quantitative significance of muscle ADH metabolism (because of the large proportion of muscle mass in the body), also remains to be determined.

In addition to ethanol, alcohol dehydrogenases also oxidize several "physiological" alcohols with high catalytic efficiency including retinol, ω-hydroxy fatty acids, hydroxysteroids, and hydroxy derivatives of dopamine and epinephrine metabolites [30, 32]. Oxidation of these alcohols can be inhibited by ethanol, and therefore the role of ethanol substrate competition is an important issue in alcohol-related toxicology. Another important issue is the regional expression of ADHs in brain and their potential role in the local formation of acetaldehyde, which may be centrally active, possessing stimulant as well as sedative/hypnotic effects [33–35].

Genetic Variation

Single nucleotide polymorphisms (SNP) have been identified at the *ADH1B* and *ADH1C* loci [15, 17, 31]. Variant alleles of *ADH1B* result in the β1, β2, and β3 subunits, while variants in *ADH1C* result in the γ1 and γ2 subunits. The resulting subunits have different catalytic activities for ethanol (see Table 2.1). Additionally, the *ADH1B* alleles appear with different frequencies in different racial groups, with the *ADH1B*1* form predominating in Caucasian and African-descent populations, and *ADH1C*2* predominating in East Asian populations (e.g., Chinese and Japanese), and also found in about 25% of Caucasians with Jewish ancestry. The *ADH1B*3* form is found in about 25% of individuals of African descent. With respect to the *ADH1C* polymorphism, *ADH1C*1* and *ADH1C*2* appear with about equal frequency in Caucasians, but *ADH1C*1* predominates in African-descent and East Asian populations [36]. Recently, a novel polymorphism was identified in *ADH1C*. This polymorphism results in an allele that codes for a subunit with a proline to threonine substitution in position 351 and has been described in Native Americans [37]. However, the catalytic activity of the isozyme coded by this variant and its effect on the overall elimination of alcohol remains to be determined.

There are additional SNPs that have been identified in the noncoding regions of the *ADH* genes. Several of these SNPs have been shown to affect the expression of *ADH* genes [31, 38] and may be

associated with alcoholism risk [39]; however, the effect of these variations on the catalytic activity of ADH and effect on the overall metabolism of alcohol remains to be established.

Aldehyde Dehydrogenase

Acetaldehyde is the first metabolic product of ethanol metabolism and is itself metabolized via oxidation by the NAD+−dependent aldehyde dehydrogenase (ALDH). Several isozymes of ALDH, differing in kinetic properties and tissue distribution, have been detected in human organs and tissues [15]. Currently, 19 putatively functional *ALDH* genes have been identified in the human genome [40, 41]. However, only the *ALDH1* (*ALDH1A1*) and *ALDH2* genes encode the class I and class II isozymes that are involved in acetaldehyde oxidation. *ALDH1* is the cytosolic form distributed ubiquitously in tissues including brain. It exhibits relatively low catalytic activity (Km ~ 30 μM) for acetaldehyde oxidation. *ALDH2* is the mitochondrial enzyme that is highly expressed in liver and stomach [42]. It exhibits high catalytic activity (Km ~ 3 μM) for acetaldehyde oxidation and is primarily responsible for acetaldehyde oxidation *in vivo*.

Genetic Variation

The best-known genetic polymorphism in *ALDH* genes is in *ALDH2*. The allelic variants are *ALDH2*1* and *ALDH2*2*, encoding for the high-activity and low-activity forms of the subunits respectively. The low-activity form arises from a single amino acid exchange (glutamine to lysine substitution at position 487) at the coenzyme-binding site of the enzyme subunit [15, 17]. This results in a 100-fold increase in the Km for acetaldehyde [43]. This very prominent variant allele has been seen in about half of the East Asian populations studied (including the Han Chinese, Taiwanese, and Japanese) [44, 45]. It has not been observed in populations of Caucasian origin. It exhibits virtually no acetaldehyde oxidizing activity in vitro and represents the "deficient" phenotype seen in these Asian populations [46]. Individuals who are heterozygous or homozygous for *ALDH2*2* show accumulation of acetaldehyde levels and the characteristic sensitivity reaction (facial flushing, increased skin temperature and heart rate) following alcohol intake [26, 28, 47, 48].

Cytochrome P450 Enzymes

A small fraction of an ingested dose of ethanol is metabolized by enzymes other than ADH. Metabolism of ethanol by the so-called microsomal ethanol oxidizing system (MEOS) accounts for the major non-ADH system [14, 49]. MEOS consists primarily of the cytochrome P450 isoform, P4502E1 (*CYP2E1*), along with other P450 enzymes, and is the major alternative system that catalyzes the NADPH- and O_2-dependent oxidation of ethanol to form acetaldehyde, $NADP^+$, and water. Like other cytochrome P450 enzymes, the primary role of *CYP3E1* is the metabolism of alcohol and other xenobiotics. While *CYP2E1* accounts for a much smaller fraction of ethanol oxidation than the ADH system under normal conditions, it represents a major adaptive response of alcohol metabolism with chronic ethanol consumption [49]. This is due to the direct effect of chronic ethanol consumption on the expression of hepatic *CYP2E1*. In humans, there is an induction of *CYP2E1* with chronic alcohol consumption that can be followed by a decrease in activity associated with generalized hepatic injury and loss of function. There are two mechanisms postulated for *CYP2E1* induction: (1) a posttranslational mechanism involving mRNA stabilization and protection of the expressed protein against degradation and (2) a

direct transcriptional regulation of *CYP2E1* expression, generally following high exposures to ethanol. The expression of *CYP2E1* is influenced by factors such as diet (lipids, carbohydrates) and hormones (thyroid hormones, glucocorticoids, steroids, pituitary hormones). The induction of *CYP2E1* may result in higher levels of toxic metabolites of other xenobiotics as well as the generation of superoxide radicals, which may contribute to the increased risk of alcohol-related liver disease as well as cancer.

Genetic Variation

A number of different *CYP2E1* polymorphisms have been identified [15, 50]. A variant allele called **5B* has been identified in the 5′-flanking region of the *CYP2E1* gene. This allele has been shown to be differentially expressed in different racial populations, and the variant allele (previously labeled as the *c2* allele) has been found to be associated with higher transcriptional activity, protein levels, and enzyme activity than the common wild-type *c1* allele [51]. The influence of this polymorphism on alcohol elimination was examined in one study in Japanese alcoholics and control and indicated that the presence of the *c2* allele (heterozygous or homozygous) may be associated with higher alcohol metabolic rates but only at blood alcohol levels greater than 0.25% (g/dL) [52]. Studies have identified additional genetic variation that may be relevant to alcohol, including the **1D* allele, which has been found at higher frequency in Chinese (23%) and African-Americans (31%) than in Caucasians (1–7%) [53, 54]. Studies in African-Americans have further shown higher levels of CYP2E1 inducibility following alcohol intake as measured by oxidation of the CYP2E1 substrate chlorzoxazone. However, the influence of this polymorphism on alcohol metabolism remains to be determined. Much work needs to be done to understand mechanisms for transcriptional and posttranslational regulation of the *CYP2E1* genes and their role in alcohol metabolism and alcohol-related liver disease [49].

Catalase

Catalase is an enzyme that catalyzes the hydrogen peroxide (H_2O_2)-dependent oxidation of ethanol yielding acetaldehyde and two molecules of water. It is found in the cytosol and mitochondria but its main expression and function is in peroxisomes. Most studies indicate that it contributes very little to total ethanol elimination because of the limited availability of hydrogen peroxide [14, 55]. However, the activation of peroxisomal catalase by increased generation of hydrogen peroxide via peroxisomal β-oxidation can lead to a hypermetabolic state and a swift increase in alcohol metabolism under some conditions [56]. This state may contribute to alcohol-related inflammation and necrosis in alcoholic liver disease. Additional studies have suggested that catalase may be involved in the metabolism of alcohol to acetaldehyde in the brain. This has led to implications of a role for acetaldehyde in mediating some of the behavioral effects of alcohol [35]. However, further research is needed to clarify the pharmacokinetics and central pharmacodynamic effects of acetaldehyde and its role in the pharmacology of alcohol.

ADH and *ALDH* Polymorphisms: Influence on Alcohol Metabolism

Functional polymorphisms of genes for the alcohol-metabolizing enzymes *ADH* and *ALDH2*, and differences in the prevalence of the polymorphic alleles in different ethnic populations, have resulted in several studies examining ethnic differences in alcohol metabolism and the influence of *ADH1B*, *ADH1C*, and *ALDH2* genotypes. The isozymes encoded by the polymorphic alleles have very different catalytic properties in vitro, as described earlier in this chapter, and would be expected to exert influences on an individual's alcohol metabolic rate.

One of the first studies examining the influence of *ADH* and *ALDH* polymorphisms on alcohol metabolism was done by Mizoi et al. [23] in 68 healthy Japanese subjects. Subjects were genotyped for *ADH1B* as well as *ALDH2* polymorphisms and alcohol disappearance rates (mg/ml/h), and elimination rates (mg/kg/h) were compared among the groups classified based on genotypes of both *ADH1B* (*ADH1B*1/*1*, *ADH1B*1/*2*, and *ADH1B*2/*2*) and *ALDH2*. Results indicated that there were no differences in alcohol metabolism among the *ADH1B* genotypes; however, there were marked differences among the *ALDH2* genotypes with regard to alcohol metabolism. Other studies in Asians have also failed to demonstrate an effect of the *ADH1B*2* allele on alcohol metabolism after controlling for the *ALDH2*2* polymorphism. This is discussed further below.

Studies in Jewish individuals possessing the *ADH1B*2* polymorphism have provided a clearer picture of the effect of this variant on alcohol metabolism, Neumark et al. [57] conducted a study in young healthy Jewish males to assess the effect of the *ADH1B* polymorphism on alcohol elimination rates measured using the alcohol clamp [58]. Results revealed a significantly higher alcohol elimination rates in subjects carrying the *ADH1B*2* allele (heterozygotes and homozygotes) compared with *ADH1B*1* homozygotes [57, 59]. As the Jewish do not show polymorphisms of the *ALDH2* genes, this appears to be a direct effect of *ADH1B* genotypes on alcohol metabolism.

Thomasson et al. [21] examined the influence of the *ADH1B*3* polymorphism on alcohol metabolism in a sample of 112 African-American subjects, selected by genotype. In this study, subjects received an oral dose of alcohol and alcohol disappearance rates were determined from the slope of the pseudo-linear portion of the blood ethanol concentration vs. time curves. Results revealed that subjects carrying the *ADH1B*3* allele (heterozygotes and homozygotes) showed a higher alcohol disappearance rate (mg% per h) for compared to *ADH1B*1* homozygotes. A more recent study in African-Americans failed to demonstrate an effect of the *ADH1B*3* polymorphism on breath alcohol concentrations following a moderate oral dose of alcohol in 91 African-Americans [60]. A study in Native Americans also showed that subjects with *ADH1B*3* alleles had a trend toward higher alcohol elimination rates than subjects with *ADH1B*1* [24]. However, this difference was not statistically significant probably because of the small number of subjects possessing the *ADH1B*3* genotype in the study and the low frequency of occurrence of this genotype (~7%) in this ethnic group. Earlier studies in Native Americans have previously demonstrated higher alcohol elimination rates compared to those reported in Caucasians; however, *ADH* genotypes were not determined in these studies [61, 62].

The influence of *ALDH2* polymorphisms on alcohol metabolism has been studied more extensively, although almost exclusively in Asian subjects, mainly because of the high frequency of the polymorphism in this population. Most of these studies have compared peak concentrations of alcohol and acetaldehyde as well as peak responses on subjective and cardiovascular measures and flushing across *ADH1B* and *ALDH2* genotypes, with generally consistent results. In general, individuals who are heterozygous or homozygous for *ALDH2*2* show increased acetaldehyde levels following alcohol administration [23, 25, 28, 47, 63–65]. Some studies have also demonstrated significant increases in ethanol concentrations and area under the ethanol concentration time curves [63, 65], possibly due to product inhibition of the ADH activity by acetaldehyde. However, other studies have shown accumulation of acetaldehyde in subjects carrying the *ALDH2*2* allele without any difference in alcohol concentrations or elimination rates [25, 26].

Given the high frequency of the *ADH1B*2* and *ALDH2*2* alleles in Asians, it is important to understand the contribution of each polymorphism to the observed differences in blood alcohol and acetaldehyde levels following alcohol administration. There are only a few studies that have actually estimated and compared alcohol disappearance rates or elimination rates among *ADH1B* and/or *ALDH2* genotypes. In the study by Mizoi et al. [23] described above, peak acetaldehyde levels, alcohol disappearance rates (mg/ml/h), and elimination rates (mg/kg/h) were compared among subjects classified into groups based on genotypes of both *ADH1B* and *ALDH2* (*ALDH2*1/*1*, *ALDH2*1/*2*, and *ALDH2*2/*2*). Results indicated that subjects homozygous for *ALDH2*1/*1* showed no increase in acetaldehyde levels regardless of their *ADH1B* genotype. There was a progressive increase in peak acetaldehyde levels in subjects with the *ALDH2*1/*2* and *ALDH2*2/*2* genotypes. Both alcohol

disappearance rates and elimination rates showed significant differences among the *ALDH2* genotypes and decreased in the following order: *ALDH2*1/*1* > *ALDH2*1/*2* > *ALDH2*2/*2*. A study in Chinese men indicated that the presence of the *ALDH2*2* allele was associated with slower alcohol metabolism following oral administration, while in individuals homozygous for *ALDH2*1*, the presence of two *ADH2*2* alleles correlated with slightly faster alcohol metabolism [66]. Studies by Peng et al. [26, 48, 63] have demonstrated a clear effect of *ALDH2* genotype on alcohol and acetaldehyde metabolism, as well as the lack of significant effect of *ADH1B* polymorphism on acetaldehyde metabolism. In fact, most studies in Asians have not demonstrated that the *ADH1B*2* allele is associated with differences in alcohol metabolism after controlling for the *ALDH2* [25, 47, 67].

A recent effort in understanding the influence of genetic variation in alcohol-metabolizing enzymes on alcohol metabolism has focused on the use of association analysis in a large cohort of twin pairs of Caucasian ancestry. In these studies, 103 SNPs spanning the *ADH* gene family were examined for association with measures of alcohol metabolism following oral alcohol challenge in this sample. Results indicated significant associations between alcohol elimination rates and *ADH1A*, *ADH1B*, *ADH1C*, as well as *ADH7* genes [68, 69]. These studies point to a role for *ADH7* in the metabolism of alcohol; however, more work is needed to clarify the influence of this isoform, and its associated genetic variation, on alcohol elimination rates in humans.

In summary, genetic polymorphisms of *ADH* and *ALDH* result in alterations in the metabolism of alcohol and/or acetaldehyde. Polymorphisms in *ADH1B* result in variants that code for isozymes that tend to show a faster rate of alcohol metabolism, while the *ALDH2*2* polymorphism results in a "deficient" form of *ALDH2* that causes an accumulation of acetaldehyde and its associated physiological effects.

ADH and *ALDH* Polymorphisms: Association with Alcohol Dependence

Functional polymorphisms of the alcohol-metabolizing enzymes ADH and ALDH2 can also exert important effects on the biological effects of alcohol [26, 70]. In fact, the *ADH* and *ALDH* genes are the only genes which have been firmly established to influence vulnerability to alcohol dependence or alcoholism [17, 36]. Studies have demonstrated unequivocally that the allele frequencies of *ADH1B*2*, *ADH1B*3*, and *ALDH2*2* are significantly decreased in subjects diagnosed with alcohol dependence as compared with the general population of East Asians, including the Japanese, Han Chinese, and Koreans [39, 44, 45, 67, 71–76]. The *ALDH2*2* allele and the *ADH1B*2* allele also significantly influence drinking behavior in nonalcoholic individuals. Association between reduced alcohol consumption or reduced risk of alcohol dependence and the *ADH1B*2* variant allele has recently been found in other ethnic groups that do not carry the *ALDH2*2* allele, including Europeans [77–80], Jews in Israel [81, 82], as well as Mongolians in China [45], and the Atayal natives of Taiwan [83]. Recent studies have also shown a protective association between the *ADH1B*3* allele and alcohol dependence in Native Americans. [84, 85] Finally, studies have indicated that the *ADH1B*3* allele may be protective against alcohol-related problems in infants born to African-American mothers who may have consumed alcohol during pregnancy [86–89].

Summary

There has been substantial progress in the field of alcohol pharmacogenetics to characterize differences in alcohol metabolism in subjects exhibiting polymorphic genotypes of the alcohol-metabolizing enzymes. The impact of functional variation in *ADH1B* and *ALDH2* genes on alcohol metabolism have been fairly well characterized; however, there are large interindividual differences in alcohol elimination

rates that still remain unexplained. Of potential significance in this regard may be polymorphisms in *ADH4* [90, 91], *ADH7* [39, 69], and *ALDH1A1* [92, 93] as well as the promoter regions of *ALDH2* [94]. Further studies are needed to evaluate the influence of these polymorphisms on the activity of ADH and ALDH and on alcohol levels and elimination rates in individuals, as well as on the physiological response to alcohol consumption and alcoholism. Recent integrated approaches examining the associations of *ADH* and *ALDH2* gene variation with alcohol metabolism, response, drinking behavior, and alcohol dependence in large samples [78] might be particularly useful in this regard.

Studies in monozygotic and dizygotic twins have shown that the heritability (i.e., genetic component of variance) of alcohol metabolic rates is about 50% [95, 96]. Further evaluation of the factors, both genetic and environmental, regulating the rates of alcohol and acetaldehyde metabolism, will help improve our understanding of the metabolic basis and consequences of alcohol's effects, including the risk and consequences of alcohol-related organ damage, developmental problems, as well as alcohol dependence.

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Chapter 3
Laboratory Models Available to Study Alcohol and Nutrition

Nympha B. D'Souza EL-Guindy

Key Points

- The adverse effects of alcohol abuse are many and affect almost every organ and system in the body.
- Understanding the mechanisms by which alcohol abuse in humans leads to the development of alcohol-induced diseases is difficult as multiple factors, including nutritional deficiencies, contribute to the development and progression of alcohol-induced diseases.
- Recent advances in our understanding of the many detrimental effects of alcohol abuse have been possible because of the availability of relevant and rigorously controlled in vitro and in vivo laboratory models of acute and chronic alcohol exposure/intoxication.
- Most of the available laboratory models of alcohol exposure also allow the flexibility to simultaneously manipulate dietary components and/or cofactors. This flexibility is important when attempting to delineate the role of nutrition both in the development and progression of alcohol-induced diseases in human as well as the attenuation.
- The various laboratory models available to study alcohol and nutrition to date are discussed in this chapter.

Keywords Laboratory alcohol models • In vitro • In vivo • Acute and chronic alcohol abuse • Nutritional deficiencies

Introduction

Alcohol-related diseases, including those of the brain, liver, pancreas, and the lung, result both from the direct toxic effects of alcohol and the indirect effects of nutritional deficiencies associated with drinking. Individuals who consume significant amount of alcohol (ethanol) derive most of their caloric intake from the alcoholic beverages and foods rich in unhealthy fats and added sugars. When alcohol intake replaces food, there can be numerous nutritional deficiencies caused by the lack of adequate

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nutrients intake [1–3]. Excessive alcohol consumption can induce deficiencies of vitamins and minerals such as riboflavin, B12, vitamin A, folate, possibly retinoic acid, Zn, and calcium. From the many studies published to date, it is evident that, in addition to the independent effects of heavy drinking, various dietary factors play a vital role in the development and progression of various diseases attributed to alcohol abuse [1, 4].

The objective of this chapter is to familiarize the reader with the various laboratory models (in vitro and in vivo) available for alcohol research. Almost all of these models can be manipulated to study the role various dietary factors and cofactors may have in the development/progression and/or in attenuation of alcohol-induced diseases. The laboratory alcohol models available, to date, expose either cells (primary or cell lines) in vitro or laboratory animals in vivo to alcohol for various durations and experimental conditions. The choice of the model selected will depend upon the nature of the question asked. The reader of this chapter is guided to a recently published review article which describes in depth all alcohol models available to date and discusses the advantages and disadvantages associated with each model [5].

In Vitro Models of Acute Alcohol Intoxication

Acute alcohol intoxication consists of taking a single intoxicating drink either in a single sitting or in a binge situation (i.e., several drinks consumed either within a few hours or consecutively for several days). Using in vitro and in vivo models, acute alcohol intoxication is shown to affect in a time- and dose-dependent manner carbohydrate, protein, and lipid metabolism and impair various aspects of the immune system when subjected to a variety of stimuli [6–9].

Exposure of Primary Cells or Cell Lines to Alcohol in Culture Medium

The model consists in incubating cells in a culture medium (complete or modified) containing alcohol of the desired concentration. Published studies have used alcohol in the range of 1–500 mM in vitro with different types of primary cells and cell lines. An exposure of cells to a concentration of 25 mM alcohol represents a blood alcohol concentration of about 115 mg/dl. Briefly, the cell suspension, prepared in a medium containing the desired alcohol concentration, is incubated at 37°C in a sealed tissue culture incubator filled with a gas mixture (95% O2 + 5% CO_2). An open Petri dish containing alcohol (twice the concentration used to incubate the cells) is placed at the bottom of the chamber [10]. Both the sealing of the chamber and the placing of alcohol-containing Petri dish in the chamber helps to maintain a constant concentration of alcohol in the culture medium. Exposure of cells ex vivo to alcohol for an hour to several hours is considered as an acute exposure to alcohol [11].

In Situ Perfusion

The organ is perfused with alcohol-containing Krebs-Ringer solution. The model is mostly used to study the effect of alcohol on liver carbohydrate, protein, and lipid metabolism [12, 13].

In Vivo Models of Acute Alcohol Intoxication

These models are usually used to study the effects of a single intoxicating drink or to mimic human binge drinking wherein alcohol is taken consecutively for a few days. The data generated using these models are likely to be more informative and extrapolate more closely to human acute alcohol intoxication. There are several other nonhuman models of acute alcohol intoxication that have been developed, but most commonly used are the ones generated using small laboratory animals [14, 15].

Alcohol Given Intraperitoneally (IP) or via Oral Gavage

Alcohol (20% w/v) is administered to rodents either as a single IP bolus injection or as an oral gavage directly into the stomach [16–18]. The control animals receive an equivalent volume of the vehicle (either water or saline). The peak blood alcohol concentration is seen around 30 min after alcohol administration. The blood alcohol concentration attained is dependent upon the amount of alcohol administered and the species used [19]. These models are usually used in studies where either the effects of a single intoxicating drink are evaluated or in situations of binge drinking where alcohol is given consecutively for a few days. The models allow manipulation of the diet to study the effects of dietary factors on intoxicating effects of alcohol.

Alcohol Given as an Intravenous (IV) Bolus Followed by a Continuous Infusion

The model involves implanting a catheter in the inferior vena cava under general anesthesia and aseptic conditions. This model is somewhat akin to binge drinking. An IV bolus injection is given via the catheter followed by a continuous infusion of alcohol at a lower concentration. The continuous infusion helps to maintain the desired blood alcohol concentration throughout the study period [20]. The control animals receive an equivalent amount of saline similarly.

The above described methods of acute alcohol intoxication superimposed with a second hit (e.g., live or cell-wall component of bacteria and viruses) are used to study the role an additional stimuli may have in augmenting the adverse effects of acute alcohol intoxication on various organs and systems.

The above described acute in vivo models of alcohol intoxication are the most clinically relevant to health conditions such as those seen in humans suffering from traumatic injuries while intoxicated. Published animal data indicate that if alcohol is in the systemic circulation before a traumatic injury, immune responses are suppressed. These adverse effects of alcohol might be further aggravated in already malnourished individuals.

Models of Chronic Alcohol Abuse

Both in vitro and in vivo chronic alcohol abuse models are relevant to decipher mechanisms by which long-term alcohol abuse facilities the development and progression of a number of diseases. Alcohol-induced organ damage in humans is multifactor and usually observed after years of alcohol abuse. Most laboratory animals, because of their natural aversion to alcohol, do not consume sufficient

amounts of alcohol voluntarily. Several rodent lines that drink pharmacologically significant amounts of alcohol have been developed and used to study alcohol drinking behavior and its consequences [21, 22]. Described below are the most commonly used chronic alcohol abuse models.

In Vitro Models of Chronic Alcohol Abuse

Exposure of Primary Cells or Cell Lines to Alcohol in Culture Medium

In in vitro model of chronic alcohol intoxication, primary or transformed cells are exposed to alcohol, as described for acute alcohol intoxication, for greater than 24 h. For long-term alcohol exposure, the alcohol content in the culture medium and in the Petri dish has to be replenished every 2–3 days to maintain the alcohol content constant [10].

In Vivo Models of Chronic Alcohol Abuse

In most commonly used in vivo models of chronic alcohol abuse, the animals receive alcohol orally (in liquid diet and/or in drinking water), enterally (via feeding tube or surgically implanted gastric catheter), or via inhalation (exposure to alcohol vapors) for extended periods.

The Liquid Diet Model

In this model, laboratory animals are fed liquid (Lieber-DeCarli) diet with or without alcohol added. Various formulations of this diet can be prepared either in the laboratory or purchased from Dyets Inc. (Bethlehem, PA) and Bio-serv (Frenchtown, NJ). The concentration of alcohol in the diet is increased gradually to constitute 36% of the total calories. The model is commonly used to study long-term drinking effects on various organs and systems [23]. While in the standard rodent chow (e.g., 2018 Teklad Global) protein constitutes 23% of the total calories, fat 17%, and carbohydrates 60%, in Lieber-DeCarli Regular Control Diet, 18% of the total calories are derived from protein, 35% from fat, and 47% from carbohydrate, respectively. In the alcohol diet, alcohol constitutes 36% of the total calories, with protein, carbohydrate, and fat accounting for 18%, 11%, and 35% of the calories, respectively. Along with this liquid diet, animals can also be allowed ad libitum access to water with or without alcohol added. The model involves pair feeding. In addition to the Lieber-DeCarli formulations, other commercial or custom made liquid diet formulations are being used in alcohol research [24–29]. The model can be used with a second hit or trigger factor such bacterial or viral stimuli to demonstrate the role second hit might have in the initiation and progression of alcohol-induced diseases [30–32]. Published studies suggest that the composition of the liquid diet, in which alcohol is administered, can influence significantly the intensity of alcohol effects. For example, the amount and type of fat in the diet will influence the intensity of alcohol-induced organ damage [26].

In brief, age- and weight-matched rodents are housed in microisolator cages. The animals assigned to the alcohol group are allowed free access to the alcohol-containing liquid diet. The alcohol content in the diet is increased gradually from 1% to a final concentration of 5% over a 7-day period. Thereafter,

the animals are maintained on the highest ethanol concentration for the remainder of the experimental duration. The animals assigned to the control group are pair-fed the liquid diet containing maltose dextrin in amounts isocaloric to the ethanol. The model is adaptable to baboons.

Other Liquid Diet Models

Sustacal (Mead Johnson, Evansville, IN) and Carnation Slender (Nestle, Vevey, Switzerland) are two other liquid diets that have been used by investigators to maintain rodents on alcohol long term [Bautista 1995]. It is important to note that a comparative study performed using Lieber-DeCarli, Sustacal, and Carnation Slender diets suggests that bioavailability of added alcohol may not be identical in all liquid diets [33].

The Intragastric Infusion Model

The model was developed based on the hypothesis that rats have a higher rate of alcohol metabolism than humans and, therefore, may require sustained higher blood alcohol levels than humans to induce liver damage that is similar to that seen in humans. In this model, liquid diet containing alcohol and/or other dietary manipulations is infused directly into the rodent stomach for several months via a catheter implanted aseptically into the stomach. The model allows manipulation of the dietary factors and to expose the animals to a second hit enterally with ease. In this model, blood alcohol levels between 250 and 500 mg/dl can be attained and sustained. The model has been shown to produce fatty liver, localized necrosis, inflammation, and mild portal fibrosis [34].

Ethanol Agar Block Model

In this model, rodents are maintained on solid chow, 5% agar blocks containing 40% alcohol and 0.5 g/kg peanut butter, and 10% alcohol supplemented water. The agar blocks are provided to the animals in Petri dishes. The alcohol concentration in the agar block is increased gradually to 40%. The pair-fed animals receive isocaloric chow, similar amount of agar without alcohol and alcohol-free water. The model is easy to handle and affordable and allows the flexibility for dietary and cofactors manipulation. The model has been used to study alcohol effects on the immune system [35].

Agar Gel Diet Model

This model consists of giving rodents the original or modified Lieber-DeCarli liquid diet prepared in agar gel. The alcohol in the diet accounts for 34.5% of the total calories. In the control diet, these calories are accounted for by addition of 40% carbohydrate. The agar gel diet is provided to the mice in Falcon tubes equipped with 2 × 2 cm opening and mounted in a tilted position inside the pellet grid of the cage using metal strings. Water is also provided to the animals. According to the authors, the loss of alcohol to evaporation is significantly less than in the original ethanol agar block model. According to the authors, the gel consumption is high enough to attain sustained high blood alcohol levels. Feeding alcohol to laboratory animals using this model is reported to result in significant liver steatosis and elevated plasma alanine aminotransferase within 6 weeks [36].

Alcohol in Drinking Water Model

The model is a more practical solution for long-term ethanol exposure, and it has been used in various species including mice, rats, and guinea pigs. Age- and sex-matched animals are allowed free access to rodent chow and alcohol in drinking water (single bottle – no choice). The alcohol concentration is increased gradually, and thereafter, the animals are maintained on the highest alcohol concentration throughout the study. Control mice are allowed free access to rodent chow and drinking water [37, 38]. Depending on the research question, the model can be modified from single bottle (no choice) to two bottles (free choice) between water and alcohol, multiple bottles (choice between water and alcohol of varying concentrations), and allowing access to alcohol only in the dark (drinking in the dark). The model closely mimics human drinking. In addition, the available variations to this model make it one of the best suited models for a wide range of studies including genetic, dependence, and behavioral [39–41].

Exposure to Alcohol Vapors

This is an effective and reliable model in which constant blood alcohol levels can be achieved night and day with clear signs of dependence. The model can be applied to mice, rats, and guinea pigs housed individually or in groups under standard conditions of 12-h light–dark cycle, 22°C to 23°C, and 55% humidity. The animals are maintained in an isolated plastic chamber (160 × 60 × 60 cm) in which a mixture of alcohol and air is pulsed via a mixing system allowing the quantity of alcohol to be increased every 2 days during the experimental period to avoid tolerance [42, 43]. The model allows to control the dose and duration of exposure precisely, and the level of intoxication can be maintained relatively stable during the entire course of exposure as well as from one cycle to another [44, 45].

Many of the parameters investigated to study alcohol-induced organ damage and immune system dysfunction are not only affected by stress but also by the nutritional status. For example, the modulatory effects of macro- and micronutrient imbalances on parenchymal and non-parenchymal cell responses are numerous and well described in the literature; therefore, it is imperative that nutritional status in the alcohol-consuming animal is maintained. This point can be further emphasized by the finding that mice given 20% w/v alcohol in the drinking water along with free access to laboratory chow exhibit suppressed natural killer (NK) cell cytolytic activity, but when the chow intake is reduced by 30–40% of control, there is no differential effect of alcohol consumption on cytolytic activity [46]. Even if nutritional adequacy is assured through sufficient diet consumption, it is well known that alcohol consumption affects the permeability of the gastrointestinal tract, which can affect the absorption of macro- and micronutrients and immune response [47]. The alcohol-nutrient interactions and their effects on the immune response is an under-explored area of investigation.

Choosing an appropriate animal model in alcohol research is vital as it can influence significantly the outcome of the proposed experiments. In humans, the secondary effects associated with alcohol intake such as nutrient availability and/or metabolism are virtually impossible to control. The laboratory models described above allow the feasibility to create experimental conditions directed at understanding the mechanisms by which alcohol abuse modulates the availability and metabolism of certain nutrients and how supplementation of the diet with certain nutrients could possibly attenuate and, maybe, even protect against the many detrimental effects of alcohol abuse.

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Chapter 4
Ethanol-Induced Lipid Peroxidation and Apoptosis in Embryopathy

Robert R. Miller Jr.

Key Points

- Reactive oxygen species [ROS; hydroxyl radicals ($^{\cdot}OH$), superoxide radicals ($^{\cdot}O_2$), and nitrite radicals ($^{\cdot}NO_2$)] are generated during ethanol exposure and cleave polyunsaturated fatty acids into shorter, less saturated fatty acids and a number of cytotoxic and reactive aldehydes. These reactive aldehydes include 4-hydroxynonenal (HNE), 4-oxo-2-nonenal (ONE), malondialdehyde (MDA), acrolein (2-propenal), and others.
- Many of these reactive aldehydes cross-link and inhibit a growing list of proteins by forming Michael adducts with cysteine, histidine, lysine, and occasionally arginine residues within targeted proteins and/or attack Schiff bases (lysine) within targeted proteins.
- Several reactive aldehydes can cross-link reduced glutathione (GSH) through glutathione's cysteine. Aldehyde-GSH adducts can then be escorted from mitochondria, into the cytoplasm, and out of the cell by glutathione-*S*-transferase (GST; EC 2.5.1.18). These ethanol-induced reductions in the intracellular GSH pool inhibit two GSH-dependent antioxidant enzymes that include GST and glutathione peroxidase (GPx; EC 1.11.1.9).
- Unlike reactive oxygen species, reactive aldehydes are more stable and can diffuse throughout a cell and act as a "second messenger." As reactive aldehydes diffuse into mitochondria, reactive aldehydes cause increased mitochondrial membrane permeability and cause mitochondria to release cytochrome *c* into the cytoplasm. Increased cytoplasmic cytochrome-*c* levels facilitate the formation of activated apoptosomes (active apoptosome: APAF-1 (apoptotic protease activating factor-1), caspase-9, and cytochrome *c*) that cleave and activate effector (killer) caspases during the intrinsic pathway of apoptosis.
- The oxidative stress that is associated with ethanol-induced lipid peroxidation can be ameliorated, or at least partially ameliorated, by a growing list of antioxidants. A list of antioxidants reported during the past 5 years to ameliorate ethanol-induced anomalies is included in this chapter.
- While ethanol-induced lipid peroxidation and apoptosis are well documented in a vast number of animals modeling ethanol-induced toxicity, this chapter discusses ethanol-induced lipid peroxidation and apoptosis within embryonic, neonatal, and occasionally juvenile animals. This discussion primarily deals with ethanol-induced alterations in neural crest cells, neural crest cell derivatives, and the nervous system.

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Abbreviations

ADH	Alcohol dehydrogenase
ALDA	Aldolase
ALDH	Aldehyde dehydrogenase
ALT	Alanine transaminase, also known as glutamate-pyruvate transaminase
ALP	Alkaline phosphatase
APAF1	Apoptotic protease activating factor-1
AST	Aspartate transaminase, also known as glutamate-oxaloacetate transaminase
Bad	Bcl-2-associated death domain
Bax	Bcl-2-associated X protein
Bid	BH-3-interacting death domain
Bcl-2	B cell lymphoma-2 protein
Bcl-XL	B cell lymphoma-extra large
BDNF	Brain-derived nerve growth factor
CAT	Catalase
CTNF	Ciliary neurotrophic factor
CYP 2E1	Cytochrome p450-2E1
DISC	Death-inducing signaling complex
EtOH	Ethanol
GDNF	Glial cell-derived nerve growth factor
Gli-1	Glioma-associated oncogene homolog-1
GOT	Glutamate-oxaloacetate transaminase
GPT	Glutamate-pyruvate transaminase
GPx	Glutathione peroxidase
GSH	Reduced glutathione
GSSG-R	Glutathione reductase
GSSG	Oxidized glutathione disulfide
GST	Glutathione-*S*-transferase
HNE	4-hydoxynonenal
I*k*B kinase	Inhibitor of *kappa* B kinase
LPO	Lipid hydroperoxide
LPOs	Lipid hydroperoxides
MDA	Malondialdehyde
MDA-TBARs	Malondialdehyde-thiobarbituric acid adducts
NFκB	Nuclear factor kappa-light-chain enhancer of B cells
NGF	Nerve growth factor
ONE	4-oxo-2-nonenal
PLC	Phospholipase C
Ptc-1	Patch-1 receptor
$p75^{NTR}$	Protein 75 neurotrophin receptor
ROS	Reactive oxygen species
SHH	Sonic hedgehog
SOD	Superoxide dismutase
TC	Total cholesterol
TG	Total triglycerides

TGF-α *(alpha)*	Tumor necrosis factor-α *(alpha)*
TrkA, TrkB, and TrkC	Tyrosine kinase receptors
VEGF	Vascular endothelial growth factor

Introduction

Lipid peroxidation is observed during both necrosis and apoptosis [1–4]. The ethanol (EtOH)-induced synthesis of reactive oxygen species (ROS), lipid peroxidation, mitochondria dysfunction, and oxidative stress has been demonstrated in adult rat brains [5, 6], neuronal cell cultures [7], glial cell cultures [6–8], embryonic chick brains [9–13], and rat placental tissues [14]. EtOH-induced oxidative stress has been well documented in alcohol-induced: liver disease [15–19], muscle disease [20], kidney alterations [21], erosion and alterations of digestive tract mucosa [22, 23], and pancreatitis [24]. Since the author last reviewed EtOH-induced lipid peroxidation in 2004 [25], this chapter will primarily concentrate on papers published after 2004.

Mechanics of Lipid Peroxidation

The mechanics of lipid peroxidation have been previously reviewed [3, 26–33] and can be quite ornate. However, in its simplest form, fatty acid peroxidation has six steps within three major stages. The first major stage is initiation. In the first step, an electron is donated by either a ferrous ion (Fe^{+2}), via the Fenton reaction, or a reactive oxygen species [ROS; hydroxyl radical ($\cdot OH$), superoxide radical ($\cdot O_2$), nitrite radical ($\cdot NO_2$)]. This appears to be the rate-limiting step [26]. Oxidation by the presence of a Fe^{+2} ion is nonenzymatic in means and is illustrated below via the Fenton reaction:

$$\text{Fenton Reaction: } Fe^{+2} \longrightarrow Fe^{+3} + \text{electron}$$

ROS are generally synthesized by enzymatic means and can also initiate lipid peroxidation. However, hydroxyl radicals ($\cdot OH$) are the preferred electron donors over superoxide radicals ($\cdot O_2$) because superoxide radicals ($\cdot O_2$) reduce ferric (Fe^{+3}) chelates and, thus, generate hydroxyl radicals ($\cdot OH$) via the Harber-Weiss reaction [26–29], as illustrated below:

$$H_2O_2 + Fe^{+2} \rightarrow OH^- + {}^{\cdot}OH + Fe^{+3} \text{ Fenton Reaction}$$

$$+ Fe^{+3} + {}^{\cdot}O_2 \rightarrow Fe^{+2} + O_2 \text{ Reduction of ferric ion}$$

$$H_2O_2 + {}^{\cdot}O_2 \longrightarrow OH^- + ({}^{\cdot}OH) + Fe^{+3} \text{ Harber - Weiss reaction}$$

During lipid peroxidation, the electron donated by either a Fe^{+2} ion or hydroxyl radical ($\cdot OH$) is absorbed by a hydrogen atom attached to a saturated carbon adjacent to a carbon-carbon double bond within the fatty acid under attack. Hence, an alkyl radical is formed. During the second of step of INITIATION, the absorption of an electron causes the formation of an alkyl radical and promotes a rearrangement of double bonds within the alkyl radical. In the third step, a reaction with molecular oxygen causes the formation of a lipid peroxyl radical and leads to propagation. In the forth step, the lipid peroxyl radical can remove an electron from another nearby alkyl radical and thus form a lipid hydroperoxide (LPO) which promotes termination. In the 5th step, the Fe^{+2} (ferrous)-dependent cleavage of a lipid hydroperoxide forms an alkoxyl radical. Finally, in step 6, cleavage of the alkoxyl radical by β-(beta) scission is observed. This creates a shorter, less unsaturated fatty acid and a number of reactive and cytotoxic aldehydes [26–33] (see Figs. 4.1, 4.2, 4.3, 4.4, and 4.5). The β-(beta) scission or cleavage of a long-chain polyunsaturated membrane fatty acid and subsequent replacement with a shorter-chain, less polyunsaturated membrane fatty acid has membrane fluidity implications that can contribute to a reduction in membrane functionality and reduced cellular viability [25].

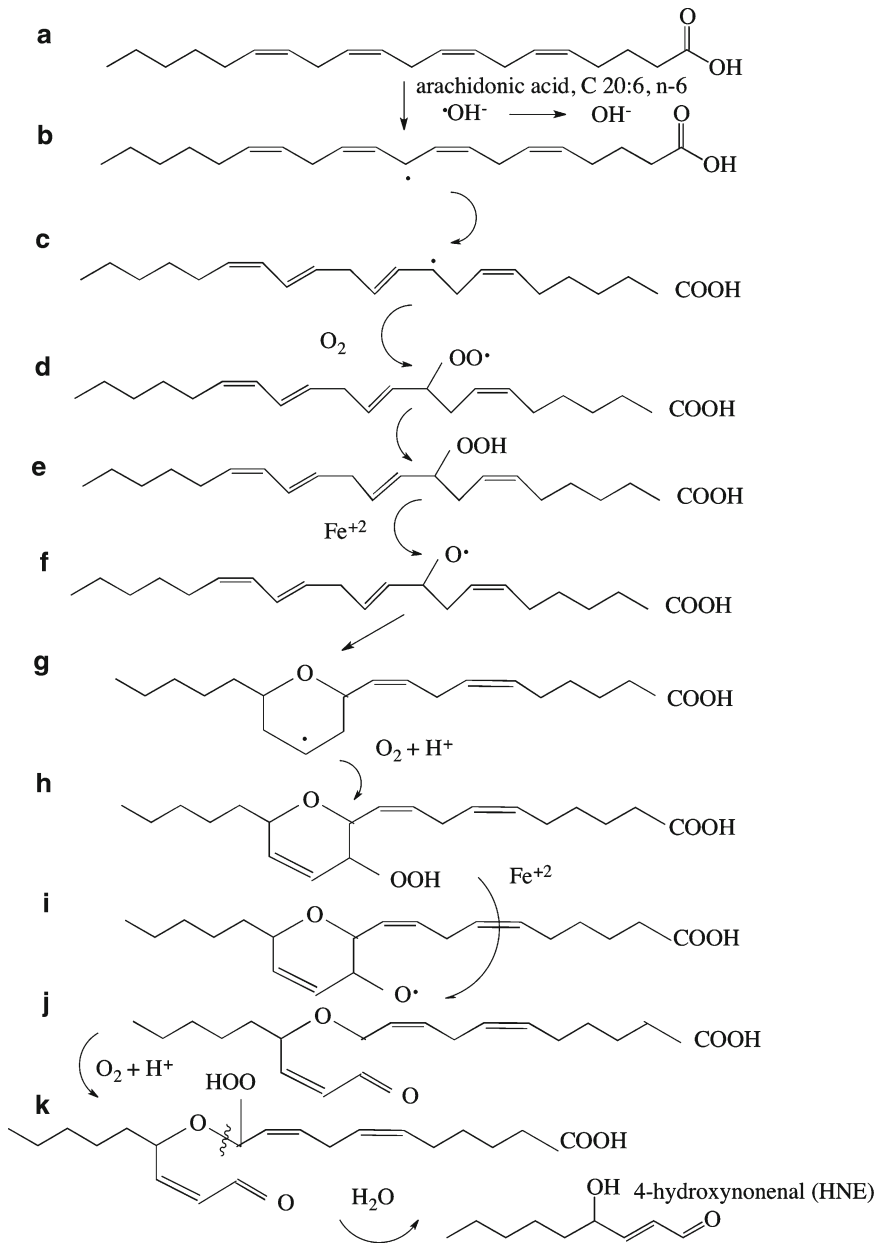

Fig. 4.1 *Lipid peroxidation and synthesis of 4-hydroxynonenal (4-HNE) from an n-6 fatty acid [arachidonic acid (C 20:4, n-6)]. A*. Initiation: Removal of an electron from a reactive oxygen species (ROS) to make an alkyl radical. *B*. Rearrangement of double bonds within the alkyl radical. *C*. Reaction with molecular oxygen to form a lipid peroxyl radical. *D*. Propagation: Removal of an electron from a second alkyl radical to form a lipid hydroperoxide. *E*. Ferrous-dependent cleavage of lipid hydroperoxide to form an alkoxyl radical. *F*. Cyclization. *G*. Electron removal and second peroxidation. *H*. Reaction with molecular oxygen and H^+ to form a second lipid hydroperoxide. *I*. Ferrous-dependent cleavage to form second alkoxyl radical. *J* and *K*. TERMINATION: β-scission (cleavage) to form 4-hydroxynonenal (Based on data from Ref. [33])

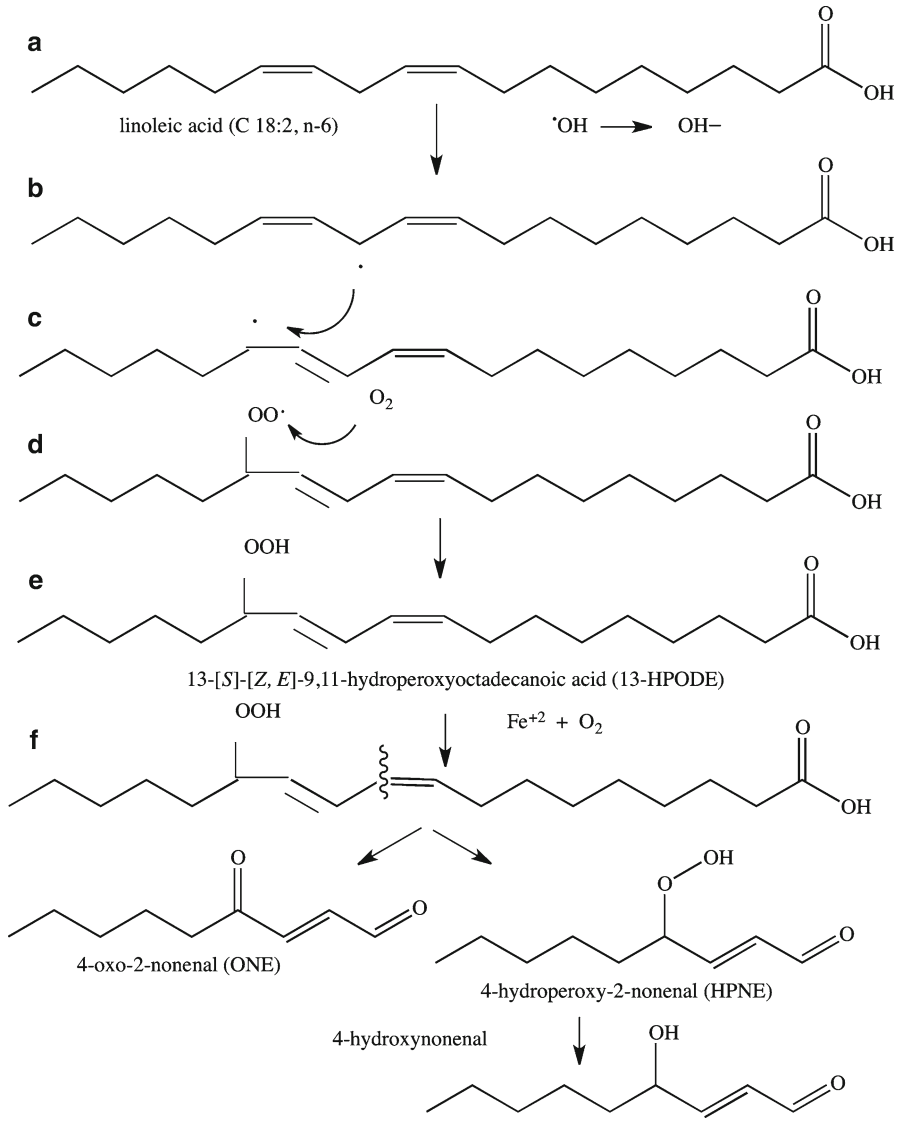

Fig. 4.2 *Lipid peroxidation and synthesis of 4-oxo-2-nonenal (ONE and 4-hydroperoxy-2-nonenal (HPNE) from an n-6 fatty acid [linoleic acid, (C18:2, n-6)]. A*. Initation: Removal of an electron from a reactive oxygen species (ROS) to make an alkyl radical. *B*. Rearrangement of double bonds within the alkyl radical. *C*. Reaction with molecular oxygen to form lipid peroxyl radical. *D*. Propagation: Removal of an electron from a second alkyl radical to form a lipid hydroperoxide. *E*. Termination: β-scission (cleavage) of 13-HPODE to either 4-oxo-2-nonenal (ONE) or 4-hydroxynonenal (HNE) (Based on data from Ref. [55])

Reactive Aldehydes

Unlike free radicals, reactive aldehydes are rather long-lived and can diffuse from their origin site and attack targets that are both intracellular and extracellular. Thus, the synthesis of reactive aldehydes via lipid peroxidation can be viewed as a "second messenger" and amplify cytotoxicity by moving from the cell membrane to the cytoplasm, from the cytoplasm into the mitochondria, and from the cytoplasm into the extracellular matrix of decomposing cells [27]. A number of reactive aldehydes have been

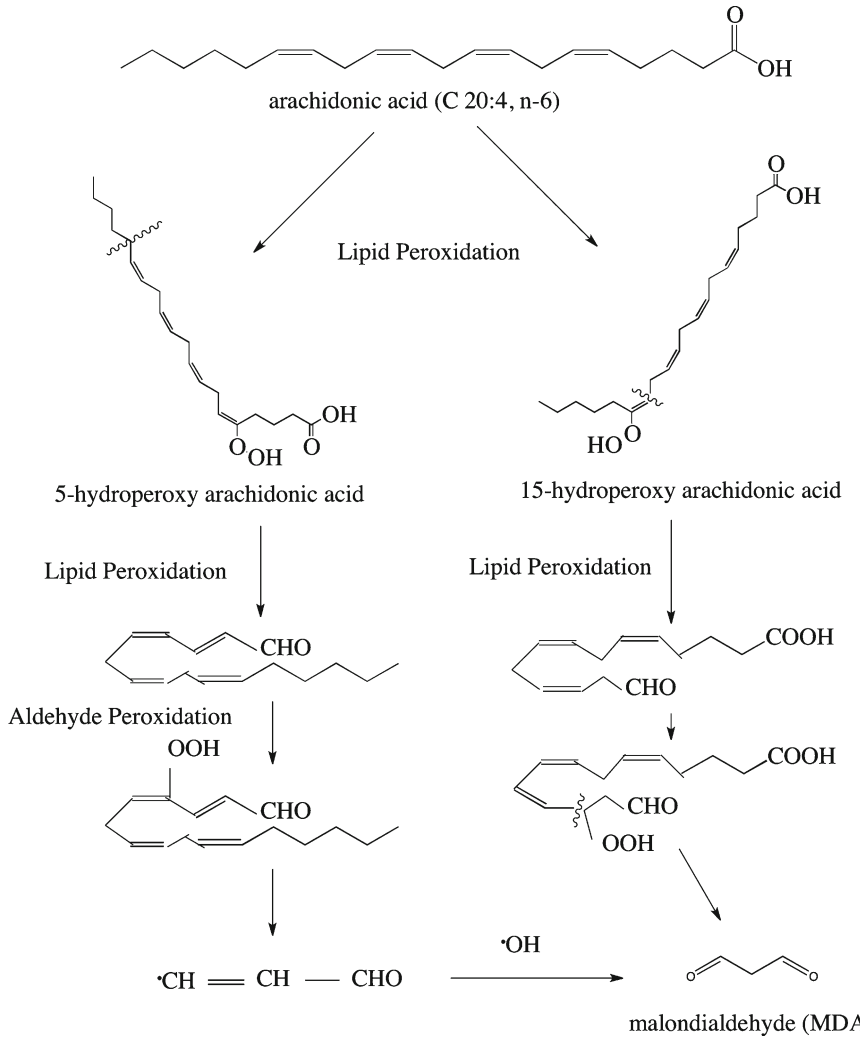

Fig. 4.3 *Lipid peroxidation of arachidonic acid (C 20:4, n-6)*. Primary peroxidation to 5-hydroperoxy arachidonic acid and 15-hydroperoxy arachidonic acid followed by secondary peroxidation to malondialdehyde (MDA) (Based on data from Ref. [27])

demonstrated to impair spinal cord and brain mitochondrial functions and can react and cross-link proteins by attacking a Schiff (imine) base (lysine) and/or forming a Michael reaction adduct by bonding to lysine, histidine, and/or cysteine residues within targeted proteins [34, 35]. Since significant differences in sensitivity between different tissues and cell types exist [27], the identification of proteins targeted by reactive aldehydes is of great interest. Presumably, the biological activity of the targeted protein is lost when forming aldehyde-protein adducts.

4-Hydroxynonenal (HNE)

The lipid peroxidation-derived synthesis of 4-hydroxyalkenals from methyl linoleate (C18:2, n-6) was discovered in the early 1960s [36], and the most cytotoxic aldehyde within the 4-hydroxyalkenals family may be 4-hydroxynonenal (HNE) [37]. The peroxidation of all lipids containing omega-6 (n-6)

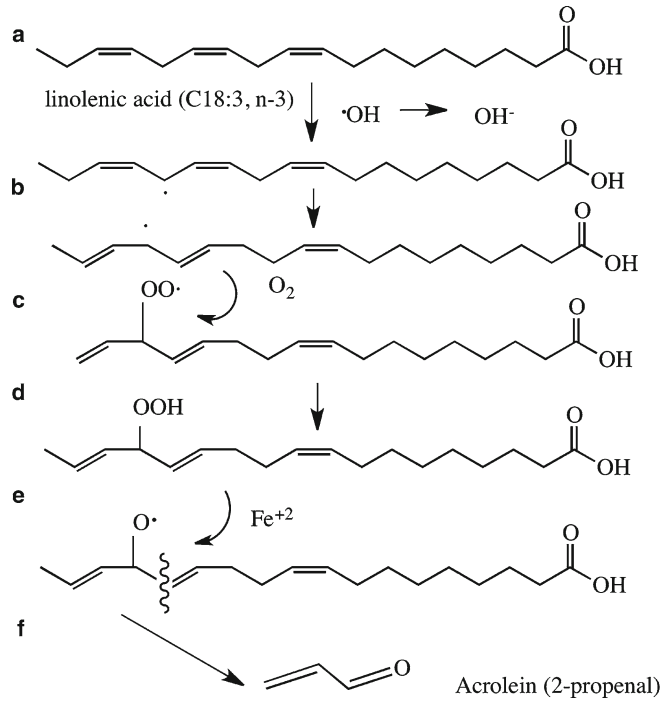

Fig. 4.4 *Lipid peroxidation of an n-3 fatty acid [linolenic acid (C18:3, n-3)] to acrolein. A*. INITIATION: Removal of an electron from a reactive oxygen species (ROS) to make an alkyl radical. *B*. Rearrangement of double bonds in alkyl radical. *C*. Reaction with molecular oxygen to form lipid peroxyl radical. *D*. PROPAGATION: Removal of an electron from a second alkyl radical to form a lipid hydroperoxide. *E*. Ferrous-dependent cleavage of lipid hydroperoxide to form alkoxyl radical. *F*. TERMINATION: β-scission (cleavage) to acrolein (2-propenal)

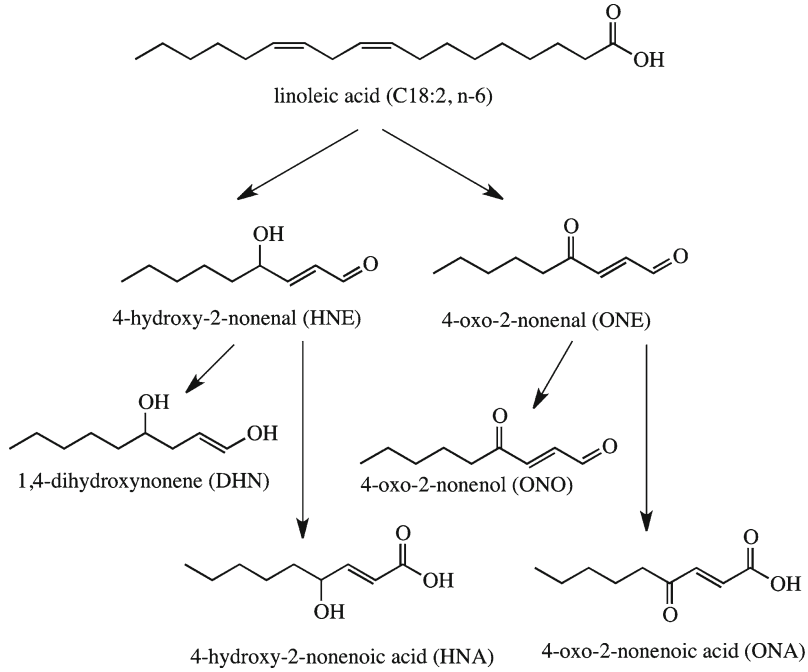

Fig. 4.5 *Lipid peroxidation of an n-6 fatty acid [linoleic acid (C 18:2, n-6)]*. Primary peroxidation to HNE and ONE followed by secondary peroxidation to DHN, HNA, ON), and ONA (Based on data from Ref. [95])

polyunsaturated fatty acids [linoleic acid (C18:2, n-6), arachidonic acid (C 20:4, n-6), and docosapentaenoic acid (DPA; C22:5, n-6)] will produce HNE and hexanal [27], and the somewhat ornate peroxidation of arachidonic acid (C20:4, n-6) to HNE is illustrated in Fig. 4.1 [33].

From a biological standpoint, HNE is known to uncouple brain and spinal cord mitochondrial respiration at concentrations ranging from 0.01 to 0.1 μM (micromolar) [34, 35]. During oxidative stress in rodent embryos, HNE can cross-link and inhibit cellular signaling proteins including I*k* (*kappa*) B kinase (inhibitor of *kappa* B kinase) [38]; heat shock protein 90 (HSP 90) and heat shock protein 72 (HSP 72); glyceraldehyde-3-phosphate dehydrogenase (GAPDH; EC 1.2.1.12); glutamate-oxaloacetate transaminase-2 (GOT-2; EC 2.6.1.2); aldolase-1 (ALDA; EC 4.1.2.13); and p300 protein/CREB-binding protein (p300/cAMP response element binding protein) [39, 40]. The HNE-p300/CREB adducts inhibit CREBP and may initiate p53-dependent apoptosis [39]. Meanwhile, in EtOH-treated hepatic tissues, the polymerization of cytoskeletal tubulin is inhibited because HNE forms adducts with both α-(*alpha*) and β-(*beta*) tubulin [41, 42]. HNE-induced inhibition of tubulin polymerization within neurons could affect developing neuron's ability to form cell processes (axons and dendrites) [42, 43]. In EtOH-treated rats, HNE cross-links hepatic ERK 1/2 (extracellular signal-regulated kinases 1 and 2), which are classical mitogen-activated kinases and are accompanied by decreased hepatic ERK 1/2 phosphorylation and decreased phosphorylation of the downstream hepatic nuclear ELK-1 kinase (E 26-like transcription factor 1 kinase) [44]. Presumably, HNE-ERK ½ adducts inhibit signaling and promote apoptosis within EtOH-treated hepatocytes [44].

Ramachandran et al. [45] conducted a time-course study on cultured fetal rat cortical neurons challenged with EtOH (2.5 mg/ml). In 5 min after the addition of EtOH, increased ROS levels were observed. ROS levels increased by 58% within 1 h ($p < 0.05$) and by 82% within 2 h ($p < 0.05$), accompanied by increased levels of mitochondrial HNE and malondialdehyde (MDA). This was followed by increased apoptosis rates as measured by EtOH-induced increased annexin-V activity associated with EtOH-induced increased caspase-3 activity, EtOH-induced release of mitochondrial cytochrome *c* into the cytoplasm, and EtOH-induced DNA fragmentation. Meanwhile, pretreatment of fetal cortical neurons with *N*-acetylcysteine (NAC) caused increased glutathione levels (GSH) and ameliorated EtOH-induced apoptosis [45]. Ramachandran et al. previously [46] demonstrated that embryonic EtOH exposure caused increased brain HNE levels, promoted increased mitochondrial membrane permeability, and promoted the release of mitochondrial cytochrome *c* into the cytoplasm and then apoptosis. *N*-acetylcysteine (NAC), which is a known antioxidant, reacts directly with electrophiles and facilitates the synthesis of reduced glutathione (GSH) [47, 48].

EtOH-induced increased HNE levels can deplete the available GSH pool [27, 31, 45, 49–52] because HNE can cross-link reduced GSH either nonenzymatically or by the enzymatic use of glutathione-*S*-transferase isozymes (GST; EC 2.5.1.18) [51]. The GST-mediated removal of GSH-HNE complexes from mitochondria into the cytoplasm is followed by efflux into the extracellular matrix [31, 51–54]. As EtOH-induced depletion of the intracellular GSH pool is observed, another GSH-dependent enzyme may also suffer reduced activity and promote oxidative stress. Glutathione peroxidases (GPx; EC 1.11.1.9) are dependent on the presence of two reduced glutathione (GSH) molecules and convert lipid hydroperoxides (LPOs), which are lipid peroxidation intermediates, into less toxic alcohols. During GPx activity, a disulfide bond between two reduced GSH molecules (GSH) forms and the oxidized glutathione disulfide (GSSH) dimer is synthesized [55]. Hence, short-term EtOH exposure depletes the intracellular GSH pool through the formation of HNE-GSH adducts, inhibits two GSH-dependent antioxidant enzymes (GST and GPx), and promotes further HNE-mediated oxidative stress and apoptosis [54].

4-Oxo-2-Nonenal (ONE)

The peroxidation of omega-6 fatty (n-6) acids is known to produce 4-hydroperoxy-2-nonenal (HPNE), 4-hydroxynonenal (HNE), and 4-oxo-2-nonenal (ONE) (see Fig. 4.2) [56]. The omega-6 (n-6) fatty

acids include linoleic acid (C18:2, n-6), arachidonic acid (C20:4, n-6), and docosapentaenoic acid (DPA; 22:5, n-6). Through the lipid peroxidation of linoleic acid (C18:2, n-6), the intermediate 13-[*S*]-[*E, Z*]-9,11-hydroperoxyoctadecanoic acid (13-HPODE) is synthesized and 13-HPODE is then further oxidized to 4-hydroperoxy-2-nonenal (HPNE) and 4-oxo-2-nonenal (ONE) [55]. While exogenous HNE, HPNE, and ONE are all known to initiate the activation of caspases, nucleosomal DNA fragmentation, and apoptosis within a human colorectal cancer cell line (RKO cells) [57], little is known about HPNE-targeted proteins and the possible role EtOH-induced HPNE synthesis may play in ETOH-treated animals. Hence, ONE-targeted proteins and the possible role EtOH-induced ONE synthesis may play in ETOH-treated animals will be discussed.

ONE and γ-ketoaldehydes are more stable and are more reactive aldehydes as compared to the more frequently studied HNE [27, 58–60]. While ONE is a 4-keto cousin of HNE, ONE can be independently synthesized from linoleic acid (C18:2, n-6) [56, 61]. In one of the few direct studies linking EtOH-treated cells to increased ONE levels, EtOH-induced lipid peroxidation caused the synthesis of both HNE and ONE in rats exhibiting chronic alcoholic liver disease [40]. Both HNE and ONE were found to form adducts with heat shock protein 90 (HSP 90) by cross-linking cysteine 576 within HSP 90 proteins [40].

Several studies indicate a possible link between ONE and neuropathy. ONE was found to be more neurotoxic as compared to HNE and forms protein adducts at a faster rate and at lower concentrations within human neuroblastoma cells [58]. Picklo et al. [35] found that ONE uncouples mitochondrial respiration, causes mitochondrial swelling, and inhibited brain mitochondrial aldehyde dehydrogenase (ALDH) activities at a faster rate and at lower concentrations as compared to HNE. Picklo et al. [35] reported that ONE enters brain mitochondria and inhibits ALDH2 activity before it uncouples mitochondrial respiration and promotes mitochondrial swelling coupled with the inhibition of ALDH5. These events, in turn, preceded a depletion of the mitochondrial GSH pool. A ONE-induced depletion of the reduced mitochondrial GSH pool was associated with cross-linkage of ONE to GSH and carnosine [62]. The ability of ONE to cross-link mitochondrial GSH [62] may mirror the previously discussed HNE story. That is, like HNE, ONE can cross-link GSH either nonenzymatically or by the use of glutathione-*S*-transferase isozymes (GST; EC 2.5.1.18) [52, 62]. Then, GST-dependent removal of GSH-ONE complexes from mitochondria to the cytoplasm followed by efflux into the extracellular matrix may be observed [31, 51–54, 62]. Like HNE, the ability of ONE to cross-link reduced GSH is due to the cysteine within GSH because ONE and HNE can cross-link targeted proteins through forming Michael adducts with cysteine, histidine, and lysine residues within targeted proteins and react with Schiff (imine) bases within lysine [27, 63–65]. Unlike HNE, ONE is also capable of forming Michael adducts with arginine residues within targeted proteins [63]. Another distinctive difference between ONE and HNE is the affect of GSH on cross-linking ability. While the ability of HNE to cross-link targeted proteins is inhibited by high concentrations of reduced GSH, the ability of ONE to cross-link targeted proteins is stimulated by high concentrations of reduced GSH [62]. However, like HNE, the neurotoxicity of ONE also involves the ability of ONE to cross-link α-(*alpha*) and β-(*beta*) tubulin and prevent microtubule polymerization [42]. ONE, like HNE-induced inhibition of α-(*alpha*) and β-(*beta*) tubulin polymerization [43], may inhibit neurite outgrowth.

Malondialdehyde (MDA)

Malondialdehyde (MDA) may well be the oldest reactive aldehyde whose synthesis was reported in 1903 [66]. It is ironic that MDA is one of the oldest known reactive aldehydes because controversy still remains concerning the biological synthesis of MDA. Esterbauer et al. [27] reported that MDA was not generated from the Fe^{+2}/ascorbate-induced oxidation of oleic acid (C18:1, n-9) and MDA could only slightly be generated from the Fe^{+2}/ascorbate-induced oxidation of linoleic acid (C18:2, n-6; 0.5 mol%). Better sources for the production of MDA included the lipid peroxidation of linolenic

acid (C18:3, n-3; 4.5 mol%), γ (gamma)-linolenic (18:3, n-6; 4.9 mol%), arachidonic acid (C20:4, n-6; 4.7 mol%), and docosahexaenoic acid (DHA; C22:6, n-3; 7.6 mol%) [27]. A mechanism for the MDA synthesis from prostaglandin-GH_2 (PGH_2) was proposed by Hecker and Ulrich [67] and included in the review of Esterbauer et al. [27]. This diverse list illustrates that MDA can be the oxidized product of either omega-3 (n-3) fatty acids, omega-6 (n-6) fatty acids, or fatty acid derivatives. The primary requirement for MDA synthesis is that the fatty acid undergoing lipid peroxidation must be polyunsaturated and possess at least three unsaturated, double bonds. The proposed mechanism by which MDA is produced from arachidonic acid (20:4, n-6) is illustrated in Fig. 4.3 [27]. While variations now exist, free-MDA is most often detected by the colorimetric or fluorescent detection of MDA-thiobarbituric acid adducts (TBARs assay) [27].

Acrolein (2-Propenal)

Acrolein (2-propenal) is generated from the peroxidation of omega-3 (n-3) fatty acids (See Fig. 4.4) [68–72]. Acrolein is mutagenic [72] and is by far the strongest electrophile among all *α-(alpha), β-(beta)* unsaturated aldehydes with the highest reactivity with nucleophiles such as the sulfhydryl group of cysteine and the imidazole group of histidine and lysine [27, 68]. While largely ignored when studying EtOH-treated animals, the in vitro treatment of adult mouse sensory neurons with either EtOH, acetaldehyde, propanol (which is another lipid peroxidation by-product), or acrolein all caused membrane pitting and a reduction in neurons bearing neurites [73]. Allyl alcohol-induced liver injury in rats was associated with reduced GSH levels and the hepatic accumulation of acrolein [74]. Coexposure of allyl alcohol with EtOH alleviated allyl alcohol-induced hepatic injuries and implied that the metabolism of allyl alcohol and EtOH may involve a common enzyme family, that is, aldehyde dehydrogenases (ALDH; EC 1.2.1.3) [74].

While acrolein accumulation and pathology has largely been ignored when studying EtOH-treated animals, a possible role may exist. Acrolein is known to form adducts with GSH [75, 76], and EtOH-induced reductions in the GSH pool are well documented [27, 31, 45, 49–54]. Acrolein accumulation is known to induce apoptosis [72, 77] via the Fas-ligand receptor [77], and EtOH-induced apoptosis via the Fas-ligand receptor is well documented [78–82]. Acrolein is also known to form adducts with ascorbic acid (vitamin C) [83], and EtOH-induced reductions in ascorbic acid levels are well documented [84–89]. Acrolein is a toxicant in cigarette smoke and causes mitochondrial dysfunction that is ameliorated by lipoic acid [90], and more than 85% of adults with a history of alcohol abuse also smoke [91]. Lipoic acid has also been used to ameliorate EtOH-induced toxicity [92–94].

Miscellaneous Reactive Aldehydes

Besides HNE, ONE, MDA, and acrolein, other reactive aldehydes exist. HNE can be further metabolized into 1,4-dihydroxynonene (DHN) and 4-hydroxy-2-nonenoic acid (HNA). Meanwhile, ONE can be further metabolized into 4-oxo-2-nonenol (ONO) and 4-oxo-2-nonenoic acid (ONA) (see Fig. 4.5) [95]. However, at this time, this author is unaware of any published paper that associates EtOH treatments with the accumulation of DHN, HNA, ONO, or ONA.

Roychowdhury et al. [60] reported EtOH-induced oxidative stress within mice livers that was associated with the ROS-dependent lipid peroxidation of arachidonic acid (C20:4, n-6) and the cyclooxygenase-dependent (COX; EC 1.14.99.1) peroxidation of prostaglandin intermediates to γ *(gamma)*-ketoaldehydes. Formation of γ *(gamma)*-ketoaldehydes due to the nonenzymatic oxidation of prostaglandin endoperoxide intermediates can represent approximately 20% of total

COX-dependent products under normal physiological conditions [96]. Two such *γ (gamma)*-ketoaldehyde products include levuglandin, known as LGE_2, and isolevuglandin, known as iso[4] LGE_2, and the synthesis pathways and detection of levuglandins and isolevuglandins have recently been reviewed [97]. *γ(gamma)*-Ketoaldehydes are more reactive than either HNE or MDA [60, 97], and LGE_2-protein adducts and iso[4] LGE_2-protein adducts have been found within the brains of Alzheimer's patients, and their levels correlate with the severity of the disease [98]. EtOH-induced liver injuries, EtOH-induced increased hepatic LGE_2-protein adduct levels, and EtOH-induced increased hepatic iso[4] LGE_2-protein adduct levels were associated with elevated serum alanine transaminase activity (ALT; EC 2.6.1.2), a marker of hepatic cell death, and elevated hepatic cytochrome p450 2E1 (CYP 2E1; EC 1.14.14.1) within EtOH-treated mice as compared to controls [60]. CYP 2E1 is well associated with EtOH-induced oxidative stress and generates superoxide anions ($\cdot O_2$) coupled with the formation of hydroxyethanol radicals [99].

Apoptosis

EtOH-induced ROS production and lipid peroxidation are important issues because they induce genetically programmed cell death (apoptosis) [9, 45, 46, 99, 100]. Initiation of apoptosis can begin by the extrinsic pathway or the intrinsic pathway [101]. The intrinsic pathway begins within the cell and can be initiated with DNA damage, oxidative stress directed against the mitochondrial membrane, and/or the transcription of oncogenes that, in turn, promote transcription of proapoptotic genes within the *Bcl-2* (B cell lymphoma 2 protein) family of genes [102, 103]. In the intrinsic pathway, DNA damage can promote the synthesis of p53, and elevated p53 levels promote the expression of proapoptotic *Bcl-2* family genes, including *Bax* (Bcl-2-associated X protein), BH-3-only proteins including Noxa (*Latin* for damage), and PUMA (p53-upregulation modulator of apoptosis). Increased Bax, Noxa, and PUMA levels and oxidative damage directed against the mitochondrial membrane all have the ability to cause mitochondria to release cytochrome *c* from mitochondria into the cytoplasm. Upon crossing into the mitochondria, several reactive aldehydes, including HNE, HPNE, and ONE, are known to cause increased mitochondrial membrane permeability and are associated with the release of cytochrome *c* from the mitochondria into the cytoplasm [34, 35, 45, 57]. Increased cytoplasmic cytochrome-*c* levels facilitate the formation of activated apoptosomes (active apoptosome: APAF-1, caspase-9, and cytochrome *c*). Activated caspase-9, within activated apoptosomes, cleaves and activates effector (killer) caspases including caspase-3, caspase-6, and caspase-7 [101–105]. Caspase-3 is a protease that cleaves any protein with a DEVD sequence (aspartic acid-glutamic acid-valine-aspartic acid) [103] and has been used as a marker of EtOH-induced apoptosis within embryos [9, 99, 100]. Thus, the rapid destabilization of the mitochondrial membrane is part of the intrinsic pathway.

The extrinsic pathway is initiated at the cell membrane with the activation of receptor proteins that possess death domains (death-inducing signaling complex; DISC) [101, 102]. The binding of a ligand, such as tumor necrosis factor, Fas-ligand, TRAIL-ligand, or Apo 3-ligand, or the deprivation of a growth factor causes the activation of membrane receptors that possess death domains (DISC), and EtOH-induced apoptosis via the Fas-ligand receptor is also well documented [78–82]. DISC signaling activates a number of adaptor molecules including FADD and caspase-8. Caspase-8 cleaves proteins that have IETD domains (isoleucine-glutamic acid-threonine-aspartic acid) [103]. Activated capase-8 activates effector (killer) caspases (caspase-3, caspase-6, and caspase-7) and/or cleaves a BH-3 protein known as Bid (BH-3-interacting death domain). Once cleaved, truncated Bid will incorporate into the mitochondrial membrane and promote the release of cytochrome *c*. Once in the cytoplasm, cytochrome *c* will activate apoptosomes and effector caspases including caspase-3, caspase-6, and caspase-7 [101–103]. It is also known that EtOH-induced apoptosis can proceed through the activation of cell membrane-bound death-inducing signaling complexes (DISC), annexin-V involvement,

poly(ADP-ribose)polymerase (PARP) involvement, p53 involvement, and PUMA (p53-upregulation modulator of apoptosis) involvement [14, 45, 78–82, 104–111]. Thus, ethanol can stimulate apoptosis by both the extrinsic pathway, which then spreads to mitochondrial dysfunction.

Examples of EtOH-Induced Lipid Peroxidation

If EtOH-induced lipid peroxidation has a major role in EtOH-induced toxicity, then the use of antioxidants should ameliorate/attenuate EtOH-induced toxicity. The use of antioxidants to attenuate EtOH-induced toxicity was covered in the 2004 review [25]. The early list of antioxidants included vitamin E (*α-(alpha)* tocopherol) [2, 9, 11, 13, 54, 89], resveratrol [13], betaine [17], *α-(alpha)* lipoic acid [23, 90, 92], melatonin [86, 87], ascorbic acid [87–89], and green-tea extracts.

Antioxidants Used to Ameliorate EtOH-Induced Lipid Peroxidation

During the past 5 years, the antioxidants used to ameliorate EtOH-induced toxicity now include an exotic list of antioxidants fount within the plant kingdom (see Tables 4.1, 4.2, 4.3, and 4.4). Several publications have dealt with the mechanics of antioxidants [33, 112], and readers should refer to the review of Hall et al. [33]. Antioxidants can be somewhat helpful in treating traumatic brain injuries because they can (1) prevent the formation of ROS including the highly reactive nitric oxide radical ($\cdot NO_2$) which is mediated by the activation of a nitric oxide synthase isozyme (NOS; EC.1.14.13.39), (2) scavenge reactive oxygen species ($\cdot O_2$, $\cdot OH$, and/or $\cdot NO_2$), and/or (3) scavenge lipid peroxyl radicals ($LOO\cdot$) or alkoxyl radicals ($LO\cdot$) [33].

Three distinct forms of nitric oxide synthases (NOS; EC 1.14.13.39) exist, and they include neuronal NOS (nNOS), endothelial NOS (eNOS), and inducible NOS (iNOS). While nNOS and eNOS are constitutively expressed and are regulated by calmodulin, iNOS is induced during oxidative stress by macrophages [113]. At low concentrations, nitric oxide can be an antioxidant and remove oxygen radicals ($\cdot O_2$) and form the powerful oxidant peroxynitrite ions ($ONOO^-$). However, high peroxynitrite levels can induce apoptosis through the decay of peroxynitrous acid (ONOOH) into hydroxyl radicals ($\cdot OH$) [114, 115]. These reactions are illustrated as follows:

$$\cdot O_2 + NO^- \rightarrow ONOO^- \leftrightarrow ONOOH \rightarrow \cdot OH + \cdot NO_2 \rightarrow NO_{3^-} + H^+$$

A fourth group of antioxidants exist which include agents that enhance antioxidant enzyme activities. This list includes compounds that contain selenium [134, 158] because selenium is a known cofactor for several glutathione peroxidases (GPx) including GPx1, GPx2, GPx3, GPx4, and GPx6. This fourth antioxidant family also includes a number of cysteine-containing compounds, including *S*-allyl cysteine (SAC), *S*-propyl cysteine (SPC), *S*-ethyl cysteine (SEC), *S*-methyl cysteine (SMC), and *N*-acetyl cysteine (NAC) [15, 112]. These compounds promote increased GSH levels. By ameliorating EtOH-induced decreases in the GSH pool, the antioxidant enzymes, GST and GPx isozymes, can continue to remove reactive aldehyde-GSH adjuncts and metabolize LPOs, respectively. Yan and Yin [112] demonstrated that in vivo exposure to SA, SEC, SMC, or SPC all alleviated EtOH-induced increased hepatic MDA levels, increased hepatic ROS levels, decreased hepatic GSH levels, and decreased hepatic GPx activities within Balb/cA mice. Meanwhile, the in vivo use of *N*-acetyl cysteine (NAC) in attenuating EtOH-induced hepatotoxicity in rats has also been reported [15].

Table 4.1 Examples of ethanol-induced lipid peroxidation from 2010 to 2011

Year	Ref. no.	Comments	Lipid peroxidation detected by	Antioxidant used for amelioration (attenuation)
2011	[116]	Wistar rat kidneys: EtOH-induced decreased activities of superoxide dismutase (SOD) and catalase (CAT) with reduced GSH levels	MDA-TBARs	*Cnidoscolus aconitifolius* (*chaya*) extract
2011	[117]	Wistar rat livers: EtOH-induced decreases in GSH levels and SOD and CAT activities	MDA-TBARs	*Cnidoscolus aconitifolius*
2011	[118]	Rat livers and serum: EtOH-induced increases in serum glutamate-oxaloacetate transaminase (GOT), glutamate-pyruvate transaminase [GPT; also known as alanine transaminase (ALT)], alkaline phosphatase (ALP), and bilirubin. EtOH-induced increased hepatic hydroxyproline levels	MDA-TBARs and lipid hydroperoxides (LPOs)	Meso-zeaxanthin (carotenoid)
2011	[119]	C57BL/6 mouse livers: EtOH-induced increased serum GOT, GPT, and triglyceride (TG) levels and EtOH-induced decreased hepatic CAT and SOD levels and increased hepatic cytochrome p450 2E1 (CYP2E1) expression	MDA-TBARs	Chestnut (*Castanea crenata*) extract
2011	[86]	Rat gastric mucosal: EtOH-induced decrease in mucosal GSH and decreased serum GSH, ascorbic acid, retinol, and β-(*beta*) carotene levels	MDA-TBARs	*Matricaria chamomilla* extract (German chamomile)
2010	[120]	Adult rat brain: EtOH-induced decreased glutathione peroxidase (GPx), glutathione reductase (GSSG-R), SOD, and CAT activities	MDA-TBARs and HNE adducts	L-carnitine
2010	[121]	Wistar rats' livers and kidneys: EtOH-induced increased levels of serum AST, alanine aminotransferase (ALT), ALP, and bilirubin	MDA-TBARs	Morin (flavonoid)
2010	[122]	Rat liver: EtOH-induced decreased hepatic mitochondrial GSH levels and increased plasma transaminases associated fatty infiltration of the liver as determined by histology	Reactive oxygen species (ROS) and aldehyde adjuncts	Wu-Zi-Yan-Zong-Wan (Yang-invigorating herbal formula)
2010	[123]	Rat hepatocytes: EtOH-induced increased CYP2E1 activities, increased caspase-3, and caspase-9 expression, associated with decreased GSH levels	Lipid peroxides	β-*(beta)* Carotene
2010	[124]	Mouse gastric mucosal: EtOH-induced gastric lesions	MDA-TBARs	Esculin (6,7-dihydroxy-coumarin-6-o-glucoside)
2010	[125]	Rat liver: EtOH-induced hepatotoxicity as determined by increased serum GOT, GPT, ALP, and bilirubin levels	MDA-TBARs and LPOs	Lutein (carotenoid)
2010	[126]	Rat hepatocyte cell cultures: Increased release of ALT and AST associated with decreased GSH, SOD, and GSSG-R activities	ROS	Phyllanthin (a component in *Phyllanthus amarus*/stonebreaker)
2010	[127]	HepG2 cells: EtOH-induced decreased mitochondrial GSH levels and increased mitochondrial membrane permeability	ROS	Wu-Zi-Yan-Zong-Wan (herbal formula)

Table 4.2 Examples of ethanol-induced lipid peroxidation from 2008 to 2009

Year	Ref. no.	Comments	Lipid peroxidation detected by	Antioxidant used for amelioration (attenuation)
2009	[128]	Human hepatocyte cell line, VL17-A: EtOH-induced oxidative stress, increased CYP 2E1 induction, decreased GSH/GSSH ratios. Cells pretreated with inhibitors for P13K, Akt, and NF-*kappa* B all exhibited ameliorated HGF-induced expression of antioxidant enzymes (SOD1, CAT, *γ-(gamma)*-glutamylcysteine synthase expression) and increased GSH /GSSG ratios. Hence HGF protects cells from EtOH-induced oxidative stress through NF-*kappa* B and PK13K/Akt signaling	Lipid peroxides	Hepatocyte growth factor (HGF)
2009	[129]	Wistar rat livers: EtOH-induced increases in serum ALT, AST, and ALP activities and reduced hepatic SOD, CAT, GST activities and reduced GSH levels	MDA-TBARs	Kolaviron (a biflavonoid from *Garcinia kola* seeds)
2009	[130]	Rat livers: EtOH-induced decreased GSH and ascorbic acid (vit. C) levels	MDA-TBARs	Exercise in elderly rats
2009	[131]	Rat livers: EtOH-induced decreases in hepatic SOD, CAT, GPx, GSSG-R, and GST activities and decreased GSH, ascorbic acid (vit. C), and *α-(alpha)* tocopherol (vit. E) levels	MDA-TBARs and LPOs	Chrysin [a flavone extracted from the blue passion flower (*Passiflora caerulea*) and honey]
2009	[132]	Rat livers: EtOH-induced increases in serum ALT, AST, and *γ-(gamma)*-transpeptidase and EtOH-induced decreases in hepatic SOD, CAT, and GST activities and reduced levels of hepatic GSH, vitamin C, and vitamin E	LPOs and protein carbonyl contents (reactive aldehyde-protein adjuncts)	Naringenin (a flavonoid in grapefruit)
2009	[133]	Wistar rat kidneys: EtOH-induced reduction in GSH and GSH/GSSG ratio associated with decrease CAT, SOD, and GPx activities	Not measured	*α-(alpha)* Tocopherol (vit. E)
2009	[134]	Rat pups: EtOH-induced increases in serum Se levels and GPx activities and EtOH-induced decreases in hepatic Se and EtOH-induced reduced hepatic CAT and GSSG-R activities	Protein carbonyl content (reactive aldehyde-protein adjuncts)	Se and folic acid
2009	[135]	Rat livers: EtOH-induced decreases in hepatic vitamin E and reduced GSH levels	MDA-TBARs and HNE	L-carnitine
2008	[136]	Rat gastric mucosal: EtOH-induced increases in vascular permeability and decreased CAT activity	MDA-TBARs	*Benincasa hispida* (winter melon) fruit extract
2008	[137]	Male fetal rat brains: EtOH-induced DNA damage, protein oxidation, and lipid peroxidation were observed within the hippocampus	LPOs and protein carbonyl content (reactive aldehyde-protein adjuncts)	*α-(alpha)* Lipoic acid

Table 4.3 Examples of ethanol-induced lipid peroxidation in 2008 continued

Year	Ref. no.	Comments	Lipid peroxidation detected by	Antioxidant used for amelioration (attenuation)
2008	[138]	Mouse livers: EtOH-induced increases in serum aspartate amino transaminase (ASP), ALT, and triglycerides (TG) and EtOH-induced increases in hepatic mitochondrial permeability and EtOH-induced decreases in hepatic SOD, CAT, GPx, and GSSG-R activities	MDA-TBARs	Diallyl trisulfide (DAT: in processed garlic)
2008	[139]	Mouse livers: EtOH-induced increases in hepatic TG content and increased serum TG levels	MDA-TBARs	Garlic oil
2008	[140]	Wistar rat livers: EtOH-induced elevated levels of serum transaminases and EtOH-induced reductions in hepatic SOD, GPx, and CAT activities	MDA-TBARs	Virgin olive oil
2008	[141]	Rat livers: EtOH-induced decreases in hepatic GSH and increased levels of hepatic protein carbonyl contents (reactive aldehyde-protein adducts)	MDA-TBARs and HNE adducts	Fenugreek (a polyphenol extract from *Trigonella foenum graecum*)
2008	[142]	Rat myocardial tissue: EtOH-induced reduction in reduced GSH and reduced Se- and non-Se-dependent GPx, GSSG-R, and GST activities	Not measured	Exercise in elderly rats
2008	[143]	HepG2 cell cultures: EtOH-induced increases in ROS levels, cytotoxicity, and release of ALT and ASP	ROS	*Soymida febrifuga* (Indian redwood) extract
2008	[144]	Wistar rat livers: EtOH-induced decreased GSH levels and decreased SOD and CAT activities associated with increased hepatic GST activities	Not measured	*Phyllanthus amarus*/stonebreaker extracts
2008	[145]	Mouse gastric mucosa: EtOH-induced increases in MDA levels and decreased levels of total sulfhydryl groups and nonprotein sulfhydryl groups	MDA-TBARs	n-Butanol extract of *Pteleopsis suberosa*
2008	[146]	Rat gastric mucosa: EtOH-induced decreased ascorbic acid levels and increased SOD activities	MDA-TBARs	Diphenyl diselenide
2008	[147]	Mouse gastric mucosa: EtOH-induced increases in ROS levels and gastric ulcerations	ROS	*Pseudarthria viscida*
2008	[148]	Mouse hepatocyte cell cultures: EtOH-induced increased SOD and CAT activities	ROS	*Usnea ghattensis* (lichen) extract
2008	[149]	Rat livers: EtOH-induced decreases in the activities of GSH-dependent enzymes	MDA-TBARs	*Hemidesmus indicus* (Indian sarsaparilla) extract
2008	[150]	Wistar rat livers and kidneys: EtOH-induced increases in serum AST and ALT activities and increased TGs and total cholesterol (TC) levels in livers and kidneys	MDA-TBARs	Ellagic acid
2008	[151]	Wistar rat livers: EtOH-induced increased hepatic HNE adducts and decreased activities of antioxidant enzymes	MDA-TBARs, LPOs, and HNE	Epigallocatechin gallate
2008	[152]	Wistar rat livers: EtOH-induced increased hydroxyproline and collagen contents	MDA-TBARs	Epigallocatechin gallate

Table 4.4 Examples of ethanol-induced lipid peroxidation in 2007

Year	Ref. no.	Comments	Lipid peroxidation detected by	Antioxidant used for amelioration (attenuation)
2007	[153]	Wistar rat livers: EtOH-induced increases in serum AST, ALT, and ALP and decreased activities of hepatic SOD, CAT, and GSH-dependent enzymes	MDA-TBARs and LPOs	*Hemidesmus indicus* (Indian sarsaparilla) extract
2007	[154]	Rat gastric mucosa: EtOH-induced gastric ulcers	MDA-TBARs	*Onosma armeniacum* extract
2007	[155]	Rat gastric mucosa: EtOH-induced gastric ulcers and reduced GSH, ascorbic acid, retinol, and *β-(beta)* carotene	MDA-TBARs	*Foeniculum vulgare* (fennel) extracts
2007	[156]	Wistar rat brains: EtOH-induced decreases in GSH within CA1 and CA3 pyramidal neurons (hippocampus) associated with reduced rates of learning a water maze	Lipofuscin pigment (end product of lipid peroxidation)	Red wine antioxidants (polyphenols)
2007	[157]	Rat livers: EtOH-induced hepatic fatty infiltration and fibrosis associated with reduced hepatic SOD and GSH-dependent enzyme activities	MDA-TBARs	Resveratrol
2007	[158]	Male Sprague–Dawley rats: EtOH-induced increases in serum TG, TC, low-density lipoprotein cholesterol (LDL-C), and TBARs	MDA-TBARs	*Ginkgo biloba* extract
2007	[89]	Rat livers and intestines: EtOH-induced serum urea, creatine, uric acid, AST, and ALT increased and hepatic and intestinal GSH levels decreased with decreased hepatic and intestinal SOD, CAT, and GPx activities	LPOs	Vitamin C, vitamin E, and sodium selenate
2007	[159]	Rat brains: EtOH-induced increased lipofuscin deposits within the hippocampal CA1 and CA3 pyramidal neurons and in cerebellar Purkinje neurons	Lipofuscin pigment (end product of lipid peroxidation)	Grape seed flavonols
2007	[160]	Rat livers: EtOH-induced decreases in hepatic GSH and decreased activities of hepatic SOD, CAT, and GSH-dependent enzymes associated with increased serum levels of ALT, ALP, and bilirubin	Not measured	Leaf extracts of *Ziziphus mauritiana* (jujube)
2007	[161]	Male Wistar rat myocardial tissue: EtOH-induced decreases in SOD and CAT activities and EtOH-induced increased xanthine oxidase activities	LPOs	Exercise training
2007	[162]	Male Wistar rat kidneys: EtOH-induced decreased activities of SOD, CAT, and GSH-dependent enzyme activities and decreased renal vitamin C and vitamin E levels	MDA-TBARs and LPOs	*Hemidesmus indicus* root extract
2007	[163]	Rat gastric mucosa: EtOH-induced gastric ulcers associated with decreased SOD, CAT, and GPx activities	MDA-TBARs	Ozonized sunflower oil

EtOH-Induced Lipid Peroxidation, Apoptosis, and Embryopathy

EtOH-induced lipid peroxidation and apoptosis have been observed during very early stages of development within the rat placenta [14]. However, EtOH-induced lipid peroxidation during vertebrate organogenesis is also well documented. EtOH-induced craniofacial, cardiovascular, and skeletal defects in *medaka (Oryzias latipes)* embryos have been observed and are associated with EtOH-induced elevated LPO levels and stage-specific reductions in mRNA levels coding for alcohol dehydrogenases (*Adh5* and *Adh8*) and aldehyde dehydrogenases (*Aldh9A and Aldh1A2*) that were associated with EtOH-impaired circulation within early-stage fish embryos [164]. This observation is of interest because diabetes-induced hypoxia, changes within the microvascular system, enhanced lipid peroxidation, and enhanced apoptosis rates have also been associated with hyperglycemia-induced embryopathy [165]. EtOH treatments of *medaka* embryos from 0 to 48 h postfertilization inhibited chondrogenesis within the neurocranium without affecting the methylation pattern of the *Aldh1A2* promoter [166]. However, EtOH treatments of *medaka* embryos from 0 to 48 h postfertilization also caused reduced expression of *Aldh9* mRNA levels within brain, eye, gill, gastrointestinal tract, liver, kidney, muscle, testis, and ovaries [166]. This early EtOH-induced delayed expression of *Aldh9* mRNA may elevate acetaldehyde concentrations and induce teratogenesis [167]. Thus, EtOH-induced teratogenesis can be observed at early stages of organogenesis.

Neurulation is one of the early substages within organogenesis. During neurulation, the invagination of presumptive ectoderm creates the neural tube, which becomes the central nervous system, and dorsally located neural crest cells. Eventually, neural crest cells migrate along a dorsal-lateral pathway and also a ventral-medial pathway through the anterior section of delaminating somites (sclerotomes) and will differentiate into a number of diverse anatomical structures. These structures include cranial nerves, dorsally located sensory nerves of the peripheral nervous system, and ventrally located motor nerves of the peripheral nervous system. Neural crest cells also differentiate into melanocytes and contribute to the aortic arch, cranium, and several endocrine glands. Because of the diverse fate of neural crest cells, EtOH-induced lipid peroxidation and subsequent apoptosis within neural crest cells cause a diverse list of malformations within embryos.

The first papers indicating EtOH-impaired development via neural crest cells appeared in 1995. Van-Maele-Fabry et al. [168] demonstrated that whole mouse embryos cultured in the presence of EtOH for a period of 48 h possessed defects in the glossopharyngeal (cranial nerve IX) and vagus (cranial nerve X) nerves. EtOH-induced alterations included absences of the dorsal root of the glossopharyngeal nerve (superior ganglion) and disorganized rootlets of the vagus nerve that were later verified in 2002 [169]. The observations of Van-Maele-Fabry et al. [168] implied EtOH-impaired migration of neural crest cells. During 1995, Rovasio and Battiato also reported EtOH-impaired neural crest cell migration associated with cranial and cardiac anomalies within the cephalic ends of chick embryos [170]. Since vertebrate neural tube closure occurs in an anterior to posterior direction, early embryonic exposure to EtOH will cause malformations more prevalently in anterior structures as compared to posterior structures. EtOH-impaired neural crest cell migration was later associated with EtOH-induced accumulation of ROS and excessive cell death by Kotch et al. [171]. During the later part of 1995, Kotch et al. [171] reported EtOH-induced increased superoxide anion ($\cdot O_2$) levels, increased rates of lipid peroxidation, and increased rates of neural crest cell death that were associated with a higher-than-normal failure rate in closing anterior sections of the neural tube within day 8 gestational mouse embryos cultured in the presence of EtOH for 36 h. Cotreatment of mouse embryos with exogenous SOD (EC 1.15.1.1) and EtOH partially ameliorated EtOH-induced teratogenesis and implied EtOH-induced free radical damage in embryos [171].

As previously stated, several lipid peroxidation-generated reactive aldehydes, including HNE, HPNE and ONE, cause increased mitochondrial membrane permeability and are associated with the release of cytochrome *c* and Fe^{+2} from the mitochondria into the cytoplasm [34, 35, 45, 57]. Cytoplasmic

cytochrome *c* can activate effector (killer) caspases including caspase-3, caspase-6, and caspase-7 via activated apoptosomes [101–105], and cytoplasmic Fe^{+2} ions can initiate and/or perpetuate lipid peroxidation. As previously discussed, Fe^{+2} ions can convert superoxide anions ($\cdot O_2$) to more reactive hydroxyl radicals ($\cdot OH$) via the Harber-Weiss reaction [26–29], as discussed in section "Mechanics of Lipid Peroxidation" of this chapter. This scenario appears likely in EtOH-treated neural crest cells. Through the use of Fe^{+2}-chelating agents, Chen and Sulik [172] demonstrated that Fe^{+2}-chelating agents partially attenuated EtOH-induced increased ROS levels and EtOH-induced cytotoxicity in mouse neural crest cells.

EtOH-induced apoptosis is not restricted to only cranial neural crest cells. Fertile chicken eggs exposed to exogenous EtOH during the first 3 days of development ($E_{0\text{-}2}$) displayed EtOH-enhanced brain membrane lipid peroxidation at 11 [12, 13] and 18 days of development [10, 11], EtOH-enhanced brain caspase-3 activities (a marker of apoptosis) at 11 [13, 100] and 18 days of development [173], EtOH-induced increased brain and hepatic homocysteine levels at 11 days of development [100, 173], and EtOH-induced decreased brain and hepatic taurine levels at 18 days of development [173]. Since chick embryos normally hatch in 21 days, embryos at 11 days of development have completed approximately 52% (11/21) of their development, and embryos at 18 days of development have completed approximately 87% (18/21) of their development. Hence, early embryonic exposure to EtOH may have long-lasting developmental consequences in chicks.

Once initiated, ethanol-induced neural crest cell apoptosis in chick embryos involves signaling pathways utilizing G proteins, Rho family GTPases, and phospholipase C [174]. In in vitro cultures of mouse first branchial neural crest cells, ethanol-induced apoptosis was associated with the formation of ceramide, which comes from PLC-dependent sphingomyelin degradation, and EtOH-induced apoptosis and was attenuated by preincubation of mouse neural crest cells with CDP-choline (citicoline), a precursor for the conversion of ceramide to sphingomyelin [175]. In vivo studies utilizing EtOH-treated mouse embryos demonstrated EtOH-induced reductions in transforming growth factor-β *(beta)*1 (TGF-β *(beta)*1) levels within mouse meninges [175]. TGF-β *(beta)*1 is a critical growth factor for both bone and brain development, and the meninges is a tissue complex derived from neural crest cells.

EtOH-induced cell death of neural crest cells, which are observed in premigratory and migratory neural crest cells, involves EtOH-induced apoptosis, EtOH-altered cell-signaling pathways, the possible EtOH-induced removal of survival/growth factors, and/or altered EtOH-induced morphogen expression. In chick embryos, EtOH-induced reductions in cranial *Sonic Hedgehog (Shh)* transcripts levels were observed which were associated with EtOH-induced cranial neural crest cell death and EtOH-impaired cranial facial growth [176]. EtOH-induced cranial neural crest cell death and EtOH-impaired cranial facial growth were ameliorated through the administration of exogenous SHH in chick embryos [176]. While chick embryos exhibited EtOH-induced reductions in cranial *Shh* mRNA levels [176], EtOH-induced increased levels of *Ptc-1* (patch-1 receptor) and *Gli-1* (glioma-associated oncogene homolog-1) mRNAs were observed in EtOH-treated mouse embryos as compared to controls [177]. Hence, species-specific and possibly evolutionary-related differences in EtOH-induced alterations in *Shh* signaling pathways exist.

Interest in EtOH-induced changes in *Shh, Ptc-1, and Gli*-1 expression was inevitable because SHH is a known morphogen that lies in both anterior to posterior and right- to left-side gradients with posterior regions possessing higher concentrations of SHH as compared to anterior sections and left-side regions possessing higher concentrations of SHH as compared to right-side regions of vertebrate embryos. Recently, *Shh, Ptc-1, and Gli*-1 expressions within early embryonic and adult forebrains were associated thyroid hormone-responsive genes because maternal hypothyroidism and hyperthyroidism bidirectionally influenced *Shh, Ptc-1, and Gli*-1 expression within fetal brains [178]. In fetal mouse brains obtained from hypothyroid dams, *Shh* expression, *Ptc-1* expression, which codes for a receptor for SHH, and *Gli*-1 expression, which is a transcription factor observed in SHH-Patch-stimulated cells, were downregulated [178]. Surprisingly, when *Ptc-1*-deficient mice were crossbred with *qk(v/v)* mice, the *Ptc (+/−)* mice exhibited enhanced *Shh* expression and was associated with

hydrocephaly and dilation of the ventricles at 5 months of neonatal age [179]. Recently, SHH pretreatment of H_2O_2-treated primary rat cortical neuron cell cultures caused an attenuation of lipid peroxidation, as measured by reduced MDA-TBAR adduct levels; increased expression of antioxidant enzymes, including superoxide dismutase (SOD) and glutathione peroxidase (GPx); increased expression of the antiapoptotic *Bcl-2* allele; downregulation of the proapoptotic *Bax* allele; inhibited H_2O_2-induced ERK (extracellular signal-regulated kinases) signal transduction pathway; and upregulated expression of neurotrophic/survival factors including brain-derived nerve growth factor (BDNF) and vascular endothelial growth factor (VEGF) [180].

Strong interest in EtOH-induced downregulation of neurotrophic/survival factors, including nerve growth factor (NGF), neurotropin-3 (NT-3), ciliary neurotrophic factor (CTNF), BDNF, VEGF, and glial-derived nerve factor (GDNF), and their possible roles within neonatal, adolescent, and adult brains exist. In 1992, Brodie and Vernadakis [181] reported that EtOH-treated cultures of neurons derived from the cerebral hemispheres of 8-day-old chick embryos exhibited enhanced choline acetyltransferase (ChAT; EC 2.3.1.6) activities and reduced glutamic acid decarboxylase activities (GAD; EC 4.1.1.15) as compared to controls. ChAT activities were used as a biochemical marker for cholinergic neurons, and GAD activities were used as a maker for GABAergic neurons. However, cultures exposed to NGF and EtOH exhibited higher GAD activities as compared to EtOH-treated cultures. Thus, the authors concluded that EtOH-altered neuronal phenotypic expressions were due to differential responses to neurotrophic/survival factors [181]. Using both in vitro and in vivo studies, Jaurena et al. [182] recently demonstrated that EtOH-induced chick neural crest cell apoptosis was attenuated by exposure to either NT-3 or CNTF. Bradley et al. [183] conducted in vivo experiments using 10–15-day-old chick embryos and reported EtOH-induced cell death among spinal cord motor neurons and that exogenous BDNF or GDNF ameliorated EtOH-stimulated motor neuron cell death. GDNF and NGF synthesis is regulated by the presence or absence of tumor necrosis factor-α *(alpha)* [TNF-α *(alpha)*]. Kuno et al. [184] demonstrated in mixed glial cell cultures obtained from C57BL/6 mice that astrocytes express both TNF-α *(alpha)* receptor 1 (TNFR1) and TNFR2 and that activation of these receptors by TNF-α *(alpha)* caused astrocytes to synthesize and release NGF and GDNF [184].

Recently, Kulkarny et al. [185] reported that EtOH exposure in juvenile Sprague–Dawley rats caused regional BDNF and GAP-43 expression differences within the hippocampus as compared to the cerebellum. GAP-43 is a "growth" or "plasticity" protein and is commonly found within neuron growth cones and during axonal regeneration. EtOH exposure caused increased GAP-43 expression within the hippocampus and decreased expression within the cerebellum. Meanwhile, ETOH exposure caused increased BDNF expression within the hippocampus but had no effect on BDNF expression within the cerebellum. In vitro studies using fetal rat hippocampal pyramidal neurons demonstrated that EtOH treatments caused increased tyrosine kinase B (TrkB) expression, the receptor for BDNF, but inhibited BDNF signaling as measured by EtOH-induced inhibited Rac 1- (Ras-related C3 botulinum toxin substrate 1), Cdc-42- (cell division cycle 42), and Rho A activities [186]. Rho A (Ras homologous member A) is a member of the Rho GTPase family and is required for axon growth cone extension. Normally, BDNF binding to its receptor, TrkB, stimulates Rac 1-, Cdc-42-, and Rho A-induced signaling and axon growth cone extension [186] and implies EtOH-inhibited BDNF signaling within fetal brains.

There are at least two receptors for BDNF which may be involved in BDNF signaling. The first receptor is TrkB, which is a tyrosine kinase [186], and the second receptor is the low-affinity nerve factor receptor, also known as protein 75 neurotrophin receptor ($p75^{NTR}$) [187]. The TrkB receptor, which binds BDNF, neurotropin-3 (NT-3), and NT-4, is related to the TrkA receptor, which binds NGF, and the TrkC receptor, which binds NT-3. TrkA, TrkB, and TrkC are survival receptors. Upon binding their respective ligands, TrkA, TrkB, and TrkC receptors autophosphorylate themselves and activate members of the MAPK family (mitogen-activated protein kinase family) and promote the synthesis of the antiapoptotic protein (Bcl-XL) and, therefore, avoid apoptosis [188, 189]. Meanwhile, $p75^{NTR}$, which is overexpressed in the sensory-motor cortex of EtOH-treated postnatal mice [190], possesses death-inducing signaling complex (DISC) [101, 102] and initiates apoptosis via the extrinsic pathway by promoting the PLC-dependent degradation of sphingomyelin to ceramide and JNK (cJun N-terminal

kinase)-dependent dephosphorylation of BAD (Bcl-2-associated death domain) [190–193]. Once dephosphorylated, BAD will form heterodimers with the antiapoptotic proteins, Bcl2 (B cell lymphoma 2 protein) and Bcl-XL (B cell lymphoma-extra large), and, thus, inactivate Bcl2 and Bcl-XL. Through the BAD-induced inactivation of Bcl2 and Bcl-XL, the outer mitochondrial membrane proteins, BAX and BAK (BAX; Bcl-2-associated X protein) (BAK; Bcl-2 antagonist/killer), can transport cytochrome *c* from the mitochondria into the cytoplasm and activate apoptosomes. Activated caspase-9, within activated apoptosomes, can then cleave/activate effector (killer) caspases including caspase-3, caspase-6, and caspase-7 [101–105]. In comparing ligand affinity, $p75^{NTR}$ has lower affinity to NGF and BDNF as compared to TrkA and TrkB receptors [190–193]. Hence, $p75^{NTR}$ initiates apoptosis when it has failed to bond its neurotrophins and inhibits apoptosis when it bonds the appropriate neurotrophin [190–193]. Therefore, EtOH-induced overexpression of $p75^{NTR}$ as compared to TrkA, TrkB, and TrkC receptors may be more important than EtOH-induced relative changes in neurotrophin levels.

Summary

It is well documented in a variety of animals that EtOH exposure causes lipid peroxidation and a growing list of antioxidants have provided some aid in alleviating EtOH-induced toxicity. As polyunsaturated fatty acids are attacked by ROS, a number of cytotoxic, reactive aldehydes are synthesized. In comparison to ROS, reactive aldehydes last longer and diffuse within and throughout cells. These reactive aldehydes can cross-link and form DNA adducts [72] and protein adducts [64, 70], and identifying proteins targeted inhibited by reactive aldehydes has become of interest. Some reactive aldehydes diffuse into mitochondria, promote increased mitochondrial membrane permeability, and cause the release of Fe^{+2} and cytochromes into the cytoplasm. As cytochrome *c* enters the cytoplasm, cytochrome *c* can activate apoptosomes. Activated caspase-9, within activated apoptosomes, can then cleave/activate effector (killer) caspases including caspase-3, caspase-6, and caspase-7 [101–105]. This form of apoptosis is known as the intrinsic pathway. While it has been well documented that EtOH-induced lipid peroxidation and apoptosis within embryonic and juvenile neural tissues involve mitochondrial dysfunction and leakage, the question has arisen as to whether or not EtOH-induced apoptosis and lipid peroxidation within embryonic, neonatal, and juvenile neural tissues can be initiated by the extrinsic pathway involving the activation of membrane receptors that possess death-inducing signaling complexes (DISC) [101, 102]. The activation of DISC-containing membrane receptors (extrinsic receptors) can cause signaling that also promotes mitochondrial permeability and the release of cytochrome *c* into the cytoplasm and subsequent activation of killer caspases including caspase-3, caspase-6, and caspase-7 [101–105]. While EtOH-induced lipid peroxidation, oxidative stress, and apoptosis as initiated by the extrinsic pathway via the release of tumor necrosis factor-α *(alpha)* is well documented in alcohol-induced liver disease [16, 19, 25, 194, 195], this chapter has discussed the possible existence of the extrinsic pathway, EtOH-induced lipid peroxidation, and apoptosis within primarily embryonic, neonatal, and juvenile neural tissues.

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Chapter 5
Alcohol Use During Lactation: Effects on the Mother-Infant Dyad

Julie A. Mennella

Key Points

- Lactating women metabolize alcohol differently, partly due to frequent breast stimulation during breastfeeding and pronounced physiological changes that accompany parturition.
- Folklore in many cultures, including the USA, relates that alcohol facilitates milk letdown, rectifies milk insufficiency, and has sedative properties that calm "fussy" breastfed babies. There is no scientific evidence to support such claims.
- Contrary to these popular beliefs, immediately after maternal alcohol consumption, the mothers' hormonal response to suckling is altered, and the infants actually ingest less breast milk, show disrupted sleep-wake patterning and motor development, and form alcohol-related memories that may affect later behavior.
- Beyond alcohol's teratogenic effects on the fetus and breastfed infant and beyond the disruptive effects on the lactational process, a growing body of experimental research suggests that during alcohol exposure, the fetus or young infant can acquire an association between ethanol's orosensory properties and pharmacological consequences, causing the animal subsequently to seek out (or avoid) ethanol.
- Knowledge about the time course of the transfer of alcohol to human milk and the potential impact that alcohol exposure via breast milk has on the infant is crucial for informing parents and health-care professionals.
- Women should not stop breastfeeding because of their concern for alcohol in their breast milk but rather can limit their infants' exposure by timing breastfeeds in relation to drinking and consuming food with alcohol to reduce the amount of alcohol transmitted to the milk. Alcohol is not stored in breast milk but parallels that found in maternal plasma, peaking approximately one-half hour to an hour after drinking and decreasing thereafter.
- Providing insights from evidence-based research on ethanol pharmacokinetics will continue to aid in the development of scientifically sound guidelines for ethanol consumption by nursing women and how nursing affects the availability and elimination of ethanol, and perhaps other drugs, during lactation.

Keywords Alcohol • Lactation • Pharmacokinetics • Oxytocin • Prolactin • Sleep • Behavioral state • Olfaction

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Introduction

Breastfeeding has increased across all socioeconomic groups in the USA, with increasing recognition of the health and psychological benefits to both mother and infant [1–4]. Despite this resurgence, and the fact that alcohol is one of the most frequently consumed drugs in women of childbearing age [5, 6], little is known about the effects of alcohol on this biologically important reproductive state in women or on the growing infant. Although lactating mothers exhibit similar drinking patterns as formula-feeding mothers [7–9], recommendations about alcohol are largely based on folklore passed down through generations, and many cultures believe that alcohol is a milk-producing substance (galactagogue).

During the past two decades, researchers have begun to systematically study the effects of moderate drinking on lactational performance of the mother and on the child's behavior and nutrition [10]. Much of this research follows from research in other animals that revealed negative effects of alcohol on the lactational process and long-term consequences of infants learning about the sensory properties of alcohol in milk. In this chapter, I review the folklore and the scientific literature, albeit limited, on the effects of maternal alcohol consumption on both maternal health and infant nutrition, state regulation, and learning about the sensory properties of alcohol (see ref [11]. for an earlier review of this topic).

The Folklore that Alcohol Is a Galactagogue

Although cultures have both differences and commonalities in the use of alcohol for medicinal purposes [12, 13], for centuries, many cultures have claimed that alcohol is a galactagogue [11]. The type of alcoholic beverage recommended is partly culturally driven [10]. In Mexico, pregnant and lactating women are encouraged to drink pulque, a low-alcohol beverage made from *Agave atrovirens [*14]; the "magic elixir" in Argentina [15] and Germany [16] is malt beer. Chicken soup flavored with sesame oil and rice wine is recommended in China [17, 18].

Beliefs in the galactogenic properties of alcohol are also deeply ingrained in American tradition (see ref. [10]). In 1895, a major US brewery produced a low-alcohol beer that was sold exclusively in drugstores and prescribed by physicians as a tonic for lactating women [19]. Even today, alcohol consumption is regarded by many authorities [20] and cultures [11, 17, 21] as compatible with breastfeeding and/or as imparting positive effects, such as facilitating milk letdown, rectifying milk insufficiency, and calming "fussy" breastfed infants [22–25]. Our own research on women living in the Delaware Valley revealed that one-quarter of the women who were discouraged from drinking alcohol while they were pregnant were encouraged to drink by their health professionals once they began breastfeeding [11]. However, no scientific studies support any of these recommendations, and most are made on the assumption that there are no dangers to drinking and that it is good for you [24, 25]. This is surprising, given the physiological and metabolic complexity of lactation and the well-known effects of alcohol on oxytocin [26] and prolactin [27] (the hormones of lactation), suggesting that alcohol likely has a profound effect on the lactational process and vice versa.

The Lactating Mother

Physiology of Lactation

Lactation is the result of highly synchronized endocrine and neuroendocrine processes that begin during late pregnancy to prepare both the body and brain for motherhood. Mammary gland development begins in late pregnancy in response to reproductive hormones (e.g., estrogen, progesterone, prolactin,

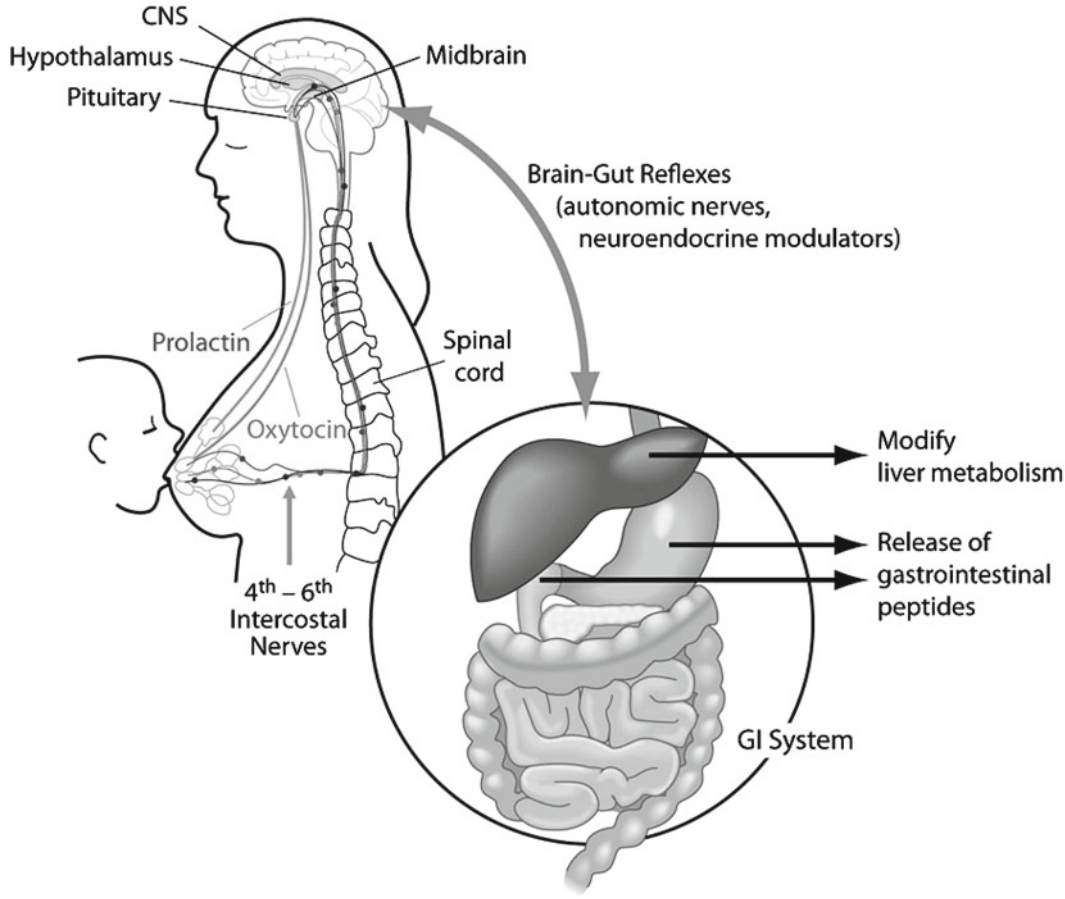

Fig. 5.1 The suckling reflex: brain-breast and brain-gut axes. Illustration by Mary A Leonard, Biomedical Art & Design, University of Pennsylvania

and oxytocin) that act directly on the mammary glands and metabolic hormones (glucocorticoids, insulin, growth hormone, and thyroid hormone) that act indirectly by altering nutrient flux to the mammary glands.

Following parturition, endocrine events that sustain lactation are triggered by infant suckling (Fig. 5.1). The production, secretion, and ejection of milk result from highly synchronized endocrine and neuroendocrine processes, which are governed partly by the frequency and intensity of the infant's suckling. This multistage process is controlled by several hormones, the most important of which are prolactin and oxytocin. Breast stimulation (by the infant or a breast pump) causes oxytocin and prolactin release [28, 29] by lactotrophic cells in the anterior pituitary and other tissues, including the breast [30]. Suckling is the most potent and best physiological stimulus for prolactin release and does so partly by increasing the release of opioids and other prolactin-releasing factors that inhibit dopamine secretion into the portal circulation [31–33]. Uniquely among the pituitary hormones, prolactin has a propensity for hypersecretion and is under tonic inhibition [31]. The amount of oxytocin released, which correlates with the amount of milk transferred from mother to baby [34], may also be involved in mother-infant interaction [35, 36]. While prolactin increases transiently in response to the suckling stimulus, no clear temporal correlation exists in humans between plasma prolactin levels and milk

yield of a particular breastfeed. However, prolactin does appear to be essential for the maintenance of lactation in the longer term [37].

Perhaps less well known than the effects of suckling on milk production is its stimulation of the brain-gut axis (Fig. 5.1). Associated with lactogenesis is an increase in the size and complexity of the mother's digestive tract [38] and altered nutrient metabolism in adipose tissues, skeletal muscles, and liver [39, 40]. Suckling stimulates vagal release of hormones (e.g., insulin, gastrin, and cholecystokinin) that regulate digestive processes such as gastric emptying [41–45]. The evolution of common neural and endocrine regulation of lactation and energy balance [46, 47] ensures a sufficiently large flux of nutrients is mobilized to mammary tissues to support milk synthesis [47, 48]. These common regulatory mechanisms suggest that suckling may exert effects upon ethanol pharmacokinetics (and perhaps other drugs) similar to those of food consumption.

Alcohol and Lactational Performance

Ethanol transfers to human milk in amounts almost identical to that in maternal blood, peaking within an hour of ingestion [49–51]. Because women are often advised to drink alcohol shortly before they nurse their babies to promote milk production, we conducted an experimental study to determine whether this advice is valid. Contrary to lore, but consistent with animal research [52–54], women produced significantly less milk after they consumed an alcoholic beverage (0.3 g/kg dose of alcohol) versus consuming a nonalcoholic beverage [55]. There were no changes in the milk's caloric content.

We then hypothesized that drinking alcohol alters lactation hormones, based on standard-of-care practice dating back to late 1960s when ethanol was used to treat premature labor [26, 56, 57]. Complete or partial blockage of the milk-ejection reflex, as assessed by either intramammary pressure [56] or uterine contractions [58] (an indirect measure of oxytocin), has been observed in peripartum women after alcohol consumption. Alcohol's efficacy in partially blocking uterine contractions during labor is partly due to its inhibition of oxytocin, a hormone that also contracts myoepithelial cells surrounding the alveoli and causes the ejection of milk from the mammary gland during lactation.

We found that moderate doses of alcohol disrupt the mothers' hormones, decreasing milk production and interfering with milk ejection [28, 29]. The slower the mother eliminated ethanol, the longer the latency for milk ejection and the smaller the milk yield [28]. The key hormones underlying lactational performance, which usually increase in response to suckling, were disrupted following moderate drinking. Oxytocin levels decreased and prolactin significantly increased during the hours immediately following alcohol consumption (Fig. 5.2). Because prolactin has a propensity for hypersecretion and is under tonic inhibition [31], alcohol may cause hyperprolactinemia in the short term by affecting extrapituitary tissues capable of producing prolactin, such as breast tissue, or through a general depression of the central nervous system [32, 59]. Alcohol may also stimulate prolactin by activating inhibitors (e.g., endogenous opioids) of the hypothalamic dopaminergic neurons.

Family History of Alcoholism

Because of the drastic neuroendocrine, hormonal, and subjective perceptual associations with family history of alcoholism [60–62], we hypothesized that there may also be differences in hormonal milieu and breastfeeding behavioral patterns between non-alcohol-dependent lactating women with (FH+) or without (FH−) a family history of alcoholism. Because prolactin is important for the initiation of lactation, and because each woman has a unique intrinsic prolactin response to suckling that tracks throughout lactation [63], we hypothesized that FH+ women who exhibited marked reductions in

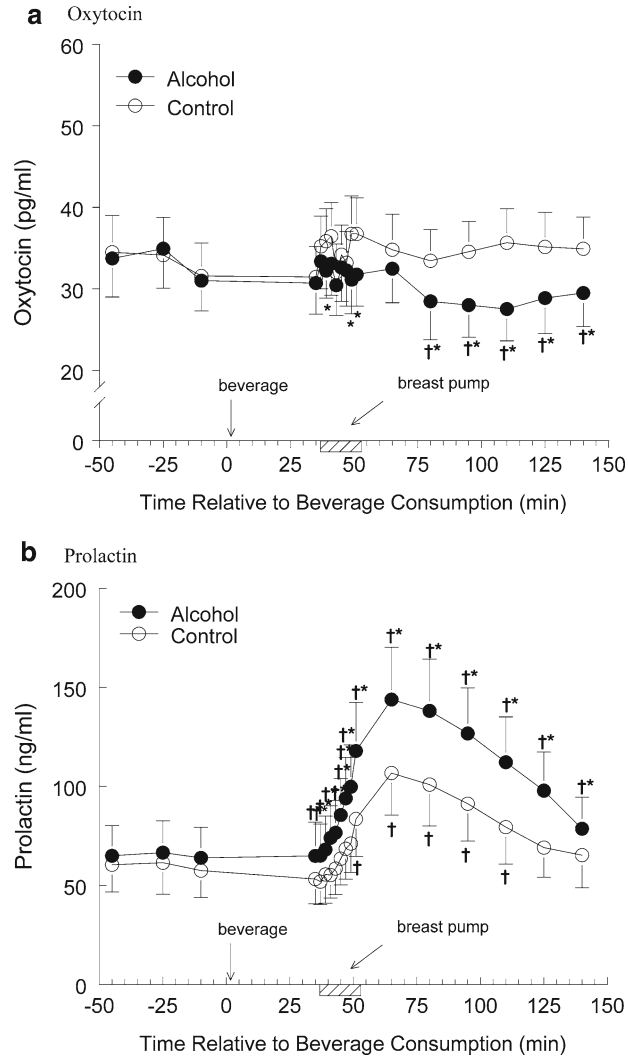

Fig. 5.2 Mean (±SEM) plasma oxytocin levels (pg/ml; A) and prolactin (ng/ml; B) in lactating women at baseline and at varying times following consumption of orange juice with (*closed circles*) and without (*open circles*) 0.4 g/kg alcohol on different test days. Women received breast stimulation with a breast pump (*hatched bars*) 35–51 min after consumption of the beverage (time point = 0 min). *Values significantly different from similar time points versus control. †Values within each test session significantly different from their respective baseline values

prolactin response to breast stimulation, and their breastfeeding infants, would have made adjustments in breastfeeding patterning to maintain successful breastfeeding [64].

We evaluated the hormonal responses to an alcohol as well as to a control challenge in lactating women of normal weight (since obesity may alter prolactin levels [65]) who were not alcohol dependent and who drank only occasionally. Although we detected no differences in alcohol pharmacokinetics, FH + women exhibited blunted prolactin responses to breast stimulation after drinking both the alcohol beverage (Fig. 5.3) and control (nonalcoholic) beverage and felt more of the stimulant-like effects of alcohol than did FH − women [64]. Interestingly, FH + women also reported that they nursed their infants more often – not in the morning hours but in the afternoon and evening, when prolactin levels are lowest.

Together, these data suggest that familial effects on the hormonal response to alcohol may directly or indirectly result in breastfeeding pattern differences throughout the day (and hence may be subject to a circadian rhythm) that must be accounted for in future studies. That the degree of prolactin increase to both breast stimulation and alcohol consumption was *blunted* in FH + lactating women and that moderate drinking magnified the prolactin differences between them and FH − lactating women

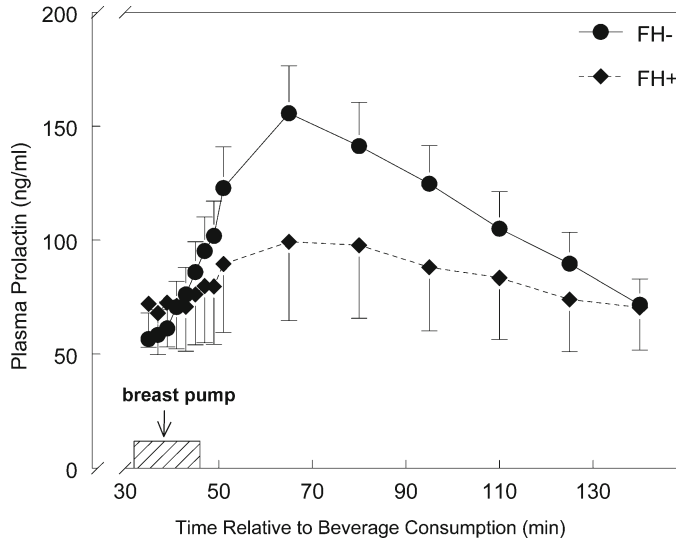

Fig. 5.3 Mean (±SEM) plasma prolactin (ng/L) among lactating women without (FH−; *circles*, *solid lines*) and with (FH+; *diamonds*, *hatched lines*) a family history of alcoholism at baseline and at varying times following consumption 0.4 g/kg alcohol in orange juice. Women received breast stimulation with a breast pump (*hatched bars*) 35–51 min after the consumption of the beverage (time point = 0). *Values within each test session significantly different from their respective baseline values. †Values significantly different from similar time points between FH + and FH − women

lend further support that the dopaminergic system differs between non-alcohol-dependent FH + and FH − women [64].

The blunted prolactin phenotype is an important risk factor for lactation failure among obese women [66]. Although having a family history of alcoholism is not as "visible" as obesity, the hormonal phenotype associated with this family history (at least in the morning hours) is as pronounced, if not more so, as that observed in obese women [67]. Addressing the challenges that this family history imposes upon breastfeeding and studying strategies that overcome them will help develop targeted interventions for new mothers and for the health-care providers who treat them [68].

Ethanol Pharmacokinetics, Pharmacodynamics, and Milk Flavor

Research conducted at the turn of the twentieth century, and then again almost a century later, has revealed that the ethanol content in human milk, which is almost identical to that detected in the mother's blood, peaks 1 h after ingestion and declines thereafter [49, 51, 69, 70]. The amount of alcohol in mother's milk is a fraction of that consumed by the mother (generally <2% of the maternal dose). The presence of ethanol produces a significant flavor change in the milk [69, 71] (Fig. 5.4), a finding similar to that reported for a variety of foods and beverages consumed by lactating mothers (see Mennella [72] for review).

As for many other drugs, the effects of lactational state on alcohol kinetics remain unknown. In fact, most research on breastfeeding and drugs focuses on the health risks for the nursing infants, not for the mother [73]. While studies have ratiometrically quantified drug concentrations in milk and plasma at a single time point [74, 75], the transfer rate of a drug between blood and milk does not

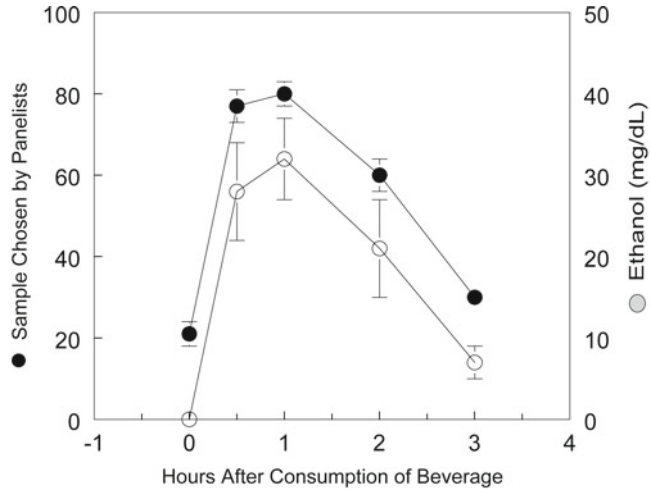

Fig. 5.4 The ethanol content of (*open circles*) and the percentage of time (*closed circles*) panelists chose milk samples obtained at baseline (0) and 30 min, 1, 2, and 3 h after the mothers consumed a 0.3-g/kg dose of alcohol in orange juice. Using a forced-choice paradigm, the panelists were presented individually with each set of milk samples and asked to indicate which of the paired smelled "stronger" or "more like alcohol." A value of 50% would be expected if there were no difference in the odor of the samples, and hence, the panelists responded at random. Values below 50% for the samples collected at baseline and after 3 h are a consequence of these samples being paired with a stronger-smelling sample (e.g., one collected 30 min or 1 or 2 h after alcohol consumption). The *bars* indicate standard errors. To convert values for ethanol to millimoles per liter, multiply by 0.2171 (Reprinted from Mennella and Beauchamp [69], with permission from Massachusetts Medical Society)

reveal how lactation affects the metabolism and clearance of the drug *over time* or how the drug affects the body and brain.

While the effects of lactation on the kinetics of ethanol have received little scientific attention, the effects (and mechanisms) of food consumption on ethanol metabolism are well described. Food increases metabolism of ethanol during its first passage through the digestive system (gut and liver) circulation, either by enhancing blood flow to the liver and/or activity of alcohol-metabolizing enzymes or by delaying gastric emptying and intestinal absorption [76–78]. As mentioned in section "Physiology of Lactation", the gastrointestinal system exhibits pronounced physiological adaptations during lactation. We, therefore, hypothesized that lactational state would be associated with alterations in ethanol pharmacokinetics and that these alterations would be most pronounced when the GI system was stimulated by co-consumption of a meal.

We compared ethanol pharmacokinetics and pharmacodynamics following consumption of a standardized amount of ethanol (0.4 g/kg) under both fed and fasted conditions in women who were exclusively breastfeeding 2–5-month-old infants and two control groups of nonlactating women: parous women who were exclusively formula feeding similarly aged infants and women who had never given birth [79]. These two control groups enable us to determine whether any differences observed were due to lactation per se and not a consequence of physiological changes that occur during pregnancy and parturition. All subjects were nonsmokers and normal weight because smoking [80] and obesity [67] affect the pharmacokinetics of many drugs, including ethanol.

Lactation was associated with significantly lower breath alcohol concentrations (BrAC) and lower systemic ethanol availability, regardless of whether ethanol was consumed in a fed or fasting state (Fig. 5.5). Despite the lower BrAC levels in lactating mothers, we found no significant differences

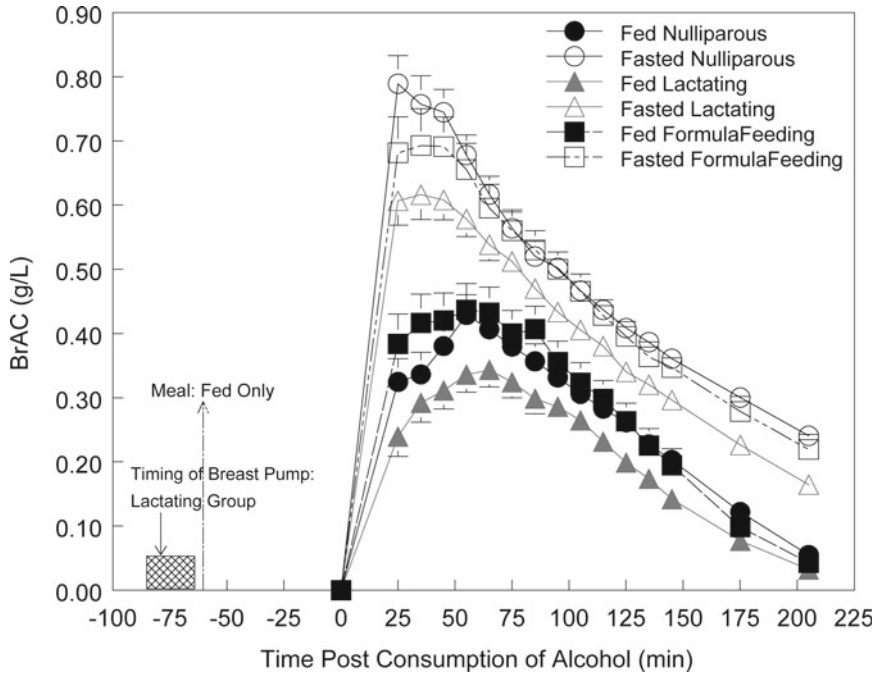

Fig. 5.5 Breath alcohol concentration (g/l) for lactating (*triangles*), formula-feeding (*squares*), and nulliparous (*circles*) women after drinking a 0.4-g/kg dose of alcohol in an overnight-fasted condition (*open symbols*) and a fed condition (*closed symbols*; 60 min after eating a standardized meal). Lactating women breast pumped for 16 min using a Medela Symphony pump; pumping occurred 1.5–1 h before drinking. Blood alcohol concentrations (BrAC) were estimated from breath and were measured before and at fixed intervals after drinking

among the three groups in the stimulating effects of ethanol. However, lactating women did differ in their reports of the sedative effects of ethanol compared with nulliparous but *not* formula-feeding mothers. That is, both groups of parous women felt sedated for shorter periods of time compared with nulliparous women. As hypothesized, the differences between lactating and nonlactating women in both ethanol pharmacokinetics and pharmacodynamics were most apparent when alcohol was consumed with food.

What mechanisms underlie these lactation-related changes in alcohol metabolism and subjective responses of alcohol? The act of suckling dramatically influences both brain and gut (Fig. 5.1) by stimulating vagal release of hormones (e.g., gastrin) that may regulate digestive processes by delaying gastric emptying [41, 43, 44, 46–48, 81–83] and by activating the brain dopamine reward system. We therefore hypothesized that breast stimulation was the underlying mechanism and predicted that the effects of breast stimulation and food consumption on alcohol metabolism would be similar.

We found that women who breast pumped 1.0 h *before* drinking exhibited reduced systemic availability of ethanol, compared with women who pumped after drinking [84, 85]. Women who pumped after drinking eliminated ethanol more rapidly and felt more of the stimulatory effects of ethanol. This supports our hypothesis that breast pumping has short-acting effects (within minutes) both on ethanol and energy metabolism and on mood, which perhaps results from suckling-induced hormonal changes and activation of brain areas involved in regulating motivation and emotions [86, 87]. As expected, eating a meal before drinking alcohol significantly reduced the systemic availability of ethanol by 38%, and if the women also breast pumped within the hour before drinking, availability was reduced by 58%.

The Breastfeeding Infant

Although the amount of ethanol transmitted to human milk is a minute fraction of that consumed by the mother [49, 51, 69–71], research in human infants suggests that exposure to ethanol via mother's milk affects breastfed infants in several important ways [10].

Nutrition

Consistent with research in other animals [52–54], human infants consumed approximately 23% *less* milk during the 4 h after their mothers drank an alcoholic beverage [69, 71]. The diminished intake at the breast was not due to infants feeding for shorter periods of time or rejecting the altered flavor in their mothers' milk [88]. Rather, as discussed above, maternal ethanol consumption significantly reduced the amount of milk produced by the mother [55].

Because breastfed infants are clearly capable of regulating milk intake, we hypothesized they would compensate for the diminished intake following ethanol exposure if their mothers then refrained from drinking alcohol. This was indeed the case; the compensation occurred within the 8–12 h following exposure and was partly due to an increased number of feedings during this time period [89]. These compensatory effects are subtle and remarkably similar to the infant's changes in active sleep that follow exposure to ethanol in mother's milk [89, 90], as described below. We have suggested that one reason why the folklore that alcohol is a galactagogue has persisted for centuries is because the breastfeeding mother, unlike the bottle-feeding caretaker who often feeds in response to the amount of formula remaining in the bottle, does not have an immediate means of assessing whether her infant consumes more milk in the short term, making her particularly vulnerable to such a lore [11].

Sleep

Contrary to lore that drinking ethanol shortly before breastfeeding relaxes and sedates the infant, experimental studies revealed that infants whose mothers drank a little during both pregnancy and lactation slept for significantly shorter times during the immediate hours following consumption of ethanol in mother's milk versus mother's milk alone [89, 90]. This reduction included less time spent in active sleep, a finding consistent with that observed in the near-term fetus [91] and nonalcoholic adults [92]. That sleep-wake patterning changes in infants who breastfeed from mothers who drink a moderate dose of alcohol (or smoke 1–2 cigarettes [93]) contradict prevailing medical opinion that exposure to ethanol (or nicotine) in mother's milk would be minute and not affect infants [20]. The effect was dose dependent, and reductions in sleep were compensated by the infants during the following day [89], highlighting their ability to modulate behaviors in response to such exposure in breast milk.

Mothers were unaware of any differences in their infants' behaviors after drinking, possibly explaining why the lore that alcohol helps "fussy" babies has persisted for centuries [10]. Together with the findings on milk compensation, these data highlight infants' resiliency in modulating behaviors in response to acute ethanol exposure. Whether ethanol consumption by lactating women, like that observed in other animals [94], disrupts other aspects of maternal-infant interaction [95, 96] or infant development [97], is an important area for future research.

One epidemiologic study of breastfed human infants and their mothers suggested that regular exposure to alcohol in mothers' milk can affect the infant in the long term [98] (but see Little et al [99].). Gross motor development at 1 year of age among 400 infants, as assessed by the Bayley Psychomotor

Index, was slightly, but significantly, altered in those exposed regularly (one or more drinks per day) to ethanol in their mothers' milk. Infants whose mothers drank less than one drink per day or did not drink at all as well as infants who were formula fed showed no significant differences in motor and mental development. This association between maternal drinking and motor development persisted even after controlling for more than 100 potentially confounding variables, including maternal tobacco, marijuana, and heavy caffeine use [98, 100, 101]. Little and colleagues hypothesized that either the developing brain may be exquisitely sensitive to small quantities of alcohol or, following repeated exposure, alcohol accumulates in the infant because of slower metabolism or excretion than in adults [98]. However, a later study by Little and colleagues did not replicate the effect of alcohol exposure in breast milk on motor development [99]. Whether differences in the study populations (e.g., the later study had infants who were 6 months older and fewer with high alcohol exposure than earlier study) or methodologies used to measure motor development or both contributed to this discrepancy remains unknown.

In more recent years, Hayes and colleagues have been systematically studying the effects of pre- and postnatal alcohol exposure on the behavioral state regulation of the infant (see also [91, 102]). By examining the relationship between rates of maternal alcohol consumption with the timing, vigor, and durations of spontaneous movements, stable characteristics of an individual baby which are common during sleep, they discovered that exposure to alcohol initiates a cascade of events including sleep fragmentation, sleep deprivation, and, in turn, a reduction in spontaneous movements during sleep [103]. The authors suggest that such attenuated sleep-related movements and disruption of sleep-wake organization may be one mechanism for why infants who are chronically exposed to alcohol prenatally are not only at greater risks for sudden infant death but that such risks may be compounded by postnatal exposure to alcohol or other drugs [103].

Sensory Learning

Research in humans and animals have attempted to identify some of the developmental, experiential, and cultural factors that contribute to an individual's hedonic responses to alcohol [10]. Because of the olfactory system's intense and immediate access to the neurological substrates underlying emotion [104], the hedonic responses to sensory stimuli may provide a window into children's emotional responses and reveal information about contextual effects of learning and the role of early experience on the development of preferences and aversions. Moreover, the early state of maturity and plasticity of the chemical senses favors its involvement in the adaptive responses to the challenges of normal or atypical development.

During the past decade, animal studies have elegantly revealed that early experiences with the smell and taste of alcohol can affect later responsiveness to the drug. In addition to the learning that occurs when young mammals experience the flavor of ethanol in mother's milk [105, 106], learning occurs when they experience alcohol in amniotic fluid [107–111], as an ambient odor [112, 113], when the drug is intraorally infused [114, 115], or when they are exposed to conspecifics who are intoxicated [94, 105, 116, 117]. It should be emphasized that the amount of exposure needed to trigger fetal and neonatal sensory learning about alcohol occurs at levels of exposure that are subthreshold to that needed to produce teratogenic effects. For example, a brief (10 min) exposure to alcohol resulting from direct administration of the drug into the amniotic fluid prior to cesarean delivery (peak alcohol concentration: 100 mg%) was sufficient to establish alcohol-related memories [108].

Findings in humans are consistent with this body of research and suggests that prenatal, neonatal, and infantile exposure to even low to moderate alcohol doses set the opportunity for the growing infant to acquire memories related with the emotional context that surrounds the original contact with a particular odor or flavor (odors perceived retronasally), that is, pre- and postnatal experiences with a variety of odors, including ethanol, bias infant behaviors, and preferences during infancy and

childhood (see ref. [72] for review). Not only can infants can discriminate full-strength homologous alcohols in much the same way as adults [118], but they can also retain sensory information about ethanol when experienced in amniotic fluid [119], mother's milk [120], and/or the home [120]. Moderate consumption of alcohol during human pregnancy has been shown to be strongly associated with heightened neonatal responsiveness to the odor of alcohol. That is, Molina and colleagues have shown that day-old infants born to frequent drinkers exhibited heightened reactivity (as assessed by head and facial activity) toward ethanol odor compared with newborns of infrequent drinkers. That the infants' response did not generalize to other odors such as citral [119] suggests that the effects were not due to a generalized hyperreactivity to odors due to prenatal alcohol exposure.

Experiences with ethanol odors can continue to affect infant behaviors during breastfeeding [10]. When breastfed infants were exposed to toys that were identical in appearance but differed in their characteristic scent, infants who had more exposure to ethanol, as inferred from questionnaires about parental alcoholism and alcohol intake, behaved differently in the presence of an ethanol-scented toy compared with less exposed infants [120], manifesting as increased mouthing behaviors with the toy. This finding might be anticipated based on animal studies indicating that pups exposed to the flavor of alcohol in milk increased mouthing rates to ethanol odor and were more willing to ingest alcohol-flavored solutions [106]. More mouthing of the ethanol-odorized toy may reflect the infants' familiarity with the flavor of ethanol. These data provide circumstantial evidence that prior alcohol exposure alters the human infant's reactions to this odor. Moreover, this learning appears to be keenly selective, as it allows discrimination between alcohol and vanilla, a closely related scent.

That early experiences can generate odor memories about alcohol was evident in a study in older children [121, 122]. The children's hedonic response to alcohol odor was related to the emotional context in which parents experience alcohol and the parents' frequency of drinking. Children whose parents drank alcohol to change their state of mind or reduce dysphoria ("escape drinking") were significantly more likely to judge the odor of beer as unpleasant compared with similarly aged children whose parents did not drink to escape. In contrast, both groups were similar in their preference for bubble gum odor and rejection of pyridine odor. These findings concur with previous studies on preschool-age children of alcoholic parents [123] and are consistent with animal studies demonstrating that pups exposed to an intoxicated mother develop aversive memories for the odor of alcohol [124, 125].

Early childhood represents a "critical period" for the development of expectancies about and the affective disposition toward alcohol that may affect alcohol use during adolescence [126, 127]. Some of the early learning about alcohol is based on sensory experiences and anchor it to children's experiences at home and the frequency and emotional context in which their parents experience alcohol. Clearly, more research is needed to determine whether children who dislike the odor of alcoholic beverages and associate it with such emotional contexts display a trajectory toward or against using alcohol to escape during adolescence and adulthood. It is imperative to understand the development of these alcohol-related memories and beliefs in childhood, before drinking has begun, so that primary prevention programs can be better informed [121].

Concluding Remarks

A growing body of literature indicates that lactating women metabolize alcohol differently, partly due to frequent breast stimulation during breastfeeding and pronounced physiological changes that accompany one of the most energetically costly mammalian activities. In the past, many health professionals have interpreted the reduced systemic availability of alcohol in lactating women as an indication that lactation protects the mother and infant from alcohol exposure. Such clinical interpretations, along with the epidemiological findings that women have a greater vulnerability to alcohol than do men [128], make knowledge of alcohol pharmacokinetics during lactation particularly important.

Although there has been considerable research on the effects of prenatal alcohol exposure, scientific information on the effects of postnatal exposure to alcohol, for both the mother and her infant, is quite limited. Thus, women, and consequently their infants, have relied on a rich folklore passed down through generations. This lore relates that alcohol has galactogenic properties that facilitate milk let-down and rectify milk insufficiency and sedative properties that calm "fussy" breastfed babies. Scientific study of alcohol's effects on lactation and the infant, in both humans and animals, calls this lore into serious question.

Contrary to these popular beliefs, infants actually ingest less breast milk immediately following maternal alcohol consumption, partly due to a direct effect of alcohol on the mothers' milk production and hormones. In addition, exposure to alcohol in mother's milk disrupts infant sleep-wake patterns and motor development in ways that contradict this medical lore, and experience with the flavor of alcohol results in the formation of alcohol-related memories. Based on this information, the recommendation for a nursing mother to drink a glass of beer or wine shortly before nursing may actually be counterproductive. While mothers may be more relaxed after a drink, their hormonal response to suckling will be altered and their babies will ingest less milk, have short-term sleep alterations, and learn about the flavor of alcohol in the milk.

Because breastfeeding confers significant health and developmental benefits for mother and child, the Surgeon General's health goals for 2010 include breastfeeding initiation by 75% and continuation for at least a half a year by 50% of American women [129]. The findings of the research reviewed herein help identify factors that contribute to breastfeeding success and, in turn, the long-term health of women and their children. Greater recognition of the individual differences related to lactational success will lead to the development of timely, accurate, and appropriate interventions to enable mothers to successfully breastfeed if they so desire, as well as sound guidelines for alcohol consumption during lactation.

Women should not stop breastfeeding because of their concern for alcohol in their breast milk. A lactating woman who drinks occasionally can limit her infant's exposure to alcohol by timing her breastfeeds in relation to drinking. In addition, drinking alcohol with a meal will reduce the amount of alcohol transmitted to the milk. Knowledge about the time course of the transfer of alcohol to human milk and the potential impact that alcohol exposure via breast milk has on the infant is crucial for informing parents and health-care professionals. Providing insights from evidence-based research on alcohol pharmacokinetics will continue to aid in the development of scientifically sound guidelines for alcohol consumption by nursing women and shed light on how breast pumping and breastfeeding affect the availability and elimination of alcohol, and perhaps other drugs, during lactation, an area that has received little scientific attention despite the increasing numbers of lactating women who need to take medications [130].

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Chapter 6
Moderate Alcohol Administration: Oxidative Stress and Nutritional Status

Lorenzo Leggio, Anna Ferrulli, and Giovanni Addolorato

Abbreviations

ATP	Adenosine triphosphate
BMI	Body mass index
CVD	Cardiovascular disease
FM	Fat mass
FFM	Fat-free mass
GSH	Glutathione
HDL-C	High-density lipoprotein cholesterol
HPA	Hypothalamic-pituitary-adrenal
MDA	Malondialdehyde
MEOS	Microsomal ethanol oxidation system

Key Points

- Moderate amounts of alcohol may have beneficial effects on cardiovascular disease.
- Even at moderate doses, alcohol may alter the oxidative and nutritional status, although beer and wine, as opposed to spirits, may attenuate these effects.
- Any consumption of alcohol needs to be investigated by health-care professionals, who have to consider in a case-by-case scenario the possible need for addressing even a moderate consumption of alcohol.

Keywords Alcohol • Alcohol abuse and dependence • Moderate alcohol consumption • Oxidative stress • Nutritional status

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Introduction

Alcohol abuse and dependence are related to increased morbidity and mortality, particularly due to liver [1, 2] and cardiovascular diseases [3]. On the other hand, epidemiologic studies show both beneficial and adverse effects due to alcohol intake, i.e., a J-shaped relationship between the amount of alcohol consumed and mortality [4, 5]. Traditionally, the J-shaped relationship has been seen as a clear evidence of the protective effects of alcohol, if consumed at moderate doses. For example, consumption of moderate doses of ethanol may be associated with lower death rates from cardiovascular disease (CVD) and thrombotic stroke [6, 7]. Several mechanisms have been proposed for the protective effect of alcohol on CVD, e.g., (a) alcohol-related action on platelet aggregation [6], (b) alcohol-related action on high-density lipoprotein cholesterol (HDL-C) and other nutritional and metabolic parameters [8], and (c) increased antioxidant activity [9].

Here, we will summarize some preclinical and clinical literature on the effects of moderate alcohol administration on the oxido-reductive status and on nutritional and metabolic parameters.

Moderate Alcohol Consumption: Oxidative Stress and Nutritional Status

Studies on the effects of moderate alcohol administration on oxidative stress and nutritional status have focused their attention on possible differences among different kinds of alcohol beverages, i.e., wine, beer, and liquors. In fact, both wine and beer contain many nonalcoholic components with antioxidant properties [10, 11]. Wine is the beverage mainly investigated and has been found to contain antioxidants, vasorelaxants, and stimulants of anticoagulation mechanisms [10, 11]. Beer also contains many different substances with nutritional value, such as vitamins, minerals, organic and inorganic salts, and phenolic compounds. Among these compounds, phenols – essential in determining the taste and in maintaining the foam – are well-documented antioxidants [12–15] contributing to physical and chemical stability of the packaged beer. Animal studies suggest that beer may have a variety of beneficial effects, such as prevention of carcinogenesis and osteoporosis, protection against oxidative stress, prevention and improvement of obesity and type 2 diabetes, improvement of lipid metabolism, and suppression of atherosclerosis [16]. Furthermore, in a set of experimental studies [17], rats were fed with three different isocaloric diets for 6 weeks, i.e., a beer-containing diet (30% w/w), an ethanol-supplemented diet (1.1 g/100 g, the same as in the beer diet), and an alcohol-free basal diet. At the end of the feeding period, rats were analyzed for plasma and liver oxidative status, and liver ischemia-reperfusion to assess the additional oxidative stress determined by reperfusion. While no significant differences in plasma antioxidant status were found among the three dietary groups, lipoproteins from the beer group showed a greater propensity to resist lipid peroxidation. Furthermore, ischemia caused a decrease in liver parameters of energy and antioxidant status in all groups, but adenosine triphosphate (ATP) was lower in the livers of rats exposed to the ethanol diet. Finally, during reperfusion, lipoperoxidation increased significantly in all groups, but livers obtained from ethanol-treated rats showed the higher formation of lipoperoxides. In conclusion, this study suggested that a moderate consumption of beer in a well-balanced diet does not cause oxidative stress in rats; indeed, beer could attenuate the oxidative action of ethanol, probably via its minor components [17]. Consistent with the animal experiments, human studies also suggest some different effects of beer and wine, as opposed to spirits, on oxidative stress and nutritional status, when beverages are consumed in a moderate amount. For example, our research group performed a 30-day experimental human study testing the influence of a moderate amount of beer, wine, and spirits in healthy subjects on some parameters of oxidant/antioxidant status and on the nutritional status and body composition [18]. In this study, a moderate alcohol dose of 40 g/day (see [19, 20]) was administered to 40 social drinkers, who were Caucasian males, nonsmoking, and healthy. After 2 weeks of complete alcohol abstinence ("washout" phase), these subjects received an administration of 40 g/day of alcohol for 30 consecutive days. Specifically, subjects were assigned

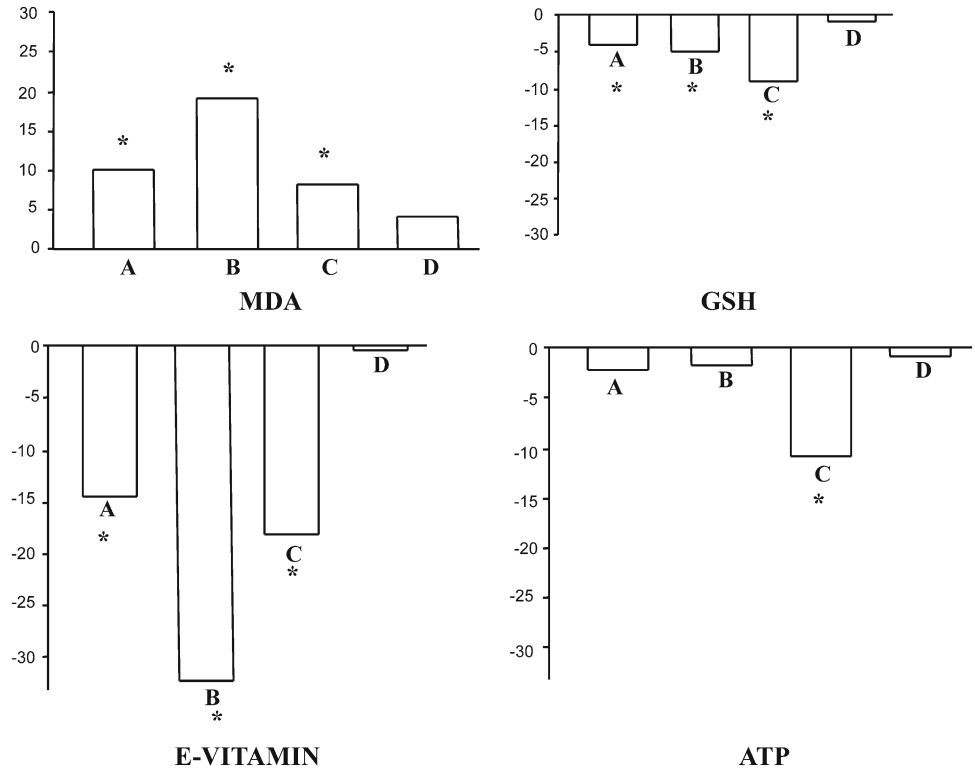

Fig. 6.1 Changes in oxidative parameters in the following groups (in Addolorato et al., 2008): Group A: healthy social drinkers who consumed 40 g of ethanol per day in lager type beer (1000 ml; 4% ethanol) during the 30-day study period. Group B: healthy social drinkers who consumed 40 g of ethanol per day in red wine (400 ml; 11% ethanol) during the 30-day study period. Group C: healthy social drinkers who consumed 40 g of ethanol per day in spirit (120 ml of distillate 40% volume) during the 30-day study period. Group D: healthy social drinkers who maintained abstinence from alcohol during the 30-day study period and served as control group. Variation between the start (T0) and the end (T1) of the 30-day study period, expressed as percentage of plasma concentrations of malondialdehyde (MDA), reduced glutathione (GSH), alpha-tocopherol (vitamin E), and adenosine triphosphate (ATP) of the four groups examined. MDA significantly increased in all subjects exposed to ethanol (group A: +9.5%, group B: +19.0%, group C: +7.3%) ($p<0.05$). A significant decrease of GSH (group A: −4.2%, group B: −5.1%, group C: −9.0%) and of vitamin E (group A: −14.5%, group B: −32.4%, group C: −17.6%) was found in all subjects exposed to ethanol ($p<0.05$). Plasmatic levels of ATP significantly decreased only in group C (−12.0%; $p<0.05$). *$p<0.05$ in T1 with respect to T0 (Reprinted from Addolorato et al. [18], with permission from Elsevier)

randomly to four possible conditions, i.e., (a) lager type beer, (b) red wine, (c) spirits, or (d) no alcohol beverage. The fourth group maintained total alcohol abstinence and served as control group, i.e., there were no significant changes in the oxidant/antioxidant status and on nutritional status before and after the 30-day period of the study. In regard to the other three groups, while plasma malondialdehyde (MDA) significantly increased, and glutathione (GSH) and vitamin E significantly decreased in all groups, on the other hand, ATP values significantly decreased only in those subjects drinking spirits. No significant changes were found in ATP levels before and after the 30-day period in those subjects drinking beer or wine. In summary, this study [18] showed a significant increase of plasma MDA, a marker of lipoperoxidation, and a significant decrease of plasma GSH and vitamin E, the two main antioxidant compounds, in all subjects exposed to ethanol for 30 days, but not in those who were abstinent during the study (control group). However, ATP was reduced only in subjects drinking spirits while no changes in ATP were found in those drinking wine or beer (Fig. 6.1). As such, this study showed that ethanol, although in low doses, determines a decrease of plasma antioxidant status. However, ATP was reduced only in those subjects exposed to spirits. Given that ATP represents a

parameter of energy level and antioxidant status [21], this result could indicate that the decrease in plasma parameters of antioxidant status is attenuated when alcohol is consumed as beer or wine, as opposed to the consumption of alcohol as spirits. Other studies, however, did not report similar results (e.g., [22]), suggesting a variety of differences across studies, such as the possible different antioxidant capacity of alcoholic beverages originating from different countries (see [23]), and/or genetic differences across individuals.

The results of our research are also consistent with a more recent study [24], which was a randomized crossover study with 40 healthy men, who received, after a 15-day washout period, 30 g/ethanol/day as either wine or gin for 28 days. Compared to gin intervention, wine intake reduced plasma superoxide dismutase (SOD) activity and MDA levels, suggesting that, compared to gin, red wine intake has greater antioxidant effects, probably due to its high polyphenolic content [24].

An additional observation in our study [18] was a significant increase in HDL-cholesterol on the three groups assigned to an alcohol condition (wine, beer, or spirits), an observation consistent with previous similar observations [25, 26]. The increase in HDL-cholesterol is consistent with the possible protective effects of moderate amounts of alcohol intake on cardiovascular diseases [3]. However, it should be kept in mind that the protective role of moderate amounts of alcohol on CVD is still controversial. For example, Beulens and colleagues [27] have suggested that in the general population, men with hypertension drinking moderately and safely may not need to change their drinking habits; on the other hand, Zilkens and colleagues [28] have reported that an intake of 40 g/day of alcohol for 4 weeks of red wine or beer could elevate blood pressure in normotensive men.

In regard to the nutritional assessments, in our study [18], there were no significant changes in body mass index (BMI), fat mass (FM), or fat-free mass (FFM). However, while FFM and FM were unmodified in the control group, FM was increased in subjects drinking beer and wine and decreased in subjects exposed to spirits. Yet, FFM was stable in subjects exposed to beer and wine and increased in subjects exposed to spirits. Ethanol represents a high-energy substrate providing 7.2 Kcal (29.7 Kj) per gram; however, these calories are defined as "empty" since they are inefficiently utilizable [29]. It is possible that the mechanisms how moderate alcohol can modify the nutritional status could be similar to those present in chronic alcoholics. In fact, "empty calories" act by displacing other nutrients in the diet and causing primary malnutrition through decreased intake of essential nutrients [29] and a decrease in FM in chronic alcoholics [30]. Different mechanisms may explain the nutritional impairment in chronic alcoholic individuals. In particular, these effects can be due to both an increase of energetic expenditure related to the microsomal ethanol oxidation system (MEOS) induction and to an increase of fat oxidation related to the mitochondrial system induction due to a free radical action [31]. More recently, our group has proposed an additional mechanism, i.e., the hypothalamic-pituitary-adrenal (HPA) axis may play a role in these nutritional and metabolic disorders [32]. Specifically, we studied a sample of chronic alcoholic individuals who were current drinkers at baseline and abstinent from alcohol for the consecutive 12 weeks. At baseline, there was a high HPA-axis activation, as reflected by high plasma cortisol levels. Additionally, plasma cortisol levels were associated with lower FM values. Conversely, after 12 weeks of total alcohol abstinence, there was a reduction in the HPA-axis activity, as reflected by a significant reduction of plasma cortisol levels, and a significant increase in FM values. Furthermore, after 12 weeks of total alcohol abstinence, the relationship between cortisol and FM was not present anymore. In summary, this study [32] suggested a role of the HPA axis throughout cortisol both in the etiology of the alcohol-related nutritional alterations and in their recovery after a period of total alcohol abstinence.

All or some of these mechanisms could also be involved in the nutritional and nonsignificant metabolic changes observed in our study, testing the effects of moderate alcohol consumption in healthy social drinkers [18]. However, it should be noted that the decrease in FM was present only in the group drinking spirits, but not in those drinking wine or beer or in the control group. This might suggest that when the same quantities of ethanol are contained in alcohol beverage such as beer or wine, the free radical action on the MEOS and mitochondrial systems could be counterbalanced by the nonalcoholic compounds with antioxidant action.

Summary

Despite the possible beneficial effects of moderate amounts of alcohol on CVD, several considerations need to be made. First, as detailed in this chapter, the administration of moderate amounts of alcohol under controlled and experimental conditions (i.e., [18]) can still turn into an increase in oxidative parameters. Second, although additional studies are needed, it might be possible that the effects of moderate amounts of alcohol on the oxidative status and nutrition are attenuated when ethanol is consumed as beer or wine. Finally, in spite of the potential benefits of beer and wine on oxidative stress, nutrition, and CVD, it is very important to keep in mind that even a "moderate" amount of alcohol can still be "too much" (see [33]) in several conditions, such as taking medications that interact with alcohol, presence of medical condition that can be made worse by drinking (e.g., liver diseases, bipolar disorder, abnormal heart rhythm, and chronic pain), being underage, planning to drive a vehicle or operate machinery, and pregnancy or trying to become pregnant. In summary, consistent with the more and more important urge for a "personalized medicine," consumption of alcohol always needs to be investigated by health-care professionals, who have to consider in a case-by-case scenario the potential need for discussing with their patients and addressing even a moderate consumption of alcohol.

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Chapter 7
Alcohol Use and Abuse: Effects on Body Weight and Body Composition

Stefan Gazdzinski and Timothy C. Durazzo

Key Points

- Alcohol/ethanol contains hundreds of empty calories that add up to the calories consumed in foods. Excessive alcohol consumption significantly increases caloric intake.
- Ethanol interferes with metabolism. Especially, it slows down the metabolism of fats.
- Consumption of alcohol before meals results in higher food intake.
- Over lifetime, higher levels of alcohol consumption appear to be associated with higher body weight and weight gain, in males but not in females. These effects are relatively small, in order of a BMI unit (on population scale).
- Genetic factors modulate the association between alcohol intake and body weight.
- Higher alcohol consumption is consistently associated with central (abdominal) fat depositions.

Keywords Alcohol • Alcohol abuse • Alcohol dependence • Obesity • Abdominal obesity • Waist circumference • Caloric intake

Ethyl alcohol (referred to in this chapter as alcohol) is one of the most widely consumed substances in the world and provides significant quantities of energy to living organisms. The energy density of pure ethanol is 7 kcal/g (equivalent to 29 kJ/g), i.e., second only to plant and animal lipids (i.e., fat), which contain 9 kcal/g and significantly higher than the energy density of proteins and carbohydrates (4 kcal/g) [1]. The energy provided by pure alcohol is often referred to as empty calories, as this simple molecule is not a source of carbohydrate, protein, fat, minerals, or vitamins. In order to meaningfully compare alcohol intake between studied individuals, regardless of type of drink, a standardized measure of alcohol consumption was defined. Its definition differs slightly between studies and ranges from 10 to 15 g of pure ethanol per drink [2, 3]. In many clinical studies, one alcoholic drink is defined as 13.6 g of pure alcohol [4]. A standard drink corresponds to 12 oz of beer (330 ml), 5 oz of wine (140 ml), or 1.5 oz of liquor or vodka (40 ml). Correspondingly, a 12-oz can of regular beer

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contains approximately 145 calories, a 5-oz glass of wine ~135 cal, and 1.5-oz serving of spirits ~130 cal [5]. However, certain alcoholic drinks such as sweet wine or beer may contain additional calories in form of simple and complex carbohydrates. In such cases, the caloric content of a drink may be significantly higher than 100 kcal. Thus, alcohol consumption may introduce a significant amount of calories into the diet. Understanding the contributions of alcohol consumption to individual's energy balance is of vital importance, as excessive consumption may significantly increase caloric intake and place the individual at increased risk for overweight or obesity and the associated biomedical conditions (see below).

Quantization of Body Fat

There are direct and indirect methods of determining the amount of body fat. Direct methods utilize unique physical properties of the evaluated tissues to provide express estimation of the amount of subcutaneous and visceral fat tissue, as well as the amount of ectopic organ fat. They include bioelectric impedance analysis (BIA), dual-energy X-ray absorptiometry (DEXA), and magnetic resonance imaging (MRI). DEXA and MRI are accurate but expensive methods, and their use is limited to small clinical studies. DEXA additionally utilizes ionizing radiation [6].

Indirect methods of calculating the amount of body fat and its distribution are based on generalized equations obtained by comparing the markers yielded by indirect methods with the results of direct methods. Indirect methods include anthropometry, hydrodensitometry (underwater weighting), and air displacement plethysmography. Hydrodensitometry and air displacement plethysmography are based on the principle that bones and muscles have higher density than fat. Both methods involve calculating the density of the body and subsequently the mass of bones, muscles, and body fat. They provide reliable results; however, they do not allow for distinguishing between various pools of fat in the body and their cost limits their use in clinics and in research.

Body Mass Index

Anthropometry is an inexpensive and relatively uncomplicated method, used both in small clinical and in large epidemiological studies involving hundred thousands of participants. The most commonly used anthropometric measure of person's weight status is body mass index (*BMI*). It is defined as body weight in kilograms divided by squared body height in meters. Body weight can be divided into several classes based on the World Health Organization's (WHO) cutoffs: underweight (BMI < 18.5), normal weight or healthy weight (BMI between 18.5 and 25), overweight (BMI between 25 and 30), and obesity (BMI > 30). The category of obesity is further divided into simple obesity (BMI between 30 and 40) and morbid obesity (BMI > 40). The morbid obesity subcategory was introduced as a guide for suitability of certain medical procedures, such as bariatric surgery. Underweight, overweight, and obese categories are associated with increased risk for certain medical conditions or premature mortality. Morbid obesity is an indication for bariatric surgery. It should be mentioned that BMI cutoff values are race specific. For Asians, the relative risks for obesity-related conditions were higher than among other races, and a new cutoff BMI index for obesity was introduced: it is 27.5 compared with the traditional WHO figure of 30. An Asian adult with a BMI of 23 or greater is now considered overweight and the normal range is 18.5–22.9.

Although BMI is relatively easy to obtain and broadly used, it suffers from multiple limitations. It does not distinguish between muscle mass and fat mass, and it does not account for bone mineral density. Thus, it may incorrectly classify, e.g., athletes as overweight or obese. BMI also does not account for body fat distribution. The location of body fat is an important factor defining risks of

medical conditions. The distribution of body fat may be depicted on a continuum spanned between the apple type and the pear type of obesity. In the former case, body fat is stored in the upper part of the body, mostly around the abdomen, whereas in the latter case, body fat is accumulated on the lower parts of the body – on hips, buttocks, and thighs. Apple-shaped individuals are more likely to develop medical conditions associated with obesity than their pear-shaped counterparts. Obese females tend to have the pear shape, whereas obese males the apple shape.

Waist Circumference

Waist circumference (WC) and waist-to-hip ratio (WHR) were introduced as measures accounting for body fat distribution. There are two major methods of measuring WC. The WHO advises measuring WC in the midpoint between the lower border of the rib cage and the iliac crest, whereas the method endorsed by the National Institute of Health (USA) involves measuring waist circumference at the superior border of the iliac crest. Both methods have separate sets of cutoff values specifying abdominal obesity, and these cutoff values are gender specific. The relationships between WC and risk of chronic diseases and premature mortality are consistent for both methods of measuring WC. Another method of measuring fat distribution is waist-to-hip ratio (WHR). WHR is defined as WC (obtained with either method) divided by hip circumference, which is measured at the widest part of the hips [7]. Use of WHR leads to same patterns of risks for medical conditions as WC; WHR does not have higher predictive value for these risks than WC.

Biomedical and Psychosocial Correlates of Overweight and Obesity

Worldwide prevalence of overweight and obesity has steadily increased over the last half of the century and has reached epidemic proportions, with more than two billion overweight and 400 million obese individuals; the number of obese individuals is projected to increase to 700 million by 2015 (WHO fact sheet No 311, 2006). Previously considered a problem only in high-income countries, overweight and obesity are now dramatically on the rise in low- and middle-income countries (WHO fact sheet No 311, 2006). Obesity increases risk for a variety of medical and psychiatric/psychological problems. There are numerous biomedical conditions associated with obesity including arthritis, sleep disturbance, type 2 diabetes mellitus, and cardiovascular and cerebrovascular disease [8], as well as Alzheimer's disease [9]. Additionally, obesity is associated with mood and anxiety disorders [10]. Obesity was also related to poorer cognitive abilities and their faster decline with aging [11, 12], as well as poorer self-esteem [8]. Additionally, elevated BMI is associated with increased medical costs [13]. Specifically, in the United States alone, the economic cost of treating conditions related to obesity was estimated at $117 billion a year (http://www.weight.addr.com/BMI.html#4) and is expected to rise [14]. Europe currently also faces increasing rates of obesity, and European societies bear ascending medical costs related to the associated biomedical and psychiatric conditions [15]. The following paragraphs will discuss short-term and long-term effects of alcohol consumption on body weight.

Short-Term Effects of Alcohol on Subsequent Energy Consumption

Short-term effects of alcohol on body weight relate to its effects on appetite and subsequent caloric intake and metabolism. They are evaluated in small clinical studies. Alcohol inhibits the body's ability to burn fat. Ethanol is converted in liver to acetate, released into the bloodstream, and used by the

body as energy source. When the levels of acetate rise, the body begins to burn more acetate than fat, thus the blood levels of circulating lipids increase [5, 16]. This mechanism may promote excessive fat storage and, due to some positive feedback loops, lead to overconsumption of fats and ethanol [16].

Although energy from alcohol is additive to energy consumed from foods, there is no evidence of reducing food intake following ingestion of alcohol; thus, alcohol may promote short-term "passive overconsumption [1]." However, this view has little support in experimental data. Most of the studies evaluating the effects of ethanol on subsequent caloric intake from foods were either underpowered (and reported only statistical trends) or reported no significant findings. Their results were often contradictory. Frequently, the time interval between alcohol ingestion and food consumption was long; thus, the effect of alcohol on caloric consumption might have worn off [1]. Nevertheless, Martin R. Yeomans in his review article [1] noted that small doses of alcohol consumed shortly prior to meals "cause a clear and consistent increase in food intake." He then demonstrated in an ensuing study that alcohol in drinks consumed prior to meal by females increases caloric intake during actual meal 30 min thereafter [3]. The effect depended on the type of alcoholic drink, with orange juice drink leading to more food consumption than beer.

However, organism is able to decrease its caloric intake following a larger meal, thus increased caloric intake following alcoholic preload does not necessarily lead to long-term weight gain.

Relationship Between Alcohol Consumption and Markers of Obesity in Epidemiological Studies

Epidemiological approaches provide information on the long-term effects of alcohol as an energy source in the diet. In the British Regional Heart Study of 7,608 males aged 40–59 years and without diabetes, the prevalence of males with BMI > 28 increased from the none-to-occasional-drinking group (16.8%) to the heavy-drinking group (20.8%) [17]. These results remained significant after controlling for cigarette smoking, social class, and physical activity. A follow-up study of 3,327 males found that consumption of more than three drinks a day was not only associated with higher BMI and higher percent body fat estimated with bioelectric impedance method but to a greater extent with central adiposity [18]. The results were not related to the type of drinks and whether alcohol is drunk with meals or not. All the results were obtained after accounting for cigarette smoking, preexisting diseases, social class, and physical activity. In both above-mentioned studies, about 87% of participants consumed alcohol primarily in beer or in spirits.

Similar findings were reported in a study that reviewed medical records of 27,030 young South Korean males. In this study, alcohol intake was proportionally related to BMI, and prevalence of overweight participants increased from 35% in a nondrinking group to 44% in the group having two or more drinks per day [19]. However, the effect was small, and the average difference in BMI between nondrinkers and those having four or more drinks per day was only 0.4 kg/m^2. Interesting results were published by French et al. [5], who evaluated independent effects of drinking frequency and the average number of drinks per drinking episode in the cohort of 32,763 males and females evaluated in the National Epidemiological Survey on Alcohol and Related Conditions (*NESARC*). In both genders, more alcohol consumed per occasion was positively associated with higher BMI. Surprisingly, higher number of drinking episodes was related to lower BMI both in males and females.

The results above were only partially confirmed by the SU.VI.MAX study of 2,691 French males and females aged 35–60 years [20]. It found that higher consumption of spirits was related to higher BMI and higher WHR, whereas males who consumed one drink of wine per day had lower BMI and WHR than nondrinkers and those who consume more than one drink per day.

In a population-based study of 1,491 males and 1,563 females by Gerona Heart Registry in Spain, Schroeder with colleagues [2] determined that consuming more than three drinks of alcohol per day

was significantly associated with risk of abdominal obesity and exceeding recommended energy consumption in males; the results in females were not conclusive, as only a small percentage of them drank at such high levels. BMI was not recorded in this study. A similar study of 8,603 middle-aged South Korean males and females, who visited health promotion centers for routine health examinations, found that higher alcohol intake was associated with elevated WC [21]. Participants consuming more than two drinks a day had the largest WC. Comparable results were obtained in Uppsala Longitudinal Study of Adult Men of 807 Swedish elderly participants [7]. After correction for confounding factors, more alcohol consumption was related to larger WC, but not BMI. This result is consistent with associations between more alcohol consumption and larger WHR in Italian alcoholics, who had normal BMI [22].

However, a series of studies found inverse associations between drinking and body weight. In a study by Gearhardt et al. [23] of 37,259 participants from the NESARC sample, participants who were at normal weight or overweight drank on average almost three drinks per week, the obese individuals had on average two drinks per week, whereas the morbidly obese individuals had only one drink per week. Positive family history of alcoholism (defined as having a biological parent with alcohol use disorder (AUD = DSM-IV diagnosis of either alcohol abuse or alcohol dependence)) was associated with more frequent alcohol consumption except for the obese category [23]. Although this study was based on a similar cohort as French et al. [5], it arrived at different conclusions, partly due to differences in statistical model. For example, Gearhardt et al. [23] used blood alcohol concentrations calculated based on number of drinks per episode, duration of the episode, and body weight, whereas French et al. [5] utilized the number of drinks per episode in their model. This example illustrates how selection of a statistical model may affect the results of a study.

A similar finding as Gearhardt et al. [23] was reported in Missouri Adolescent Female Twin Study of 3,514 young adult American female twins [24]. Obese white females were less likely to ever use alcohol, consume alcohol on a weekly basis, or engage in episodic heavy drinking compared to their normal-weight counterparts. None of the findings observed in white females were apparent in black females.

Modulating effects of family history of alcoholism on the relationship between alcohol consumption and body weight were also reported by Grucza et al. [25]. This study found in population-based samples of 39,312 and 39,625 individuals from National Longitudinal Alcohol Epidemiological Survey and NESARC, respectively, that positive family history of alcoholism (defined as having a biological parent or sibling with AUD) was associated with 49% higher odds of obesity than those with negative family history. This association remained significant after adjustment for covariates including cigarette smoking, alcohol and (illicit) substance use, major depression, and sociodemographic factors. These findings might be partially attributable to increased preference of sweets among individuals with positive family history of AUD [26].

The long-term effects of alcohol consumption on changes in weight gain and changes in WC were evaluated in a few longitudinal, population-based studies.

In the British Regional Heart Study of 7,608 males aged 40–59 years and without diabetes, 6,832 participants were reevaluated after 5 years [17]. In this group, alcohol consumption of three or more drinks per day was directly associated with body weight gain, regardless of type of drink. These results remained significant after correction for cigarette smoking, social class, and physical activity. Heavy drinking was also associated with weight gain over 5 years of follow-up: participants who continuously drank at levels of three drinks per day and participants who started to drink at this level experienced the greatest weight gain and had the highest prevalence rates of high BMI. Similar findings were reported by French et al. [5], who found that an increase in the average numbers of drinks per episode was associated with small increase in weight (fraction of BMI unit) over 3 years in males, but not in females.

Similarly, in a lightly drinking cohort of 3,032 Chinese adults aged 25–95 years and participating in a community-based Shanghai Diabetes Study, alcohol consumption of more than half a drink per

day was related to higher risk of becoming overweight or obese over 3.6 years only in males [27]. On the contrary, higher alcohol consumption (drinks per day) in a cohort of 19,220 American middle-aged and postmenopausal females, participating in Women's Health Study, was associated with lower risk of becoming overweight or obese over 13 years [28]. These results were adjusted for potential confounding factors, such as physical activity, nonalcohol energy intake, etc. Finally, a Finnish population-based study of 5,563 twins (FinnTwin16) in their late adolescence found that abstinence in males in late teens was associated with smaller BMI increase of 0.62 kg/m^2 over 5–9 years [29]. Other associations, including positive relationship between more drinking and larger self-measured WC in females, were explained by confounders that included cigarette smoking, diet, physical activity, and socioeconomic status.

Finally, a diagnosis of AUD (which has a hereditary component) appears to be related to weight status and weight changes. Barry et al. [30], using NESARC data of 40,364 participants, found that overweight and obesity in males were associated with higher risk for AUD. Similarly, in a longitudinal study, diagnosis of AUD in a group of 383 young American adult females aged 24 years predicted development of obesity 3 years later [31]. No such relationships were observed among males.

Taken together, gender and unaccounted genetic factors appear to modulate the relationship between alcohol consumption and body weight. Higher levels of alcohol consumption in males are generally associated with higher BMI and more weight gain, whereas the opposite is often noted among females. Similarly, the longitudinal studies with male participants generally demonstrate a direct relationship of more alcohol consumption to larger weight gain, whereas the studies of female participants provide mixed evidence on the relationship between alcohol use and weight gain. Genetic factors reflected as positive family history of alcoholism appear to be related to higher body weight.

Potential Limitations and Caveats

There were few limitations that may have obscured the potential associations between amount of consumed alcohol and body weight. None of the reviewed studies accounted for genetic factors. Family history of alcoholism is a measure of heritable factors that may lead to alcohol abuse or alcohol dependence, and it was shown in two studies to modulate the relationship between severity of alcohol consumption and BMI [23, 25]. Multiple studies [5, 23, 24, 28, 29] used self-reported body weight and height, which may have biased the results due to underreporting of weight among individuals at higher BMI [32]. The studies also differed in the ways for accounting for differences in lifestyle choices. It is especially important, as behaviors such as heavy alcohol consumption, cigarette smoking, sedentary lifestyle, and poor nutrition are not independent but tend to cluster [33]. It is not surprising given neurobiological abnormalities observed in the reward system in AUD and obesity [34], as well as cigarette smoking [35]. Since these behaviors are not independent, potential interactions between them or nonlinear effects could have affected the reported results. This case will be illustrated on the example of cigarette smoking. Cigarette smoking is associated with lower BMI; however, increased amount of smoking tends to be related to higher BMI and larger WHR [36]. Nicotine, independent of lifestyle characteristics, is associated with increased metabolic rates [37]; thus, corrections for caloric intake cannot be accurate. Chronic cigarette smoking is associated with poorer diet, i.e., higher intakes of total and saturated fat and lower intakes of folate, vitamin C, and fiber [38]. Finally, the increasing costs of cigarettes may act indirectly on the diet by decreasing available funds to buy food in those who are economically challenged [39]. The last statement may be also applicable to some consumers of alcohol in countries that levy a high tax on ethanol products. Finally, overweight and obesity are associated with psychiatric disorders, such as major depressive disorder and anxiety disorder [40]; however, most of the results were adjusted for comorbid major depressive disorder.

Although the relationship of alcohol consumption to BMl and weight gain appears to be modulated by gender and genetic factors, the proportional relationship of more drinking to larger WC is apparent in all studies, regardless of BMI. Thus, excessive alcohol consumption is not necessarily related to increased body weight but to excessive abdominal fat depositions. These findings have to be supported by longitudinal studies. Unfortunately, the only study that evaluated relationships between alcohol consumption and changes in WC suffered from very low levels of alcohol consumption. Additionally, WC in this study was self-measured, which likely resulted in some deterioration of data quality.

In general, abdominal obesity is strongly related to insulin resistance and metabolic syndrome [41] and predicts type 2 diabetes [7]. The effects of insulin resistance in alcoholics will be discussed in Chap. 39.

Conclusions/Summary

Despite large energy density of ethanol and its appetizing effects, consumption of more alcohol seems to lead to small increases in BMI. Higher intake of alcohol is associated with abdominal fat depositions and increased risk for abdominal obesity. The relationship of frequency and average number of drinks per occasion to impaired fasting glucose, insulin resistance, and type 2 diabetes remains to be evaluated.

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Chapter 8
Alcohol: Nutrition and Health Inequalities

Adrian Bonner and Margherita Grotzkyj-Giorgi

Key Points

- Health inequalities result from poor nutritional status exacerbated by heavy drinking. In low socio-economic households and individuals, a combination of dietary intake and heavy alcohol consumption is associated with increased alcohol-related harm.
- Metabolic dysfunction resulting from these interacting factors includes oxidative stress, imbalances in amino acid ratios and vitamin deficiencies, all of which have direct pathological effects and maladaptive behavioural influences on lifestyle.

Keywords Alcohol • Nutritional status • Socio-economic profile • Oxidative stress • Tryptophan metabolism • Vitamins

Introduction: Epidemiology of Alcohol-Use Disorders

Alcohol-use disorders are common in all developed countries and are more prevalent in men than women, with lower but still substantial rates in developing countries [1, 2]. Although rates of these disorders are lower in the Mediterranean countries (e.g. Greece, Italy and Israel) and higher in northern and eastern Europe (e.g. Russia and Scandinavia), they are responsible for a large proportion of the health-care burden in almost all populations [1, 2].

In developed countries, around 80% of men and 60% of women drink at some time during their lives [1]. In any year, between half and two-thirds of individuals who drank are likely to consume alcohol; recent abstainers are most likely to have stopped because of medical concerns ('sick quitters') [2].

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Between 30% and 50% of people who drank in the past year experience at least one adverse alcohol-related problem during their lifetime, such as missing work or school, driving after drinking or interpersonal problems [1, 3].

The lifetime risk of alcohol-use disorders for men is more than 20%, with a risk of about 15% for alcohol abuse and 10% for alcohol dependence [1, 4, 5]. The risk of developing an alcohol-use disorder in the previous year is about 10% overall [1, 4, 5].

More than a fifth of European adults admit to binge drinking (five or more drinks on any one occasion) at least once a week; of all World Health Organization regions, Europe has the greatest proportion of alcohol-related ill health and premature death, and the overall social cost of alcohol to the European Union is around €125bn (£110bn; $180bn) a year [6]. In Scotland alone, adults drink the equivalent of 46 bottles of vodka, or 537 pints of beer, or 130 bottles of wine each year [7]. In England, more than a quarter of adults drink at hazardous levels, and the NHS spends £2.7bn a year on treating alcohol-related conditions [8], whilst the overall cost to society of alcohol use each year amounts to around £20bn [9].

Nutrition, Alcohol and Health Inequalities

Alcoholism remains one of the major causes of nutritional deficiency syndromes in the developed world. Millions of people across the world seek treatment for alcohol misuse and dependence each year. Chronic alcohol ingestion may lead to impaired absorption, transport, storage and metabolism of nearly all nutrients [10, 11]. To compound the problem, people abusing alcohol may consume as much as 50% of their daily calories in alcohol [12]. The consequences of chronic alcohol abuse and dependence are expressed in a wide range of pathological indications which can include muscle, bone and major organ systems including the brain, cardiovascular system, digestive system and the liver [13]. The nutritional perturbations underpinning this range of pathological effects should be addressed as prerequisite of any treatment regime. The provision of a restorative nutritional environment is confounded by the effect of alcohol on eating behaviour and other socially mediated behaviours. The high frequency of alcohol problems in the socially marginalised increases the probability of poor nutrition and the negative influences on cognitive performance and a decrease in social functioning [14].

In 2008, the Scientific Advisory Committee on Nutrition (SACN) conducted a comprehensive analysis of British dietary surveys [15]. Results from the analysis indicate that there have been positive changes in the diet of the British adult population over the last 15 years. For example, the evidence shows a fall in the intake of fat and saturated fat; a decrease in the consumption of red meat, processed meat and meat-based dishes; and an increase in fruit and vegetable consumption. These all reflect moves towards healthier patterns of intake. However, there are further improvements needed in the diet of the British population, especially in those groups of the population who are particularly vulnerable, i.e. children, adolescents and those in low-income groups. People in the group with the lowest mean intakes or biochemical status of all nutrients, except for iron, were more likely to be smokers, to live in households receiving benefits and to have had the highest consumption of soft drinks, savoury snacks and alcoholic beverages [15]. A higher consumption of sugar, preserves and confectionery was associated with low nutrient intake and low biochemical status [15].

Given this evidence, individuals in lower socio-economic groups have been identified as being at increased risk of poor dietary variety, low nutrient intake and low biochemical status. SACN recommends increasing nutritional monitoring of this group and actuation of focused health initiatives to encourage a healthy lifestyle [15].

In general terms, the promotion of a balanced, nutrient-dense diet and improvement in the quality and variety of the diet would contribute to reduce health inequalities, to a better overall health and well being and to reduce the risk and burden of nutrition-related ill health and disease (such as obesity, diabetes, coronary heart disease, stroke, cancer and alcohol dependence). These initiatives should be

set in the context of a healthy lifestyle and reinforce existing measures to stop smoking, to maintain a healthy body weight and to take part in regular physical activity. Strategies to achieve behavioural change should be targeted particularly at young adults, older adults living in institutions and people in lower socio-economic groups.

Whilst a multitude of health problems have been attributed to poor diet and heavy alcohol consumption, morbidity and mortality resulting from heavy alcohol drinking disproportionately affects people of lower socio-economic status [16], even when controlling for level and pattern of alcohol consumption [17]. Additionally, households with higher income are more likely to have better quality diets, consuming more fruit and vegetables [18, 19].

Lower socio-economic status, or income, has been linked to poorer overall health, negative lifestyle behaviours, such as smoking and alcohol misuse, and to shorter life expectancy. Individuals living in less deprived areas of the UK can expect to live 10 years longer than more deprived areas and to spend more of their life free from long-standing illness and disability [20, 21]. Lifestyle factors such as diet and alcohol consumption may partly explain such health differentials.

Poor diet can affect physical and mental health in many ways; diets low in fruit and vegetables have been associated with increased risk of cardiovascular disease, diabetes and cancer; diets high in salt with increase blood pressure and risk of coronary heart disease; foods high in saturated fatty acids with high blood cholesterol; diets high in sugars with increased risk of diabetes and tooth decay [22]. The evidence associating mental health and nutrient intake is still in its infancy. However, a recent report jointly composed by food campaigners Sustain and the Mental Health Foundation [23] suggests that research is increasingly reporting a plausible link between diets poor in essential fatty acids (omega-3 fatty acids) and, for example, lack of concentration, poor academic performance and increased risk of developing behavioural disturbances, such as anxiety and depression, in children, adolescents and adults (for reference to the original research articles please consult [23]). Given the ever-increasing evidence of a link between socio-economic status and diet, unhealthy diet and drinking at harmful level seem to cluster in households of lower socio-economic status, thus exacerbating health inequalities.

'Diet' shows a very distinct gradient in socio-economic differences, such that families who are less affluent, less educated or employed in less prestigious jobs have diets that are least concordant with official recommendations, both in general and specifically in relation to fruit and vegetable consumption in Europe and in the United Kingdom [15, 24, 25].

Lower socio-economic position has been extensively associated with poorer health outcomes [26, 27]. Socio-economic position shapes many health behaviours, such as dietary patterns, physical activity and tobacco and alcohol consumption. Evidence suggests that diet, particularly lower intake of fruit and vegetables, partially explains the higher rates of cardiovascular disease and overall mortality in low socio-economic groups [28, 29]. Research has in fact shown that fruit and vegetable consumption proportionally increases with education level and income [30]. Smoking has been associated with structural, material as well as perceived dimensions of socio-economic disadvantage [31]. A recent investigation by Gell and Meier indicated that harmful alcohol consumption tends to cluster in lower socio-economic groups [32].

Disparities in fruit and vegetable consumption are very important because increased intake of vitamins, minerals and antioxidants from fruit and vegetables reduces risk of chronic conditions, including type 2 diabetes, cardiovascular disease, stroke, cancer and obesity. The international epidemic of obesity [33] raises the possibility that heavy alcohol intake and obesity could be working in unison to elevate risk of liver disease. The mechanisms by which alcohol and obesity affect the liver are not fully understood but biochemical and pathological evidence suggests that common pathways exist [34]. The high prevalence of people who consume excess alcohol and are overweight or obese implies that a better understanding of their prognosis is of clinical importance. Additionally, as obesity, harmful drinking and liver disease seem to cluster in lower socio-economic groups, primary and secondary prevention strategies, specifically tailor-made for this segment of population, are of paramount importance to reduce the burden of disease.

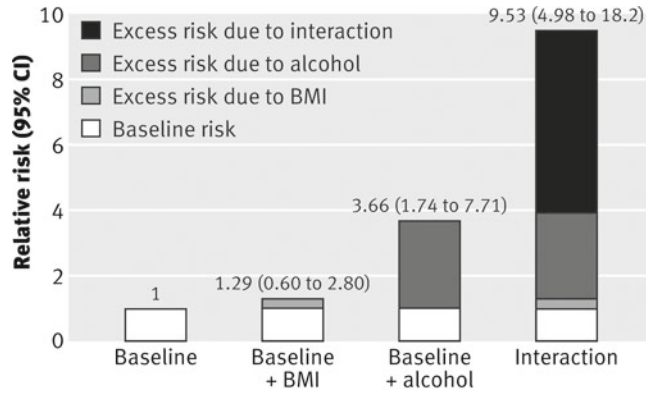

Fig. 8.1 Relative risks of contributions of BMI and alcohol to liver disease mortality (adjusted for all risk factors) (Reprinted from Hart et al. [35], with permission from BMJ Publishing Group Ltd)

A recent meta-analysis conducted by Hart and collaborators investigated whether obesity had an additive effect on liver disease caused by harmful drinking [35]. Authors analysed data from two prospective cohort studies, 'main' study and the collaborative study. Authors concluded that raised BMI and alcohol consumption are both related to liver disease, with evidence of a supra-additive interaction between the two (Fig. 8.1).

Following on the findings by Hart and collaborators, Morleo, Bellis et al. highlighted the need to recognise that a historically strong, underlying relationship exists between alcohol and food [36]. Following on Morleo's suggestion of a link between food and alcohol, Gell and Meier investigated the nature of this relationship in the form of household expenditure on alcohol and/or food [32]. Authors concluded that as adult-only households' expenditure on alcohol increases, spending on food proportionally decreased. In accordance with the Danish study on supermarket expenditure on food and type of alcoholic beverages purchased (wine versus beer and spirits) [37], households that prefer to purchase wine have healthier expenditure patterns than those that prefer to buy beer or spirits, even after controlling for income.

Given this evidence, individuals from those from low-income groups and from those households that purchase more beer or spirits than wine, and in particular children and adolescent, should be targeted for health promotion interventions to help them reduce their risk of negative health outcomes resulting from the clustering of heavy alcohol consumption and unhealthy diet.

Public health strategies should be implemented to tackle the ever-growing dysfunctional relationship of the British population with food, alcohol and tobacco.

Tryptophan Metabolism and Its Role in Alcohol-Related Disorders

The concentration of serotonin in the brain influences mental state. Both acute and chronic ethanol intake alters serotonin system either directly, by ethanol action on serotonin axons and axon terminals [38], or indirectly, via tryptophan metabolism, the metabolic precursor of serotonin. Tryptophan metabolism controls not only the synthesis of serotonin but also the metabolism of a family of neuroactive compounds collectively known as kynurenines [39]. Kynurenines are mainly produced in the liver and to a lesser extent in the brain. However, kynurenines can easily cross the blood–brain barrier (BBB).

Kynurenines have been suggested to play a key role in the neurotoxicity associated with pathology of a wide variety of inflammatory brain diseases [40] and in modulation of alcohol and drug-seeking behaviours [14, 39]. In fact, they modulate a variety of physiological cognitive functions either positively, through the action of the neuroprotectant compound kynurenic acid (KA), or negatively, through the action of neurotoxic molecules 3-hydroxykynurenine (3OHKYN), quinolinic acid (QA), anthranilic acid (AA) and 3-hydroxyanthranilic acid (3OHAA) [40].

Oxidant action of some kynurenines (3OHKYN, AA, 3OHAA, QA) are further enhanced by the fact that brain essentially relies upon glucose metabolism for its functioning [41], hence producing an excess of free radicals, and that brain cells are naturally more vulnerable to free radicals damage because of the lower presence of endogenous antioxidant defences [42]. Thus, to effectively counteract the damaging effects of oxidative stress, brain cells need constant exogenous supply of antioxidants, vitamins and minerals. Consuming a diet rich in fruit and vegetables will ensure a good supply of vitamins and minerals to effectively protect the body and the brain from oxidative damage, ever more so if individuals are drinking alcohol at harmful level.

SACN report identified individuals in lower socio-economic groups as being at increased risk of poor dietary variety, low nutrient intake and low biochemical status [15] and thus at increased risk of oxidative damage.

When an increase in free radical production and a lack of exogenous antioxidant substances concomitantly occur, neurotoxicity may result [14]. In this regard, Bonner et al. [14] proposed an interesting model in which increase in neurotoxic kynurenines concentration together with a decrease in B vitamins, free radical scavengers and neuroprotectant KA could cause a metabolic imbalance and thus cause neurodegeneration; vice versa, when levels of KA, free radical scavengers, vitamins and minerals are sufficient enough to counterbalance the negative effect of kynurenines, neuroprotection may occur. Thus, there is a need to study the possible role of serotonin, kynurenines, minerals and vitamins in relation to neuroprotection/neurodegeneration and cognitive decline often observed in alcohol misusers.

Recently, a randomised controlled trial was conducted by the authors to investigate whether tryptophan and micronutrients supplementation had an effect on Trp: LNAAs ratio and on kynurenines concentration of 43 alcohol-dependent patients undergoing detoxification [43]. Results indicate that tryptophan supplementation not only altered Trp: LNAAs ratio in favour of tryptophan, so to possibly increase cerebral serotonin concentration, but concentration of the neuroprotective KA was also increased in the two supplemented groups (trp-only and trp + vitamins groups). No effect on Trp was observed for LNAAs ratio and on kynurenines in the placebo group. Cognitive tests (Bexley-Maudsley Automated Psychological screening test, BMAPS) were performed on study participants before entering the trial and every day until the end of trial.

Results show an improvement, albeit small, in visuospatial memory of those participants who were fed tryptophan-only and on tryptophan + vitamins supplements. Specifically, participants who had tryptophan + vitamins supplementation for a week showed a 1.5-fold improvement in visuospatial memory test results when compared to the placebo group.

The effects of acute ethanol intake on circulating levels of tryptophan have been also studied in alcoholics. Here, acute alcohol load did not lower plasma tryptophan levels [44]. One possible explanation for this apparently contradictory result is that chronic ethanol consumption inhibits liver tryptophan pyrrolase (TP) activity, thus preventing activation by an acute dose [39]. Badawy and Evans demonstrated that chronic ethanol consumption inhibits liver tryptophan pyrrolase activity, thus enhancing cerebral serotonin synthesis, and that subsequent withdrawal causes a rebound enhancement of the enzyme [44]. This can be regarded as the biochemical mechanism underlying the psychological and behavioural disturbances often observed in chronic alcoholics and patients experiencing the alcohol withdrawal syndrome.

Serotonin synthesis modulation by chronic alcohol consumption is difficult to investigate in humans due to a variety of interpretational and methodological differences [45]. Nevertheless, some authors suggested that brain serotonin activity is likely to be increased during chronic long-term alcohol consumption [46]. This is consistent with changes in kynurenine levels reflecting decreased hepatic tryptophan catabolism by chronic alcohol intake, thus potentially leading to diversion of tryptophan metabolism towards serotonin synthesis.

During alcohol withdrawal, both free and total serum tryptophan concentrations were increased in alcoholic patients [47] as a consequence of TP induction. Induction of liver tryptophan pyrrolase activity during alcohol withdrawal may be an important feature of the alcohol withdrawal syndrome: the

timecourse of induction of tryptophan pyrrolase activity and gene expression pattern have been found by Oretti and co-workers to mirror very closely that of the behavioural features of alcohol withdrawal syndrome [48]. Accordingly, with enhanced hepatic tryptophan pyrrolase activity, serotonin synthesis and turnover are decreased during alcohol withdrawal in association with decreases in precursor tryptophan availability to and within the brain [49].

Although the maximum changes in tryptophan disposition and in serotonin synthesis observed seem to be modest, maintenance of such changes over long periods, together with individual, genetic predisposition factors, all strongly suggest that modulation of tryptophan and serotonin status by alcohol could exert important physiological, behavioural and psychological effects in subjects exposed to it.

Dietary Micronutrients and Their Role in Neuroprotection and Neurodegeneration

Chronic alcohol misusers often suffer from a wide range of nutritional deficits because of their reduced intake of thiamine (vitamin B1) and vitamins due to high alcohol intake and their poor intestinal absorption [14, 50]. Inadequate levels of antioxidants and depleted vitamins stores will result in oxidative stress, a dyshomeostasis between endogenous and exogenous antioxidant defences, and increased free radical production. Oxidative stress, together with increased neuroactive tryptophan metabolites production, can be regarded as some of the aetiological causes of alcoholic brain damage and alcohol-related cognitive impairments [14]. People from lower socio-economic groups have poor diet variety and very low consumption of fish, fruit and vegetables, the main sources of essential fatty acids, vitamins and minerals, respectively. Additionally, harmful drinking seems to cluster in the lower socio-economic groups. Taken together, individuals from lower socio-economic background are more likely to have lower intake of antioxidants and protective micronutrients so they are more prone to the brain-damaging effects of alcohol.

An overwhelming body of evidence suggests that among the prime candidates responsible for producing neurodegenerative disorders are free radicals and the resulting imbalance between them and the endogenous antioxidant defences [51, 52].

Acute or chronic alcohol toxicity is mediated primarily via the generation of damaging free radical species in various tissues (muscle, liver, brain) [53, 54] . As protection becomes less efficient, ROS may damage critical biological molecules in the brain, such as proteins [55], cell membrane lipids [56] and nucleic acids [57]. Of all organs of the body, the CNS is particularly vulnerable to oxidative abuse because of its high content of polyunsaturated fatty acids (PUFAs) in the membranes and low levels of enzymatic and non-enzymatic antioxidant defences [57].

Alongside the neuroprotection exerted by endogenous antioxidant enzymes, micronutrients (vitamins and minerals) are particularly important to protect neurobiological structures associated with cognitive functions [58]. Vitamins play important roles in the human body, not only as antioxidants but also as essential enzymatic co-factors and gene regulators [14]. Water-soluble vitamins include B group vitamins (thiamine [B_1], riboflavin [B_2], nicotinamide [B_3], pantothenic acid [B_5], pyridoxine [B_6], biotin [B_8], folic acid [B_9], cyanocobalamin [B_{12}]) and ascorbic acid (vitamin C).

Thiamin

Thiamin (vitamin B_1) is extremely important for the brain because it facilitates the use of glucose, thus ensuring the production of energy. In rat, thiamine deficiency results in selective neuronal cell death in thalamic structures [59]. Moreover, it has been extensively demonstrated that this vitamin modulates

cognitive performance [60]. Particularly relevant is the role of thiamin deficiency in the aetiology of alcoholic brain disease and in Wernicke-Korsakoff syndrome [61, 62]. Riboflavin (vitamin B_2), niacin (vitamin B_3) and the folates (vitamin B_9) improve the level of abstract thought and lead to more favourable biochemical status [63].

Between depression due to thiamin deficiency and the excitation induced by deficiency of niacin, the appropriate balance can be found with the assistance of riboflavin, which ensures the harmonious use of the other two vitamins [64].

Pyridoxine

Pyridoxine (vitamin B_6) is required as an enzymatic co-factor for the absorption and metabolism of amino acids and neurotransmitters and is involved in the production of red blood cells [65]. It is rapidly taken up by circulating erythrocytes and converted into pyridoxal phosphate (PLP), the active form of pyridoxine. PLP acts as coenzymes in the biosynthesis of neurotransmitters GABA, dopamine and 5-HT [65]. Serotonin may be physiologically altered due to deficiency in decarboxylation of 5-hydroxytryptophan (5-HTP), the immediate precursor of 5-HT [66]. This deficiency may have an impact on functioning of NMDA receptors, important glutamatergic receptors involved in learning and memory.

Folic Acid

Folic acid (vitamin B_9) is essential for correct elaboration of the nervous system during foetal development [67]. In the elderly, deficiency decreases intellectual capacity and impairs memory [68]. Chronic alcoholism has long been known to adversely affect those vitamins involved in one-carbon metabolism, notably folates, pyridoxine and cyanocobalamin (vitamin B_{12}) [69–71]. Gloria and coworkers [72] reported that alcoholics PLP serum levels were lower in the study group than in the control, non-drinkers group. Red blood cell folates were also lower in the alcoholic group when compared with the control group [72]. In contrast, both folate and cyanocobalamin levels in serum were higher in the alcoholics group than in the control group; this inconsistency can be explained as poor retention of folate and cyanocobalamin by people suffering from chronic alcohol misuse [72].

Choline

Choline has long been recognised as playing an important role in alcohol-related brain damage [73]. Choline is an essential nutrient in humans and is an important methyl-group donor [74]. Its role in the body is rather complex. It is needed for neurotransmitter synthesis (acetylcholine), cell membrane signalling (phospholipids), lipid transport (lipoproteins) and methyl-group metabolism (homocysteine reduction) [75]. It is the major dietary source of methyl groups via the synthesis of S-adenosylmethionine (AdoMet) [76]. At least 50 AdoMet-dependent reactions have been identified in mammals, and it is likely that the number is much higher [76]. Such methylation reactions play major roles in biosynthesis of lipids, regulation of several metabolic pathways, and detoxification in the body [76].

It plays important roles in brain and memory development in the foetus and appears to decrease the risk of the development of neural tube defects [77]. One of the likely mechanisms for these effects of choline on foetal development is epigenetically mediated [78]. Choline is in fact a major source of

methyl groups [74], and methylation of DNA and histones are important components of the epigenetic code [79]. Thus, DNA methylation is altered by the availability of choline [79].

Choline is an essential nutrient that influences brain and behavioural development. Alcohol exposure disturbs the metabolism of choline and other methyl donors [73]. Studies on animals have shown that when pregnant rat dams, for example, are fed alcohol, their pups develop abnormalities characteristic of foetal alcohol spectrum disorders (FASD), but if these rat dams were also treated with choline, the effects from ethanol were attenuated in their pups [80].

Recent animal research indicates that prenatal choline supplementation leads to long-lasting cognitive enhancement, as well as changes in brain morphology, electrophysiology and neurochemistry.

In 2009, Thomas and collaborators [80] highlighted the importance of choline during the perinatal period in particular if pregnant women are actively drinking alcohol. Their results indicate that choline supplementation significantly attenuates ethanol's effects on birth and brain weight and most behavioural measures in rats born from ethanol-fed rat dams. In fact, behavioural performance of ethanol-exposed subjects treated with choline did not differ from that of controls [80]. These data indicate early dietary supplements may reduce the severity of some foetal alcohol effects, findings with important implications for children of women who drink alcohol during pregnancy.

Decreased choline availability to the foetus decreases hippocampal neurogenesis and increases apoptosis [81, 82]. Exposure of the foetus to alcohol also decreases hippocampal neurogenesis and decreases cell survival [83], resulting in reduced numbers of hippocampal pyramidal cells [84]. Though there are differences in the genes and tissues studied in both models, both choline deficiency and ethanol alter genes of cell cycling by altering DNA methylation of these genes [81, 85].

Choline, folate and methionine metabolism are highly interrelated, and these pathways intersect at the formation of methionine from homocysteine [74]. Acute ingestion of alcohol in humans lowers brain concentrations of choline as measured by magnetic resonance spectroscopy (the choline/creatinine ratios measured in such imaging likely measure a mixture of choline-containing compounds in brain) [86].

In alcoholic liver disease, methionine metabolism is impaired, and S-adenosylmethionine (formed from methionine) concentrations in liver are decreased [87]. S-adenosylmethionine is the methyl donor needed for methylation of DNA and histones. Alcohol exposure also diminishes the availability of methyltetrahydrofolate, thereby increasing the demand for choline. Diets of alcoholics are especially deficient in folate [11]. Very low dietary folate intake (<180 μg per day) was 2.5-fold more common among women who drank 30 g alcohol regularly [88]. Heavy alcohol users malabsorb folate [89] and increase the loss of folate in the urine through a reduction in renal tubular reabsorption [90].

Vitamin C

Ascorbic acid (vitamin C) is an important antioxidant, enzymatic co-factor and neuromodulator in the brain [91]. Its presence is required for the biotransformation of dopamine into noradrenaline. Moreover, the synthesis of catecholamines occurs in tissues rich in ascorbic acid like the brain and the adrenal glands [91]. In recent studies, ascorbate was found to buffer glutamate-generated ROS and limit consequent cell death in cultured neurons [92]. Additionally, ascorbate has been shown to be a neuromodulator of both dopamine- and glutamate-mediated neurotransmission, as reviewed in [93, 94]. Ascorbate is also an essential co-factor in the synthesis of many neuropeptides [95], and it promotes myelin formation by Schwann cells [96].

Zinc

Zinc ions play a major role in a plethora of normal brain functions, which include LTP and synaptic plasticity, cognitive functions, gene regulation and transcription and antioxidant response [97]. Consequently, zinc metabolism and homeostasis have been suggested to play a major role in many processes related to brain ageing and in the onset of age-related neurodegenerative diseases [97, 98]. Higher zinc concentrations are found in grey than in white matter, and the highest ones are present in the hippocampus, amygdala and neocortex [99], which are regions involved in higher cognitive functions. The zinc homeostasis is maintained dynamically, by increasing zinc uptake when in presence of low zinc concentrations in the blood and decreasing it when high blood zinc concentrations are present [100]. A critical zinc depletion induces apoptosis due to increased oxidative damage and activity of pro-apoptotic enzymes (i.e. zinc inhibits their activities) [101]. On the other hand, an excess of zinc also causes apoptosis [102], in particular in the hippocampus [103].

Zinc acts as a neuromodulator at excitatory synapses and has a considerable role in the response to stress and in functionality of zinc-related proteins contributing, as such, to maintain brain compensatory capacity [104].

Zinc has been described to modulate a number of neurotransmitter systems, mainly glutamate receptors [99] and GABA synaptic transmission [105]. Interestingly, the release of zinc with glutamate reduces the ability of the latter to activate post-synaptic NMDA receptors [97, 99, 106]. Glutamate neurotransmission plays a very important role in memory formation.

In conclusion, zinc plays a key role in NMDA-receptor regulatory process; indeed, zinc can be considered to counterbalance the actions of excitotoxins like QA and alcohol, by modulating glutamate-induced NMDA receptor excitability. On the other hand, zinc deficiency may synergistically act with neurotoxins to cause neuronal death by enhancing excitotoxicity damage brought by an excess of glutamate.

Thiamine, together with the other vitamins B, A, C, E and zinc, forms a complex network of exogenous (diet-derived) antioxidant protection, which has been demonstrated to be essential in preventing age-related and alcohol-caused neurodegeneration, by acting in close collaboration with our endogenous antioxidant protection systems, such as the GSH system [42, 51].

In summary, all vitamins, water- and lipid-soluble ones, and minerals are important in maintaining the correct functioning of the brain, but extensive research has been conducted specifically on the role of thiamine deficiency, and other B vitamins, in relation to long-term alcohol consumption. Vitamin B1 (thiamine) plays a central role in preventing the development of Wernicke-Korsakoff psychosis, a neurodegenerative disorder affecting mainly alcoholics [14, 61, 107]. Generally, the vitamin B complex is essential for the overall cerebral cognitive performance as a lack of pyridoxine (vitamin B6) and riboflavin (vitamin B2) overloads the γ-amino butyric acid (GABA) shunt, thus resulting in an excess of glutamate production and neuronal death caused by a glutamate overexcitement (excitotoxicity) [14].

Conclusion

Social class differences in health are seen across the entire lifespan, with lower socio-economic groups having greater incidence of premature and low birth weight babies, cardiovascular disease, stroke and some cancers in adults. Risk factors include lack of breast-feeding, smoking, physical inactivity, obesity and hypertension. Harmful drinking and poor diet are clustered in the lower socio-economic groups. The diet of the lower socio-economic groups provides cheap energy from foods such as meat products, full cream milk, fats, sugars, preserves, potatoes and cereals but has little intake of vegetables, fruit and whole wheat bread. This type of diet is lower in essential nutrients such as calcium,

iron, magnesium, folate and vitamin C than that of the higher socio-economic groups. New nutritional knowledge on the protective role of antioxidants and other dietary factors suggests that there is scope for enormous health gain if a diet rich in vegetables, fruit, unrefined cereal, fish and small quantities of quality vegetable oils could be more accessible to poor people.

Even within the lower socio-economic groups, there is a clear consumption gradient. Families that have their food spending power very restricted, such as around 20% of income support claimants who face compulsory rent and/or fuel deduction, have nutrient intake far below the reference nutrient intake for iron, calcium, dietary fibre, folate and vitamin C; it is even lower for smokers [108].

Households that contain at least one heavy drinker are likely to have a reduced amount of money to spend on good quality food and also are more vulnerable to a range of health and social problems.

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Chapter 9
The Effect of Diet on Protein Modification by Ethanol Metabolites

Simon Worrall

Key Points

- Ethanol metabolism generates reactive substances either directly through the enzymes carrying out the reactions or indirectly through free radical damage to unsaturated fatty acids.
- Reactive substances generated during ethanol oxidation can react with cellular components such as proteins to generate unstable and stable modifications (adducts).
- Modification can alter the functionality of proteins and/or make it a neoantigen to become a potential target for immune attack.
- Modified proteins and antibodies reactive against them are found in animals fed ethanol and in human alcoholics.
- Diet is an important facet of some forms of alcohol-related injury, particularly to the liver. In liver injury, unsaturated fats promote liver injury and saturated fats are protective.
- Limited research on the interactions between dietary components and adduct formation has been carried out. However, α-tocopherol supplementation to ethanol-fed rats decreased the generation of hepatic adducts.

Keywords Alcoholic liver disease • Alcoholic myopathy • Alcoholic cardiomyopathy • Alcoholic brain injury • Alcoholic cerebellar degeneration • Acetaldehyde • Malondialdehyde • 4-hydroxy-2-nonenal • α-hydroxyethyl radicals • Lipid peroxidation • Modified proteins • Adducts • Neoantigens • Antibodies • Immune response • Dietary components • Unsaturated fat • Saturated fat • α-tocopherol

Introduction

Alcohol (ethanol) is the most widely abused drug in Western societies and, as such, is a major cause of morbidity and mortality, leading to major social and economic costs. Despite an intensive research effort over many years, the main mechanisms by which alcohol exerts its toxicity remain largely elusive or unclear. This seems strange for such a simple molecule which has been associated with disease since at least Roman times. However, what is becoming clear is that alcohol-related tissue injury and

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disease is clearly multifactorial in nature, with some damage by direct toxicity while other damage occurs through indirect mechanisms. Further, it appears that at least some individuals appear to be genetically predisposed to injury, particularly to the liver, that gender can play a role, and that dietary components can influence the severity of the injury. The main tissues affected by long-term alcohol abuse include the liver, brain, skeletal muscle, cardiac muscle and the pancreas.

Many different pathological processes have been implicated in the aetiology of alcohol-related injury to various tissues and organs. One mechanism, for which there is a growing body of evidence, is the modification of cellular macromolecules such as proteins by reactive substances produced during the oxidative metabolism of ethanol. This chapter will focus on the modification of proteins in alcohol-affected tissues and show that dietary components such as unsaturated fatty acids are important in liver injury and that antioxidants may help to determine the amount and types of modifications produced.

Alcohol Abuse Is Associated with Cell and Tissue Injury

Chronic alcohol abuse results in injury to the brain, liver, skeletal and cardiac muscle and the pancreas. Long-term alcohol abuse can lead to brain damage with accompanying cognitive and motor deficits. In vivo imaging techniques have shown ventricular enlargement and brain shrinkage, particularly of the white matter, occur in human alcoholics. Animal models of chronic abuse have shown that injury to several brain areas, especially the hippocampus and cerebellum, occurs [1]. The damage observed in animals includes a loss of neurons and a reduction in dendritic spines and branches [2–4]. Long-term chronic alcohol administration to animals also decreases long-term potentiation [5], a process thought to be involved in learning and memory formation, and may be responsible for some of the cognitive deficits seen in alcoholics. Although the nature and location of the toxic effects of alcohol on the brain have now been well described, the pathologic mechanisms leading to the damage are still to be delineated [6–8]. However, neuroscience research has shown that several mechanisms including oxidative stress, free radical formation and excitotoxicity may underlie alcohol brain injury.

Alcohol-induced liver injury can be divided into three main stages based on histological observations [9]. Initially, alcohol abuse leads to the formation of alcoholic steatosis, a relatively benign state in which hepatocytes accumulate intracellular lipid droplets. This can be wholly explained by perturbation of normal fat and other metabolism by NADH produced during the oxidative metabolism of ethanol. If drinking ceases, the fat droplets disappear as normal metabolism reasserts its effects on the cells. However, continued heavy drinking results in the formation of centrilobular (zone 3) foci of necrotic and ballooning hepatocytes with an associated characteristic neutrophil infiltrate, together with the formation of intracellular keratin-containing Mallory bodies. These pathological observations define alcoholic hepatitis, a state thought of as the transition between reversible and irreversible liver injury. Cessation of drinking in alcoholic hepatitis generally results in full recovery, whereas continued drinking leads to the transition to alcoholic cirrhosis. The cirrhotic state is characterised by small regenerating nodules of liver cells which are surrounded by regions of fibrous tissue. Alcoholic hepatitis is often superimposed on alcoholic cirrhosis and is indicative of continued heavy drinking. Despite an intensive research effort over many years, the mechanisms responsible for the progression from reversible to irreversible liver injury are still not fully understood. However, there is now a growing body of evidence that genetic factors, nutrition and an aberrant immune response all have potential roles in this progression [10].

Excessive alcohol intake also results in damage to skeletal and cardiac muscle. Skeletal muscle myopathy is characterised by atrophy of type II fibres, whereas the type I fibres are relatively spared, only being affected in the most severe cases. Alcoholic myopathy can result in the loss of 20–30% of the musculature, leading to difficulties in gait and frequent falls [11]. The incidence of alcoholic

myopathy is often under-reported, but it seems likely that up to 65% of alcoholics may suffer from this form of muscular injury [12]. The exact mechanisms involved in the aetiology of the injury are not well understood, but malnutrition, altered muscle protein synthesis and breakdown [13–15], free radical damage [16] and concomitant liver disease may all play a role [17].

Similar damage can also occur in heart muscle leading to alcoholic cardiomyopathy. The pathology of this form of heart injury has been well characterised and consists of fibrosis, increased deposition of lipid and inflammatory changes. Mitochondrial and sacrolemmal changes are also observed together with changes in the architecture of myofibrils [18] including variable size, loss of cross striations, vacuolisation and oedema [19, 20]. These changes result in diastolic dysfunction, atrial fibrillation, myofibrillary disarray and altered cardiac enzyme activities [21–23]. Furthermore, acetaldehyde has been shown to have a direct depressive effect on cardiac contractile function [24]. Despite these observations, the aetiology of alcoholic cardiomyopathy is still unclear.

How Alcohol Metabolism Produces Reactive Metabolites

The primary route of ethanol metabolism in humans is through enzyme-mediated oxidation (Fig. 9.1) in the liver. About 90% of the imbibed ethanol is metabolised by this route at a rate of 10–15 g/h [25], a small amount is metabolised by extrahepatic tissues, and the remainder is excreted unchanged in exhaled air and in urine.

The hepatic enzymes involved in ethanol metabolism have been studied in the greatest detail. Hepatocytes, which account for about 85% of the mass of the liver, contain two main alcohol oxidising systems. One is located in the cytosol and involves alcohol dehydrogenase [26–28] (Fig. 9.2). The alcohol dehydrogenases are a widespread large family of enzymes which have varying affinities for ethanol [29].

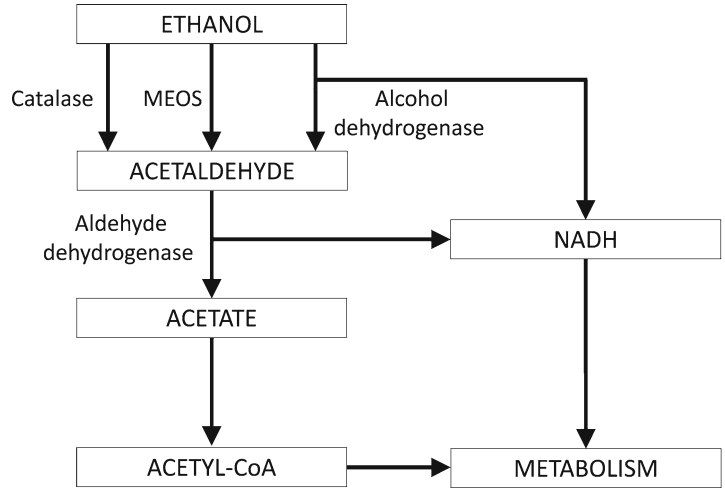

Fig. 9.1 *Potential pathways for the oxidative metabolism of ethanol*. There are several ways in which the oxidative metabolism of ethanol can occur. These pathways can be the only one operating in a cell or may operate in concert with others. For example, in the liver, alcohol dehydrogenase operates at low blood alcohol concentrations, but as the concentration increases, the microsomal ethanol oxidising system (*MEOS*) becomes induced and predominates. However, in the brain, alcohol dehydrogenase and MEOS are minor contributors to ethanol oxidation which is carried out by catalase, an enzyme which has no role in the liver

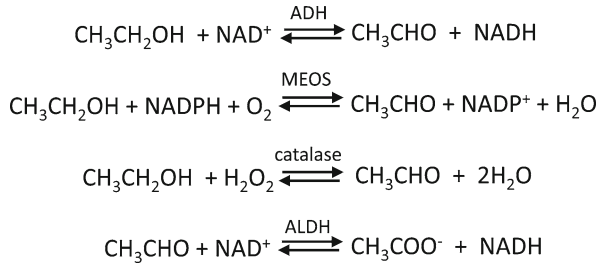

Fig. 9.2 *Reactions of the main enzymes involved in ethanol and acetaldehyde oxidation*. Ethanol can be oxidised to acetaldehyde via the actions of alcohol dehydrogenase (*ADH*), the microsomal ethanol oxidising system (*MEOS*) or catalase depending on the tissue in which it occurs. The acetaldehyde produced by these enzymes is oxidised by aldehyde dehydrogenases (ALDH) to acetate which can then enter metabolism as acetyl-CoA

The main forms in the liver are referred to as class I and class II alcohol dehydrogenases, with the class I form carrying out the majority of ethanol oxidation at low blood alcohol concentrations. Alcohol dehydrogenases are not inducible and oxidise ethanol to acetaldehyde (ethanal) with the concomitant reduction to NAD^+ to NADH. Continued ethanol metabolism results in a change in the hepatic redox state (NADH/NAD^+), leading to dramatic changes in intermediary metabolism by inhibiting pathways that require NAD^+ [30]. The other main system for oxidising ethanol is found in the smooth endoplasmic reticulum, is based around the enzyme cytochrome P450 2E1 (CYP2E1) and is known as the ***m***icrosomal ***e***thanol ***o***xidising ***s***ystem (MEOS; Fig 9.2) [31–34]. This enzyme uses molecular oxygen and the reducing agent NADPH to oxidise ethanol to form acetaldehyde. This system has a lower affinity for ethanol than alcohol dehydrogenase but is inducible, only becoming important after several weeks of heavy drinking when it can account for up to 70% of ethanol metabolism. As well as producing acetaldehyde, there are several other reactive species such as α-hydroxyethyl and hydroxyl radicals that can be formed due to the enzyme's "leaky" catalytic cycle. Ironically, the metabolism of ethanol, a relatively unreactive compound, produces the much more reactive compound acetaldehyde which must be further metabolised before a less toxic, unreactive metabolite is formed.

Acetaldehyde, produced by the action of alcohol dehydrogenase and MEOS, is further oxidised by aldehyde dehydrogenases [26, 35–37] located in the cytosol and mitochondria to generate acetic acid which can enter intermediary metabolism as acetyl-CoA (Fig. 9.2). These enzymes are also non-inducible such that acetaldehyde can accumulate at concentrations up to 1 mM in cells undergoing chronic ethanol oxidation [38]. Acetaldehyde and acetic acid are the main metabolites produced by the direct action of enzymes during ethanol oxidation. Acetaldehyde can react with metabolic intermediates such as dihydroxyacetone phosphate to generate 5-deoxyxylulose-1-phosphate, another reactive species. Furthermore, the production of small amounts of free radical radicals can also lead to the production of other reactive species through reactions with cellular components. The main target for the free radicals is the double bonds in the hydrocarbon chain of unsaturated fatty acids [39], leading to the production of lipid hydroperoxides which spontaneously break down to generate a series of reactive aldehydes including malondialdehyde and 4-hydroxy-2-nonenal. These compounds not produced by the direct action of the enzymes involved in ethanol oxidation can be thought of as indirect metabolites of ethanol metabolism.

In comparison to the liver, the brain has a relatively poor metabolic capacity for ethanol oxidation, being able to oxidise ethanol at an estimated rate of 1/1,000–4,000th that of the liver at physiological pH [40, 41]. In rats, the ability of various tissues to metabolise ethanol decreases in the following order: liver, intestine, heart, spleen, brain and skeletal muscle [42]. Analysis of alcohol dehydrogenase activity in various regions of bovine brain indicated that the distribution is highly variable, with the

highest in the cerebellum, followed by the white matter of the cerebral hemispheres, the grey matter and the lowest in the subcortex [41]. In human brain, the only form present in significant amounts is class III alcohol dehydrogenase [43], which has a low affinity for ethanol and is unlikely to play a major role in ethanol metabolism. This enzyme is widely distributed in the brain including the cortex, subcortex and cerebellum but is only expressed in a small number of cells in each region [44]. There does not seem to be any class I alcohol dehydrogenase in human brain.

There are other enzymes capable of metabolising ethanol in brain tissue. In particular, it is known that the brain contains cytochrome P450 enzymes [45]; albeit in very small amounts. The form responsible for major metabolism in the liver, CYP2E1, is found in glial cells, nerve cell bodies, terminals and fibres, but maximal activity is found in the pyramidal neurons of the frontal cortex and hippocampus, in the neuronal cell bodies and neuropile of the striatum and in neurons of the substantia nigra, several nuclei, the central grey substance and the reticular formation [46]. However, it is another enzyme, catalase [47] (Fig. 9.2), which does not play a role in ethanol oxidation in other tissues that is the major enzyme involved in metabolism in the brain. In the brain, catalase can oxidise ethanol in a H_2O_2-dependent manner to generate acetaldehyde [48]. The enzyme is localised in small cellular organelles called microperoxisomes [49] and is found throughout the cerebellum, medulla and cerebrum of rats [50]. In small regions of these parts of the brain, the difference in activity between the most and least active areas was only twofold [51]. However, much greater heterogeneity between cell types and microregions is seen using histochemical techniques [52]. The human brain also contains multiple types of aldehyde dehydrogenase [53, 54].

In other extrahepatic tissues, the metabolism of ethanol is less well understood. There is some evidence that cardiac muscle expresses alcohol dehydrogenase [55], but it is at a very low level when compared with the liver. It is also thought that catalase may play a major role in cardiac ethanol oxidation [56]. Another study has shown that CYP2E1 mRNA could be detected in all regions of the human heart and major vessels, whereas other CYP mRNA was more regionally expressed [57]. Less is known about ethanol metabolism in skeletal muscle, but CYP-mediated oxidation does appear to occur in the sarcoplasmic reticulum [58].

Ethanol Metabolites React with Cellular Components

Metabolites produced directly, or indirectly, during the oxidation of ethanol can react with macromolecules both in vitro and in vivo to produce covalent modifications (Fig. 9.3). These reactions can occur with any of the major types of macromolecules including nucleic acids, carbohydrates, lipids and proteins. The best understood set of reactions is those between the metabolites and proteins, and they will be the focus of this section.

The electrophilic nature of the carbonyl group of acetaldehyde makes it able to react with nucleophilic groups in proteins [59]. The main targets are the α-amino group of the N-terminal residue in polypeptides or the ε-amino group on the side chain of internal lysine residues. These groups readily react with acetaldehyde to initially form Schiff bases [60, 61], unstable adducts, that either break down to regenerate a free amino group and acetaldehyde or are stabilised through a variety of mechanisms to generate stable adducts. In theory, acetaldehyde could react with any amino group in a protein, but this is unlikely because some amino groups appear to be more reactive than others. Indeed, in vitro incubation of proteins with supraphysiological concentrations of acetaldehyde only modifies about half of the available amino groups. This is probably because their local environment makes them more reactive and because they are favourably exposed to the environment. If the Schiff base forms on the α-amino group of the N-terminal residue, then stabilisation can occur through reduction to generate an ethylated amino group or by cyclisation to produce a 2-methylimidazolidin-2-one derivative [62, 63]. However, if the Schiff base forms on the ε-amino group of a lysine residue, then stabilisation can occur

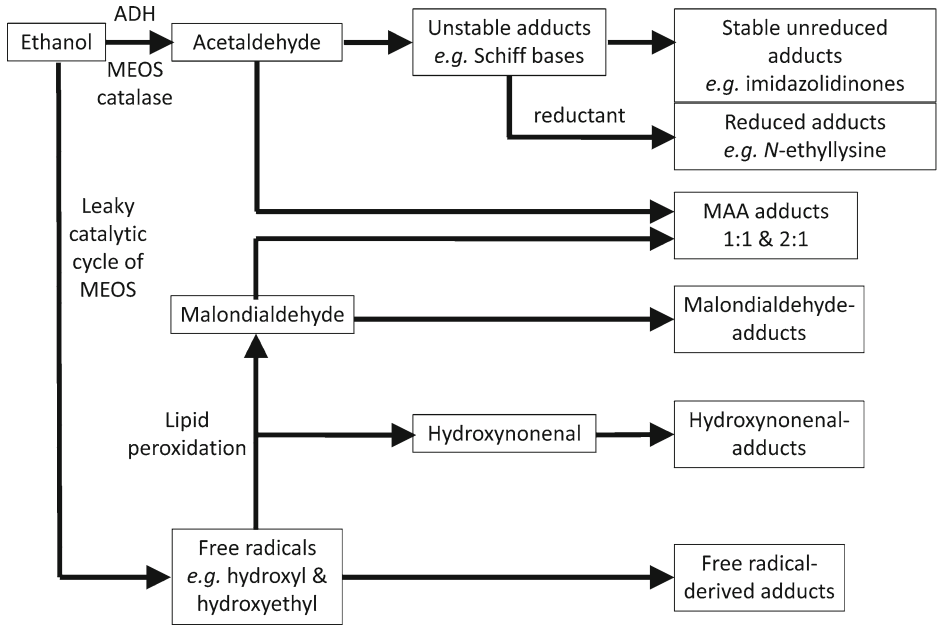

Fig. 9.3 *Formation of modified proteins by reactive metabolites formed during ethanol oxidation*. The metabolism of ethanol generates reactive metabolites, either directly or indirectly, through the production of free radicals. These reactive metabolites can interact with proteins to generate many different types of modification

through addition across the double bond, either through reduction or nucleophilic addition by a thiol group. Originally, it was thought that reduction was the most likely form of stabilisation to occur in chronic ethanol oxidation in vivo. However, this is now being questioned [64, 65]. The adducts formed in vitro in the absence of strong reducing agents have been shown to be both chemically [61] and immunologically [64] different to the ethylated amino groups formed in their presence. Proteins lacking free thiol groups together with polylysine have been shown to form large number of adducts when incubated with acetaldehyde in vitro in the absence of reducing agents [61].

The reaction of acetaldehyde with thiol-containing amino acids and peptides has been studied in vitro at physiological temperature and pH. Analysis of the products formed when acetaldehyde reacts with free cysteine or peptides with an N-terminal cysteine residue showed that a cyclic thiazolidine derivative was rapidly formed [66]. However, when acetaldehyde was incubated with peptides containing an internal cysteine residue, analysis by NMR showed that the expected hemimercaptal residue either did not form or was not stable under the experimental conditions [66].

Acetaldehyde can condense with the glycolytic intermediate dihydroxyacetone phosphate in a reaction mediated by the enzyme aldolase to form the sugar 5-deoxyxylulose-1-phosphate [67]. This compound has been shown to react with haemoglobin in vitro to produce stable modifications [68] but little else is known about its reactivity. These modifications probably form in a similar manner to those formed by glucose through non-enzymatic glycation. Initially, the sugar forms a Schiff base on an amino group which then undergoes Amadori rearrangement to form a ketoamine product. The adduct formed contains an α-hydroxyketone group which can then react with another amino group through the same reactions. If the second amino group is on a different peptide, then cross-linking can result.

The reactivity of α-hydroxyethyl radicals with amino acids, peptides or proteins has not yet been studied. However, since these radicals are extremely reactive, it is expected that they will react with numerous sites on proteins and other macromolecules.

Malondialdehyde is formed in large amounts during chronic ethanol oxidation as a result of the breakdown of lipid peroxides formed by free radical attack on unsaturated fatty acids present in membranes. It is also formed during the oxidative stress generated by many other agents, and because of this, its reactivity with cellular components has been intensively studied. Malondialdehyde is generally considered to be a highly reactive compound, but it actually exists mainly as an enolate anion, which has low reactivity, in aqueous solution at physiological pH [39]. As the pH drops, the β-hydroxyacrolein form, which has much higher reactivity, predominates.

This form of malondialdehyde can undergo Michael type 1,4-addition in a similar manner to other α, β-unsaturated aldehydes such as 4-hydroxy-2-nonenal which is also formed through the breakdown of lipid peroxides. At low pH, this type of reaction is favoured through resonance stabilisation, generating a β-substituted acrolein derivative [69] (a 1:1 adduct). These adducts can then react at low pH with another amino acid through their carbon-carbon double bond to give a 2:1 adduct. One study on the reactivity of malondialdehyde with amino acids at pH 4.2 showed that histidine, tyrosine, tryptophan and arginine reacted extensively through their α-amino groups to give 1:1 adducts [70]. Formation of the 2:1 adduct was not observed under these conditions even when the amino groups were present in large excess. When cysteine reacted with malondialdehyde under these conditions, a derivative containing two molecules of cysteine and three molecules of malondialdehyde was formed. Neutral pH did not favour reactivity with amino groups but did allow reactions with thiols to occur [39]. Thus, derivatives of thiols would be expected to be the major adducts formed in vivo.

The reaction of malondialdehyde with proteins at neutral pH cannot be totally predicted using the data gleaned from studies using amino acids. For example, malondialdehyde did not react with glutathione (a thiol-containing peptide) but reacted extensively with bovine serum albumin under the same conditions. Proteins appear to be much reactive than amino acids at neutral pH. It has been suggested that this is because proteins present amino acids in more favourable environments, making them more reactive in a peptide than they are as single amino acids in solution. It has also been proposed that it is the condensation products of malondialdehyde rather than malondialdehyde per se that are responsible for adduct formation. Reaction of malondialdehyde with polylysine resulted in the formation of three different derivatives of ε-amino groups [71]. Approximately 20% were unstable aminopropenal derivatives, around 1% were dihydropyridine derivatives, and the remainder were stable cross-linked forms based on amino-imino-propen derivatives. A similar distribution of adducts was seen when bovine serum albumin was reacted with malondialdehyde at neutral pH, with about 40% of the total ε-amino groups in the protein being modified. Another study suggest that histidine, tyrosine, arginine and methionine residues were also modified but to a much lesser extent [72].

4-Hydroxyalkenals such as 4-hydroxy-2-nonenal have three functional groups, namely, an aldehyde group, a hydroxyl group and a carbon-carbon double bond. These three functional groups can react alone or in sequence with other species. This makes the reactivity of hydroxynonenal much more complicated [39] than any of the other metabolites discussed in this section. The addition of hydroxynonenal to cells or tissues results in a rapid loss of thiol groups, suggesting that they are the initial targets of this family of molecules. The product formed is a saturated aldehyde covalently bonded to the thiol-containing target via a thioether linkage at carbon-3. This can then undergo rearrangement to generate a five-membered cyclic hemiacetal derivative. If excess thiol is present, the initial adduct can react with a second thiol to produce a thiazolidine derivative [39].

Hydroxynonenal is known to react with a variety of amino acids in proteins. For example, when 5 mM hydroxynonenal was incubated with human apolipoprotein B, a series of residues were modified: 2 cysteines, 45 lysines, 23 serines, 7 histidines and 51 tyrosines [73]. The binding to the lysine residues was reversible, suggesting that unstable Schiff bases were formed. These Schiff bases can be stabilised by loss of water to become pyrrole derivatives, but this is unlikely to be a major stabilisation reaction. Hydroxynonenal can also react with amino groups by nucleophilic Michael addition of the amino group to the carbon-carbon double bond. This derivative can then lose water to become a stable cyclic hemiacetal.

The binding of acetaldehyde to proteins is remarkably increased when incubated in the presence of malondialdehyde [74]. It is now clear that acetaldehyde reacts in concert with malondialdehyde to produce at least two different adducts, one of which is fluorescent. These have been termed malondialdehyde-acetaldehyde adducts (MAA) and have been identified as 2-formyl-3-(alkylamino) butanal (MAA 1:1; nonfluorescent and derived from one molecule of malondialdehyde and one molecule of acetaldehyde) and 4-methyl-1,4-dihydropyridine-3,5-dicarbaldehyde (MAA 2:1; fluorescent and derived from two molecules of malondialdehyde and one molecule of acetaldehyde) derivatives of protein amino groups [75]. It now appears that MAA 1:1 adducts are the initial products formed which then react with malondialdehyde-derived Schiff bases to generate the MAA 2:1 adduct [76].

Ethanol Oxidation-Derived Metabolites Form Adducts In Vivo

The previous section shows that direct and indirect metabolites of ethanol oxidation can react with proteins in vitro to form both stable and unstable modifications (Fig. 9.3). This data has been useful in giving insights into the likely adducts formed in vivo and in the generation of reagents for their detection in biological samples.

Initial studies on ethanol metabolism-derived modification concentrated on the liver, the principal site of metabolism in humans (~90% of total body metabolism). Thus, it is the tissue which will contain the highest concentrations of metabolites and therefore the highest concentration of modified macromolecules including proteins.

Early studies using cell-free homogenates [60] and liver slices [77] showed that acetaldehyde generated during ethanol oxidation reacted with cellular proteins to generate several types of acetaldehyde-protein adduct. From these experiments, it could be inferred that the acetaldehyde initially formed unstable adducts, most likely Schiff bases, which underwent stabilisation over time to generate stable adducts. More conclusive evidence for adduct formation came when polyclonal antisera were generated against proteins modified by acetaldehyde in vitro. These antisera were initially used to detect modified proteins in liver from rodents fed ethanol. However, the number and identity of the proteins modified varied between the reports. Two studies using Western blotting showed that single proteins were modified, namely, CYP2E1 [78] and a 37-kDa cytosolic protein [79–82] later identified as Δ^4-3-ketosteroid-5-reductase [83]. In contrast, two other studies identified multiple proteins including the 37-kDa cytosolic protein seen by Lin and colleagues [84, 85]. Later studies using ELISAs demonstrated that cytosolic α-tubulin, glyceraldehyde-3-phosphate and calmodulin all carried acetaldehyde-derived adducts [86] and that adducts could also be detected in mitochondrial and membrane fractions [86].

Other studies using immunohistochemistry showed the presence of acetaldehyde adducts in the cytosol of hepatocytes in liver tissue from alcoholics [87], with a greater level of modification being observed in the centrilobular regions (zone 3) of the liver [88, 89]. This region of the liver has the highest capacity for ethanol oxidation and is the region most damaged in alcoholic liver disease. Other studies using liver tissue from ethanol-fed rats showed a similar centrilobular adduct distribution [90].

Adducts formed by the other metabolites have not been studied in as much detail. Hydroxyethyl radicals have been shown to be generated by CYP2E1 [91–93] and to react with microsomal and other proteins. Recently, proteins modified by these radicals have been detected in the liver and other tissues of ethanol-fed rats [65, 94] and human alcoholics [95]. Malondialdehyde and hydroxynonenal are the major end products of free radical attack on unsaturated fatty acids. An increased concentration of malondialdehyde has been detected in samples from human alcoholics drinking about 100 g of ethanol per day, with elevated concentrations persisting for several weeks after the cessation of drinking [96]. Malondialdehyde-modified proteins have been detected in vivo under a wide variety of conditions. For example, rats with iron overload exhibited elevated levels of malondialdehyde- and hydroxynonenal-derived adducts in their

plasma and hepatic cytosol [97]. In animal models of alcoholic liver disease, several studies have shown that malondialdehyde- and hydroxynonenal-derived adducts are associated with areas of inflammation and necrosis [98–101] and with iron deposits probably as markers of oxidative stress [102].

Two forms of MAA adduct have between shown to form in vitro, but only the MAA 2:1 adduct has been detected in vivo in the liver and other tissues of ethanol-fed rats [65, 74, 103]. There is some indirect evidence that the 1:1 adduct was also formed. In vitro studies have shown that the 2:1 adduct could be formed by reacting the 1:1 adduct with excess malondialdehyde. When ethanol-fed rat liver was perfused in situ with malondialdehyde, the amount of MAA 2:1 adduct was found to increase, indicating that the 1:1 was also probably present [76]. No increase in MAA 2:1 adduct content was found after similar treatment of control animals.

Evidence is also mounting that modification of proteins by ethanol metabolites occurs in other tissues as well as the liver. Ethanol metabolite-modified proteins have been detected in skeletal [104] and cardiac muscle [105, 106] of rats fed ethanol as the Lieber-DeCarli diet for 6 weeks. This feeding regime produces pathological changes in heart muscle similar to those seen in human alcoholic cardiomyopathy including a decrease in contractile protein content. Ventricular muscle from the ethanol-fed animals showed increased generation of unreduced- and reduced-acetaldehyde adducts and MAA 2:1 adducts. No increase in the formation of other types of adduct was seen, including those derived from malondialdehyde or hydroxyethyl radicals. The same feeding regime also showed an elevation in unreduced-acetaldehyde adducts when compared to pair-fed controls. Immunohistochemical analysis showed that the sarcolemmal and subsarcolemmal regions were the most heavily modified, probably due to acetaldehyde production occurring in, or close to, these regions. The levels of modification were similar in plantaris (type II fibre-predominant) and soleus (type I fibre-predominant) muscles despite only the plantaris being affected by ethanol feeding [104]. This study did not identify the targets of modification, and it is possible that different sets of proteins are modified in each muscle. It is not clear whether these data implicate acetaldehyde in the aetiology of alcoholic skeletal myopathy.

There is also evidence that similar protein modification occurs in the brain. Rats fed ethanol for up to 2 years were tested for acetaldehyde adducts in their liver and brain. The majority of animals exhibited elevated levels of adducts in their liver, and around half of the animals also exhibited adducts in their brain [107]. Control animals had no such modifications in their tissues. These adducts were located in some of the large neurones of layers 4 and 5 of the frontal cortex and in the molecular layer of the cerebellum [108]. Mice fed ethanol were shown using immunohistochemistry to contain acetaldehyde-modified proteins in their cerebral cortex. Similar modification was also seen in rats fed ethanol for 12 months, with adducts being found in cortical neurones, the molecular layer of the dentate gyrus, neurons in the midbrain and in the granular cell layers of the cerebellum [109]. Unlike the liver, where the site of greatest modification was the cytosol, modification in the brain was often confined to the mitochondria. More recently, elevated levels of acetaldehyde-derived adducts have been detected in *post-mortem* tissue from alcoholics [110] and in cerebellar tissue from individuals suffering from alcoholic cerebellar degeneration [111], implicating acetaldehyde in the aetiology of this condition.

Modification Can Have Negative Consequences

Protein modification can lead to two major consequences which can occur alone or in concert. Modification can alter the functionality of a protein [112–115], either totally robbing it of its activity and/or alter its immunogenicity such that it becomes a *neoantigen* and a target for immune attack. Evidence is accumulating for both of these effects occurring in alcohol-fed animals and alcoholics.

One of the most important examples of acetaldehyde affecting protein function is its affect on microtubular function. Several studies have shown that the incubation with acetaldehyde with microtubular proteins in vitro leads to a decrease in microtubule formation [116, 117] and that modification of as little as 5% of the α-tubulin monomers could result in complete inhibition of polymerisation [118, 119]. It is interesting to note that thiol groups have been implicated in the polymerisation of tubulin monomers into microtubules [120]. Given their reactivity with acetaldehyde, it is possible that modification of these thiol groups may be involved in the inhibition of polymerisation. Acetaldehyde is also known to react with a highly reactive lysine residue in α-tubulin which is only accessible in the monomeric form. It is believed that this residue may also be important in polymerisation.

Two important symptoms of alcoholic liver damage are liver enlargement and the accumulation of lipids. Initially, the liver enlargement was assumed to be due to the accumulation of fat, but later studies suggest that it accounts for only about half of the increase in weight, with the remainder due to an increase in the protein content of liver cells [121]. This increase in protein content is probably due to impaired microtubule-mediated protein secretion [122, 123]. In hepatocytes from ethanol-fed animals [122] and alcoholics [124], and hepatocytes treated with ethanol in vitro, the number and size of microtubules was shown to be decreased. A concomitant increase in tubulin monomer concentration was also seen. The consequences of disrupted microtubular function can also be seen in the accumulation of secretory vesicles and disrupted protein trafficking. This is reflected in the increased amounts of transferrin, a protein normally secreted into plasma, seen in hepatocytes from alcoholics with liver damage [123]. This accumulation is not seen in patients with non-alcoholic liver disease. Pulse-chase techniques showed that ethanol blocked the exit of proteins from the liver by altering their processing in the Golgi complex or later parts of the secretory pathway [117, 125–127].

Disruption of protein trafficking can also have more subtle effects on liver cell function. The trafficking of vesicles is responsible, at least partly, for the delivery of enzymes, transporters, receptors and structural and cell recognition proteins. Thus, alterations in protein trafficking could alter the composition of the plasma membrane, potentially leading to widespread alterations in cellular metabolism and functionality. For example, receptor-mediated endocytosis is particularly deranged in the centrilobular regions of the liver, the region of the liver most damaged by alcohol abuse [128]. The plasma membrane of ethanol-affected hepatocytes appears very different under electron microscopy to that of untreated ones. The ethanol-affected plasma membrane is more labile, leading to the leakage of the enzyme alkaline phosphatase [129, 130]. Acetaldehyde has also been shown to completely inhibit many membrane-bound enzymes including 5′-nucleotidase, Na^+/K^+ ATPase and Mg^{2+} ATPase at high concentrations in vitro [131]. Whether this inhibition occurs in vivo is unclear.

Some other proteins that accumulate in the ethanol-affected liver probably reflect the metabolic changes caused by chronic ethanol metabolism. For example, there is a large increase in the concentration of fatty acid-binding protein in ethanol-affected hepatocytes such that it accounts for up 33% of the total protein content of these cells [132]. This accumulation may protect the cells against the toxic, detergent-like effects of nonesterified fatty acids.

There is also extensive evidence that proteins modified by reactive metabolites such as acetaldehyde are immunogenic, acting as *neoantigens* to illicit immune responses against the modification and the parent protein. The delineation of the immune response has largely been confined to the detection and measurement of antibodies generated against the adducts. However, there are two reports of a cellular response against acetaldehyde-modified proteins [133, 134].

In 1986, a seminal paper by Israel and co-workers [135] demonstrated the production of antibodies reactive with acetaldehyde-derived epitopes in mice chronically fed ethanol. Later, a study using rats showed that the magnitude of the response was related to the length of ethanol-feeding and probably to the cumulative ethanol load [136]. Another study showed that antibodies were generated against at least two broad types of adduct, namely reduced and unreduced acetaldehyde adducts [137]. Similar studies using rodents have now shown the generation of antibodies reactive with

hydroxyethyl radical- [65, 93, 138], hydroxynonenal- and malondialdehyde-derived epitopes and MAA 2:1 adducts [65, 139].

Many studies have also shown that a similar immune response to ethanol metabolite-modified proteins occurs in humans. Initial studies concentrated on adducts derived from acetaldehyde and showed that antibodies were generated against these epitopes [140–143]. This established that these modifications must be present in humans. It was only later that their presence was directly demonstrated. The initial studies used ELISAs to measure plasma or serum reactivity with proteins modified by high concentrations of acetaldehyde under reducing conditions in vitro. However, the conditions used to modify the proteins, together with the protein used, varied widely between studies, probably resulting in the production of different populations of adducts and hence the detection of different immunoreactivities. In the experiments using rodents described above, there was a clear difference between the responses seen in ethanol-fed animals when compared to those of the controls. In studies using human samples, the picture was not as clear as social drinkers (people imbibing <50 g ethanol per day for males and <30 g per day for females), patients with non-alcoholic liver disease and alcoholics all exhibited responses to acetaldehyde-modified epitopes. However, the highest responses and the highest number of responders were always in the alcoholic groups [140–143]. While the major focus of interest has been on reactivity with modified proteins, it should be noted that reactivity with modified phospholipids has also been reported [144, 145]. These studies also showed that the same antibodies are reactive with both modified proteins and modified lipids.

In the immune response against a modified protein, antibodies are generated which react solely with the modification, with the modification and the protein and with the protein alone. Most studies have concentrated on reactivity with the modifications, but an early study did show elevated immunoreactivity with unmodified proteins in alcoholics when compared to other groups [141]. There is also evidence for antibody reactivity with the modification and the part of the protein. Koskinas and coworkers observed that 70% of patients with alcoholic hepatitis generated antibodies that reacted with a 200-kDa cytosolic protein when it was modified by acetaldehyde under reducing conditions [146]. Only 25% of patients with non-alcoholic liver disease or controls had similar reactivity. The antibodies generated against this protein must have some degree of specificity since they only reacted with the 200-kDa protein in a mixture that contained many other modified proteins. However, they did not recognise the protein when unmodified, suggesting that the epitope to which they bound must be largely, but not wholly, generated during the modification process.

The early studies measured total immunoreactivity and did not dissect the antibody-based responses against the modified proteins. Later studies used immunoglobulin class-specific reagents, allowing the determination of IgG, IgA and IgM reactivity to be determined. Indeed, later studies showed that alcoholics have elevated reactivity against acetaldehyde-modified proteins and that measurement of IgA reactivity could be used to identify alcoholics [142]. The reason for the elevated IgA response is unclear, but (1) serum levels of IgA are elevated in alcoholic liver disease, (2) a "continuous pattern of IgA deposition" is commonly seen in the livers of alcoholics, (3) IgA and IgG reactive with liver membranes have been detected in plasma from alcoholics, and (4) circulating immune complexes containing IgG and IgA and ethanol metabolism-derived antigens are found in blood from alcoholics [147].

Studies have now implicated antibody-based immune responses in the aetiology of alcohol-related liver injury. An early study using guinea pigs fed an ethanol-containing diet for 40 days while being injected with haemoglobin modified by acetaldehyde under non-reducing conditions. The ethanol-fed animals injected with modified haemoglobin showed hepatic necrosis with an associated mononuclear cell infiltrate and elevated markers of liver injury [148]. In contrast, ethanol-fed animals injected with unmodified haemoglobin only showed steatosis similar to that seen in unimmunised animals. Control-fed animals did not show any pathological signs regardless of the type of protein injected. Increasing the period of feeding to 90 days leads to hepatic fibrosis developing around individual hepatocytes in the terminal hepatic venule associated areas, in the portal area. A concomitant increase

in hepatic proline content indicative of increased collagen synthesis was also seen [149]. This model demonstrates that antibodies reactive with modified proteins may play an important role in the generation of the inflammation, necrosis and fibrosis seen in alcoholic liver injury. Later experiments using rats and a similar treatment regime in which animals were immunised with cytosolic proteins derived form their own livers with or without acetaldehyde modification showed similar results [150]. This study enabled the degree of damage to be related to the strength of the antigenic stimulus and to the time of exposure. For example, animals injected with protein modified by 240 mM acetaldehyde exhibited major liver injury within 10 weeks, whereas those injected with protein modified using 1 mM acetaldehyde required 30 weeks to generate much less damage. The antibodies generated in this study had a similar class and reactivity profile to those generated by human alcoholics [150].

There is little evidence for the role of adducts in extrahepatic tissues. Acetaldehyde has been observed to bind to actin in vitro with the G-form being more reactive than the F-form [114], potentially implicating it in alcohol-induced muscular dysfunction. Acetaldehyde has also been shown to alter the contractile properties of cardiomyocytes in culture. This suggests a role for acetaldehyde in alcoholic cardiomyopathy. There is little direct evidence for the role for acetaldehyde in brain injury, but acetaldehyde has been shown to affect neurotubulin in a similar manner to liver tubulin, and elevated levels of acetaldehyde-derived adducts are associated with alcoholic cerebellar degeneration.

Nutrition Plays a Role in Alcohol-Related Injury

Undernutrition/malnutrition has long been considered part of the aetiology of alcohol-related tissue injury. For example, undernutrition is common in some alcoholics and is a major precipitator of injury in them. Generally, disturbances in nutrition do not cause similar pathology to that seen in alcoholics, with the exception of thiamine deficiency. However, alcohol toxicity can impair nutrition by impairing absorption, transport and utilisation of essential nutrients.

Alcohol makes up an appreciable percentage of the total caloric intake (4–6%) in Western societies. Ethanol itself can be efficiently used by the body, particularly the liver, as a fuel at intakes of up to around 45 g per day, but the efficiency of utilisation decreases at higher levels probably due to the induction of MEOS which does not produce NADH for ATP synthesis [151]. Morphological changes in mitochondria induced by ethanol consumption may also decrease the efficiency of ATP production. Many nutritionists describe ethanol as being “empty” calories since it often lacks important minerals and micronutrients.

The diet of alcoholics is often suboptimal, making the potential role of dietary deficiencies in the sequelae of long-term alcohol abuse an area of intensive research effort. For most forms of alcohol-related tissue injury, there is little evidence to support a role for the diet in the pathology of these conditions. For example, studies on vitamin D [152–154], riboflavin, pyridoxine, vitamin B_{12}, folate and general nutrition [154–156] have shown that while all are associated with alcohol abuse, none were associated with the aetiology of alcoholic cardiomyopathy. Similarly, decreases in muscle and plasma α-tocopherol and selenium concentrations are also associated with alcoholism, but supplemental α-tocopherol did not prevent acute or chronic muscle injury in rat models of alcoholic myopathy [157]. Paradoxically, muscle antioxidant status does seem to be affected by alcohol abuse, perhaps putting the muscle at greater risk of lipid peroxidation [158].

There is strong evidence to link thiamine deficiency with Wernicke’s encephalopathy, a serious neurological disorder with high morbidity and mortality, encountered in chronic alcoholics and persons with grossly compromised nutritional status. The activities of thiamine-dependent enzymes such as α-ketoglutarate dehydrogenase and transketolase are significantly decreased in affected brains and may be involved in the pathogenesis of brain injury [159]. Nicotinamide deficiency, leading to alcoholic pellagra, is also seen in alcohol abusers, albeit at a much lower incidence than Wernicke’s encephalopathy [160].

The incidence of malnutrition in alcoholics without liver disease is relatively modest but is much greater in individuals with alcoholic hepatitis or cirrhosis. In alcoholic hepatitis, some of the symptoms of the condition such as anorexia, malabsorption and altered metabolic state are related to the malnutrition but are probably not underlying causative factors [161]. Detailed dietary analysis of about 250 chronically alcoholic men showed that only the lifetime alcohol load could be associated with cirrhosis and other complications [162]. Further analysis of these individuals revealed that only 10% had evidence of calorie malnutrition, 6% had protein malnutrition and 6% had both.

A dietary component that does appear to be important in the development of alcoholic liver disease is not a micronutrient but rather a macronutrient: fats [163]. Dietary fat appears to be an important factor in the pathogenesis of alcoholic hepatitis, and cirrhosis as fatty infiltration (steatosis) appears to be an important risk factor for the development of cirrhosis [164]. The generation of free radicals (reactive oxygen species, α-hydroxyethyl and hydroxyl radicals) leading to lipid peroxidation is one of the main processes believed to underlie the development of alcoholic liver injury. Measurement of lipid peroxidation products has demonstrated that their concentration is related to the amount of alcohol consumed and with the severity of cirrhosis in actively drinking alcoholics [165] and animals fed ethanol [100]. Triacylglycerol rapidly accumulates in the liver of rats fed ethanol when the fat content of the diet exceeds 25% of the caloric intake [166]. If a low fat (5% of caloric intake) is given with ethanol, the animals did not develop steatosis or show observable lipid peroxidation [167]. Animals given diets with 36% of the calories as fat developed severe steatosis and showed elevated α-hydroxyethyl radical formation [168].

Although there is evidence that the amount of dietary fat plays a role in the development of alcoholic liver disease, it is clear that the composition of the fat also plays a role. Comparison of the cirrhosis mortality rates in countries with similar per capita intake of alcohol revealed that it was higher where the intake of unsaturated fat was high and lower where the intake of saturated fat was high [169]. The effect of dietary fat composition has been examined in rodent models of alcoholic liver injury. The use of the Tsukamoto-French intragastric feeding technique [170] on rats has shown that feeding a high-fat diet for 85–120 days leads to fatty infiltration, necrosis, polymorphonuclear and mononuclear cell infiltration, stellate cell activation and fibrosis [171]. In other studies where this paradigm was used to feed rats ethanol for 6 months, it was found that inclusion of unsaturated fat (corn oil) leads to severe injury, and inclusion of pork fat (lard) leads to moderate injury, with no injury being seen when beef fat (tallow) was included [172]. The degree of injury was found to correlate with the linolenic acid content of each diet. Furthermore, supplementation of the tallow-containing diet with linolenic acid leads to the development of severe injury in these animals [173]. This effect may be associated with the induction of CYP2E1 activity [174]. Rats fed fish oil exhibit even more severe injury than those fed corn oil [175]. This is likely to be because fish oil contains many fatty acids with two or more double bonds which are highly susceptible to lipid peroxidation. This increased injury correlated with the induction of CYP2E1 and increased lipid peroxidation. The injury in these animals could be reversed by replacement of the dietary fat with tallow [176].

Ethanol is also known to alter the phospholipid composition of membranes by decreasing the amounts of palmitic and oleic acids and increasing the amounts of stearic and arachidonic acids [177]. How this affects cellular function is still unclear. However, the administration of soybean lecithin containing phosphatidyl choline prevents the development of cirrhosis in ethanol-fed baboons, probably due to the phosphatidyl choline correcting changes in membrane composition, making the membranes more stable and resistant to lipid peroxidation [178]. This positive effect of unsaturated fat is in direct contrast to the negative effects it has in the Tsukamoto-French model.

The relationship between diet and the formation of modified proteins has not been extensively studied. One study has looked at the effect of α-tocopherol supplementation on adduct formation in the liver of ethanol-fed rats [179]. Supplementary α-tocopherol was found to reduce the formation of adducts derived from the products of lipid peroxidation such as malondialdehyde-derived and MAA 2:1 adducts (Table 9.1). This was unsurprising given α-tocopherol's role as a membrane antioxidant. However, more surprisingly, it also reduced the formation of unreduced and reduced

Table 9.1 The effect of α-tocopherol supplementation on the formation of protein adducts in rat liver. Rats were fed control, ethanol-containing and ethanol-containing plus supplementary α-tocopherol at 30 mg/Kg/day for 4 weeks (n = 6; ± std)

Adduct type	Control diet (Abs 405 nm)	Ethanol diet (Abs 405 nm)	Ethanol diet + α-tocopherol (Abs 405 nm)	Change in adduct after α-tocopherol supplementation (%)
Unreduced acetaldehyde	0.05 ± 0.02	0.32 ± 0.03	0.19 ± 0.03	−40.7
Reduced acetaldehyde	0.03 ± 0.03	0.63 ± 0.02	0.53 ± 0.02	−15.9
Hydroxyethyl radical-derived	0.08 ± 0.02	0.48 ± 0.02	0.35 ± 0.03	−27.1
Malondialdehyde	0.13 ± 0.03	0.39 ± 0.04	0.16 ± 0.02	−59.0
MAA 2:1	0.02 ± 0.03	0.23 ± 0.03	0.15 ± 0.01	−34.8

acetaldehyde adducts. This may be because the aldehyde products from lipid peroxide break down compete with acetaldehyde for removal through the action of aldehyde dehydrogenases. Thus, when supplementary α-tocopherol decreases the formation of malondialdehyde and hydroxynonenal, the aldehyde dehydrogenases more efficiently metabolise acetaldehyde, reducing the amount of adducts that can be formed.

Summary

There is considerable evidence that proteins are modified by reactive metabolites derived directly, and indirectly, from ethanol oxidation. This modification can lead to altered function or the protein acting as a neoantigen, providing targets for immune attack. The relationship between dietary composition and adduct formation has not yet been extensively investigated, but it is clear that α-tocopherol supplementation decreases adduct formation which may decrease cell and tissue injury.

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Chapter 10
Vitamin B12 Deficiency in Alcoholics

Alberto Fragasso

Key Points

- Measurement of total serum cobalamin (Cbl) is the standard investigation for this vitamin deficiency, but a diagnostic "gold standard" for this purpose is still lacking.
- Falsely increased Cbl values are caused by alcohol abuse.
- Some alcoholics with megaloblastic anemia may respond to Cbl treatment despite normal or borderline Cbl serum levels.
- In clinical practice, caution is urged in the interpretation of Cbl assays in alcoholics.

Keywords Vitamin B12 deficiency • Alcoholic liver disease

Introduction

Vitamin B12 (also referred as cobalamin) has a crucial biological role, because its intracellular availability is necessary for DNA synthesis. Cobalamin (Cbl) and folic acid are closely related; both are involved in a common metabolic pathway. The clinical pictures of these vitamin deficiencies are overlapping. Cobalamin deficiency is a significant public health issue, because it is estimated to affect 10–15% of people over the age of 60 [1] and is generally caused by malabsorption, in most cases resulting from pernicious anemia (PA). On the contrary, folate deficiency is often caused by insufficient intake [2]. Typical clinic manifestations of this vitamin deficiency are megaloblastic anemia with variable degrees of pancytopenia, glossitis, malabsorption, and neurological signs and symptoms. In some patients with Cbl and folate deficiency, the classic hematologic, neurologic, or biochemical abnormalities are lacking [3]. The early diagnosis of vitamin B12 and folate deficiency is critical since neurologic disease of Cbl deficiency may be irreversible if treatment, safe and inexpensive, is delayed [4]. Measurement of total serum Cbl is the standard screening test for assessing vitamin B12 deficiency, but a diagnostic "gold standard" for this purpose is still lacking, especially in cases with borderline values. There are major limitations with this approach, and the type of assay used may be relevant. Sensitivity is about 97%, and specificity is limited. In a study, specificity is 90% in patients with Cbl levels below 100 pg/ml but only 60% with Cbl levels <200 pg/ml [3]. Falsely increased values are

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caused by myeloproliferative disorders, liver diseases, intestinal bacterial overgrowth, congenital transcobalamin (TC) II deficiency, nitrous oxide, and seldom by circulating antibody to TC II, high serum vitamin B12 binding protein, and analytical problems [5–9]. Falsely low values can be seen with folate deficiency, pregnancy, myeloma, AIDS, and TC I deficiency. Serum folate levels decrease within a few days of low-folate diet; therefore, the determination of red blood cell (RBC) folate levels has been advocated as a better measure of folate tissue stores. These assays also lack specificity and sensitivity. Serum folate levels increase in patients with Cbl deficiency and with hemolysis; falsely low RBC folate levels also occur in vitamin B12 deficiency. In anemic megaloblastic patients, evaluation of all these parameters is recommended. Vitamin B12 deficiency increases the concentration of total plasma homocysteine (tHcy) and methylmalonic acid (MMA), while folate deficiency only increases the concentration of tHcy.

Megaloblastic Anemia and Alcoholism

Many authors recognize tHcy and MMA as the most sensitive and early indicators of vitamin B12 and folate status; the two metabolite determinations combined have a sensitivity of 99.8% [10, 11]. In these studies, the two metabolic markers are more specific than are serum Cbl levels; this opinion is not unanimous [12, 13]. Increased MMA and tHcy together can be found with primary metabolic defects, renal insufficiency, and hypovolemia, while tHcy alone can increase in alcohol abuse and vitamin B6 deficiency [14]. Furthermore, in the ambulatory care setting, not only Cbl but also MMA and tHcy levels fluctuate with time and neither predict nor preclude the presence of Cbl-responsive hematologic or neurologic disorders [15]. Vitamin B12 in serum is bound to proteins called transcobalamin (TC): most cobalamin is carried on TC I, also called haptocorrin (HC); 20–30% is carried on TC II. The TC II-cobalamin complex is called holotranscobalamin (HoloTC) that is the metabolically active fraction. The HoloTC RIA is the first available method for measurement of HoloTC [16]; recently, an automated assay for measuring HoloTC on the Abbott AxSYM analyzer has been introduced [17]. HoloTC, or "active" B12, contains the biologically available Cbl; several studies have shown that HoloTC is the earliest and most specific marker of vitamin B12 deficiency [18, 19], but further studies are needed to establish the role of this metabolite. Alcohol has a variety of pathologic effects on erythropoiesis: induces macrocytosis, sideroblastic anemia, hemolytic anemia, and megaloblastic anemia that result from nutritional deficiency and/or a direct toxic effect on erythroid precursor [20] and may particularly disturb folate metabolism [21, 22]; this vitamin deficiency may be ascribed to dietary inadequacy, intestinal malabsorption, decreased hepatic uptake and retention, and increased urinary excretion. In a previous study, low serum folate levels were found in more than two-thirds of alcohol abusers [23]. Vitamin B12 metabolism in alcoholics was investigated in the past years [24], and it is thought that Cbl deficiency is not common in these patients. In many reports, serum Cbl levels were found higher in alcoholics than in the control group but generally remain in the reference range [22, 25]. Falsely increased Cbl values are caused by liver diseases [5]; particularly elevated serum vitamin B12 levels were found in alcoholics with liver disease [26], also associated with a lowered liver tissue Cbl concentration [27]. Measurements of serum B12 levels also include metabolically inactive Cbl analogs (HC); therefore, Cbl depletion in tissue may be masked by normal to high serum vitamin B12 levels [28]. Elevated Cbl levels are also found in acute hepatitis; the hepatocellular necrosis may cause the release of stored Cbl following tissue depletion. Alcoholic liver disease leads to elevated Cbl levels in serum despite lowered liver tissue total vitamin B12 concentration accompanied by a lowering of HoloTC distribution. Possible explanations for this phenomenon may be the failure of the damaged liver to take up Cbl from the serum and/or a defective storage that causes vitamin B12 to leak out of the liver into circulation, where it predominantly binds to HC; on the other hand, a diminished concentration of TC II and a reduced clearance of HC may be the result

of an impaired synthesizing liver capability [27–29]. Moreover, a specific role for alcohol abuse may be assumed in inducing a hematologic significant "functional" Cbl deficiency, as nitrous oxide exposure (which oxidizes cob(I)alamin inactivating methionine synthase) does. In the same way, for patients with Cbl-responsive neurologic disorders despite normal serum Cbl levels, Solomon considered a pathophysiologic role for oxidant stress (as alcohol abuse) leading to "functional" Cbl deficiency [30]. A significant positive correlation between serum Cbl and hepatocellular enzymes GGT, AST, and ALT was found [25, 29, 31]. With increasing hepatocellular damage, serum Cbl also tends to be higher and reflects the degree of liver injury by alcohol; increased serum vitamin B12 titers correlate with disease severity, and declining levels were found during remission of the disease [32, 33].

Conclusions

In alcoholics with elevated hepatic enzyme levels, a tissue vitamin B12 deficiency is possible despite normal or elevated serum Cbl levels [31]. In a previous report of megaloblastic anemic patients, we found falsely normal serum Cbl levels only in alcoholics [34]; out of 101 adult patients with megaloblastic anemia, normal Cbl serum levels and normal serum and RBC folate levels were found only in three patients, all alcohol-dependent, while in another alcoholic, borderline vitamin B12 serum levels were found. All the four patients responded to cobalamin treatment. In this series, serum Cbl levels always decreased when liver disease (cryptogenetic, HCV, or alcohol related) was associated with pernicious anemia (PA). Pathophysiologic mechanism of PA probably overcomes the hypothetically affected Cbl uptake caused by hepatocellular damage and/or alcohol-related oxidative stress and produces not only tissue or functional deficiency but also serum lowered vitamin B12 levels. These findings may have an impact on the diagnosis of Cbl deficiency in alcoholics. Measurement of total vitamin B12 serum levels might therefore be misleading in these patients, because alcohol consumption may cause falsely normal Cbl serum levels. In a report, MMA concentration in serum is not affected in hepatic disease; this assay may be useful for evaluating vitamin B12 status in hepatic disease with falsely normal or high concentration of Cbl in serum [35]. HoloTC measurement may be also a suitable option for this subset of patients [36]. If MMA and/or HoloTC measurements are not available, in alcoholics with suspected vitamin B12 deficiency, one may use the pragmatic *ex iuvantibus* criterion, with empirical treatment to assess any clinical response. Caution is needed in the interpretation of Cbl assays in alcoholics, because some patients may respond to Cbl treatment despite normal vitamin B12 serum levels.

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Chapter 11
American Indians/Alaskan Natives and Alcohol: Biology, Nutrition, and Positive Programs

Felina M. Cordova, Michael H. Trujillo, and Roger Dale Walker

Key Points

- The American Indian (AI) is a diverse population consisting of various tribes in various locations across the United States.
- Alcoholism has been a problem for AI since its introduction and continuing into modern times.
- American Indians have risk factors and protective factors that are culturally specific.
- Conflicting reports have been published regarding the differences in biological process of alcohol by AI versus other populations, with more recent data pointing to the existence of genetic differences.
- There has been no research done on nutrition-related effects and the American Indian population in reference to alcohol intake.
- Programs (intervention and recovery) have begun to become more culturally tailored to the AI population with the bulk of research being done on AI youth.

Keywords American Indians • Alcohol • Biology • Nutrition • Culture • Tradition • Positive programs

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Background

American Indians/Alaskan Natives

There are currently 4.1 million people in the United States that self-identify as being American Indian (AI) or Alaskan Native (AN) that area also another race; while 2.5 million that are only AI/AN [1]. There are 560 tribes recognized by the United States, with Cherokee being the largest followed by Navajo as the second largest tribes in the nation [2]. The state with the most American Indians in the country is California with Oklahoma next and Arizona ranking third [2]. The regions of the United States with the most to least AI/AN are West (48%), South (29.3%), Midwest (16%), and Northeast (6.6%) [3]. US census 2010 data has not become available to update population data currently, but predictions of population have the AI population in the year 2020 getting up to 3.5 million [4]. Sixty-three percent of AI/AN live in urban locations of the United States versus those that reside on reservation/native land [1].

Alcoholism

AI/AN History, Risk Factors

Colonists in the United States help set the precedence of alcoholism. Colonists brought with them alcohol and a temperament for drinking large amounts of the substance while here [5]. The AI population was unprepared for this biologically, culturally, and socially when introduced to alcohol [5]. In addition, the historical trauma that the AI/AN people have faced has also contributed to alcohol use. Being forced off their land as well as removed from their homes contributes to the historical trauma [4]. The AI/AN population is also discriminated for their heritage, and reports have shown that approximately half of AI/AN between the ages of 8 and 20 report having been through a traumatic (psychological or physical) life event with posttraumatic stress disorder resulting in some cases [4]. In addition, child abuse and neglect have also been shown to be factors in AI women's consumption of alcohol [4]. Depression has also been associated with increased alcohol usage as well as lacking a supportive family unit and living alone (for the elderly) [6]. Alcoholism can also be perceived by some AI to be due to their biological makeup and genetics and less of their own control [6].

Prevalence

Alcoholism is a problem for every community regardless of ethnicity/race. American Indians/Alaska Natives had a lower average (43.9%) of alcohol use than the national average's 55.2% from 2004 to 2008 [7]. In addition, alcohol was statistically significantly lower than the national average for all age groups of AI/AK: 18–25 with 52% versus 61.1%, 26–49 with 51.3% versus 60.5%, and 50 or older with 31% versus 46.9% [7]. For binge drinking although lower, AI/AN between the ages of 26 and 49 were the only age group to produce statistically significant higher results at 39.4% versus the 28.9% national average [7]. Both female and male AI/AN have a statistically significantly lower percentage of alcohol use with 38.6% and 49.5%, respectively, versus the national average of 48.5% and 62.3% [7]. Binge drinking alcohol use is statistically significantly higher for adult women than the national average (24.2% vs. 15.9%), although male AI/AN report 3.8% higher use than the national average of 33.8% [7]. As such, AI/AN are less likely to consume alcohol on a moderate basis [8]. Alcohol use during the time period of 2007–2008 increased by 4.8% for AI/AN over the age of 18 who reported

consuming alcohol during the past month [7]. For those AI/AN under the age of 18, between 12 and 17 years old, they have a 1.2 higher reported percentage of alcohol use within a year than those of other ethnicities [9]. AI/AN are also more likely to have alcohol use disorder with 8.5% of AI/AN versus 5.8% other ethnicities in the age category of 12–17 [9]. As for rural versus urban AI, various researchers have reported that urban American Indians consume alcohol more than reservation AI [4, 6, 8]. Actual rates per tribe vary, and although generalized data is available, it does not necessarily apply to each tribe.

Alcohol Consequences

The consequences of alcohol on the AI/AN population appear to be more severe. Alcohol-related deaths (accident, suicide, vehicle related) are higher among the AI/AN population than the rest of the United States [5]. Liver problems such as cirrhosis are also more prevalent in the AI population versus the general US population [5]. Alcohol consequences are also apparent in both AI reservation youth and nonreservation American Indians who face troubles with the law, at school, and at home with higher percentages than Caucasians [6]. Legal problems and intoxication also occur for AI adults, as 25% of AI adult females taken into police custody have been found to have been consuming alcohol at time of their arrest, while 22% more AI men than women also report this same type of incident [10]. AI also go to the hospital with alcohol-related health problems more than others in the United States [11]. Babies born with fetal alcohol syndrome is also a major concern with the AI community. American Indians have three times the rate of fetal alcohol births than that for the North American population rate and even higher than African American FAS rates [10].

Biology, Alcohol, and AI

Several research studies have found genetic differences in AI and the way their bodies process alcohol while others have not. Studies have looked at the alcohol dehydrogenase (ADH) and acetaldehyde dehydrogenase (ALDH) genes and found AI populations to be lacking the protective alleles ADH1B*47HIS and ALDH2-2 [12]. Osier et al. have also found a genetic variation in AI (Cheyenne and Arizona Pima) at codon 351 of ADH1C that leads to an ADH1C Pro351Thr substitution that could have an impact on alcohol processing [13].

In a study by Ehlers and Wilhelmsen of California Mission Indians that were at least 1/16 native and alcohol dependent, a genetic desire to imbibe alcohol was found on chromosome 5 via a genomic scan [14]. AI (Mission Indians of at least 25% AI heritage) have also been shown to be more susceptible to alcohol addiction as shown by decreased P3a (upon alcohol ingestion at 0.56 g/kg alcohol) which is a portion of the event-related potential measured by an electroencephalogram [11]. In this same AI population, researchers correlated the allele ADH2-3 and alcoholism via this decreased event-related potential [11]. A decreased event-related potential increases risk as it can be indicative of less of a biological response to alcohol and has also been found in other studies involving other populations to be positively associated at follow-up with alcohol dependence in subjects followed 8 years from baseline [11].

There have also been conflicting reports on ethanol metabolism in AI. Studies have found that Alaskan Natives process ethanol at a decreased rate while other studies have an increased metabolism [6]. Facial flushing findings have been at odds as Sioux and other AI have not been found to have the isoenzyme associated with this condition, while several Oklahoma-based AI have been found to possess the isoenzyme [6].

Alcohol and Nutrition Among AI

There has been no research looking at how alcohol intake affects American Indian nutrition thus far. Alcohol and nutrition intake often looks at the Caucasian population or does not stratify results based upon ethnicity. Alcoholics have been found to not only be malnourished but to also have specific differences than the general population when it comes to nutrition. Increased alcohol consumption increases the storage of fat as well as body weight [15]. A higher percentage of obesity is seen in people that consume more drinks per week; in a study, it was found that those who drank 21 drinks or more per week had a higher percentage of obesity and larger waist circumference than those who drank none to 20 drinks per week [15].

Leptin, vitamin levels, and LDL are examples of some nutrition parameters that are altered in alcoholics. Both active alcoholics (6.78 ± 0.51) and alcoholics with cirrhosis (6.91 ± 1.37) have statistically significant higher levels of serum leptin versus controls (4.70 ± 0.32) [16]. In alcoholics with liver disease, deficiencies of vitamins A, B1, B2, B3, B6, B12, C, D, E, and K have been cited [17]. Folate is also a vitamin that is commonly lowered in alcoholics versus nonalcoholics with their RBC folate levels having been shown as 128.7 ± 56.8 nmol/L and controls being 162.7 ± 54.5 nmol/L [18]. In this same study, homocysteine was found to be statistically significantly elevated in comparison to controls [18]. Alteration of minerals in alcoholics include decreased calcium, magnesium, phosphorous, potassium, and zinc and increased copper and iron [17].

Traditional Medicine and Other Factors

In a survey study among AI/AN conducted in Seattle, 70% reported using traditional medicine [19]. Slightly more users of traditional medicine versus nonusers reported being employed, and almost double the number of users had attained an education past high school [19]. Southwest AI have been found to be more likely to use traditional medicine only than AI in the northern plains according to a study conducted with a nonspecified southwestern tribe and a nonspecified northern plains tribe with a total of 2,595 participants [20].

Sweat lodges are a component of culturally specific alcohol treatment interventions for American Indians. The sweat lodge consists of American Indians sitting in an enclosed structure where steam is created off of heated rocks for an extended period of time, allowing participants to sweat out bodily toxins [21]. Peyote has also been used to help AI quit or abstain from alcohol [22]. Traditional ceremonies and dances have also been used in the alcoholism healing process [23].

In addition, many AI programs that help in prevention and alcohol support look toward cultural beliefs. The basis of Alcoholics Anonymous has been adapted for the AI community by incorporating cultural aspects such as traditional beliefs in a "creator" versus Christian religious terms used in AA [23]. Additionally, traditional AA places more of an emphasis on modern ways and modern medicine, while AI AA focuses more on AI traditional medicine [24]. Traditional tobacco pipes as well as sweat lodges have also be utilized in AI-specific AA meetings [24].

Positive Tribal Programs

There are many tribal-specific health facilities that have been created to deal with the problem of alcoholism. According to the IHS, there are 46 facilities in the states of Arizona, Nevada, Utah, and California. IHS has even created alcohol treatment facilities in detention centers that house youth in highly populated AI locations such as Tuba City, AZ, as well as Stroud, Oklahoma, and four other

locations in the United States [6]. Groups like Alcoholics Anonymous do not culturally tailor their programs, whereas in addition, it has been found that some AI have a low comfort level with AA programs that were open to the general public and not AI-specific AA groups [25]. In accordance with this sentiment, programs that have been successful among AI/AN have incorporated cultural elements and cater to the AI population.

Specific Programs

There are numerous programs across the United States that offer support services to American Indians. The Tucson Indian Center in Tucson Arizona is one American Indian facility that uses traditional methods in alcohol prevention and support. The Tucson Indian Center is not a tribal-specific facility but one that is open to members of all tribes. Prevention services occur with AI youth via the arts and crafts program "the Native Pride Project." Their alcohol support programs are the "White Bison" adult sobriety group as well as a talking circle group. The White Bison program is a 12-step program that contains traditional and culturally tailored content, and the talking circle program is a support group; both programs are for adult AI men and women.

Specific Studies

The majority of research on prevention and interventions that currently exists for American Indians is on the youth portion of the community. There is a lack of research occurring for the adult AI population in the areas of alcohol prevention and intervention. The majority of the adult research focuses on epidemiology, factors of alcoholism, mental illness, and negative health comorbidities, with surveys being used in many cases to collect the data [4, 5, 26].

Teens: Prevention/Intervention/Relapse

Rural

- A study by Schinke et al. looked at reducing alcohol consumption among AI youth living on reservations in five states. In this three-arm study, an intervention at school consisting of life skills with American Indian cultural principles was given, intervention with culture + community involvement and the control group [27]. Communities were given prevention awareness, as such materials were given to schools, parents, etc. in the intervention + community arm by the study [27]. The intervention arm consisted of a 15-week program with youth in the grades of 3, 4, or 5 for 50 min each week [27]. During sessions, they were taught how to communicate when talking about substances via cultural material, role-playing, and take-home assignments [27]. The total study period consisted of 42 months with 1,199 participants. For alcohol use, the skills or skills + community intervention groups reported lower use at all time points of 6, 18, 30, and 42 months, while the skills + community only reported lower alcohol use at the 6-month checkpoint [27]. Results were statistically significant at the 30- and 42-month time point with skills alone reporting 15.89% and 22.87%, respectively; skills + community reporting 17.18% and 25.44%; and control reporting 19.06% and 30.17% alcohol use [27].

Reservation

- In a community-based alcohol and drug intervention 5-year program in a nonspecified western state reservation in the United States, alcohol use was found to have been reduced [28]. Through the Community Health Promotion Grants Program, there were several interventions that operated within the community and included classes (some took place at homes), conferences, carnivals, skill development, employing high school students in the summer and leadership training that occurred over the course of 5 years and targeted various age groups [28]. The data collection tool that was used to analyze the effectiveness of the intervention was surveys that were completed by 9th and 12th graders [28]. The results displayed absolute change decreases (nonstatistically significant) of 15.9% in binge drinking, 12.8% drank alcohol in the past month, 5.3% getting drunk before the 9th grade, and 17.1% passenger in car when driver had been drinking for reservation AI [28].

Urban

- The Seventh Generation Program implemented in Colorado AI urban youth combined both culturally specific ideas on alcohol interventions with those already in use in other populations via a community-based participatory research design. The program consisted of 57 evaluable AI youth of various tribal backgrounds participating in a program that lasted 14 weeks and took place after school with 4th–5th grade students [29]. The curriculum of the program consisted of modules that were designed specifically for AI youth and included topics such as AI cultural beliefs, and the last of the seven modules was a commitment ceremony. This ceremony included the cultural elements of spiritual leaders as well as storytelling, a staking ceremony, and dance in the AI youth's commitment to alcohol abstinence with their families in attendance. [29] A percentage decrease of 4.5% for drinking in a day was seen in those who took part in the program (although not statistically significant) [29].

Mixed Populations

- Although there are several studies available on AI youth alcohol prevention/intervention, not all research with AIs targets this population solely. Other research has been conducted with other populations in addition to AI such as Project Northland in Minnesota who had 3.7% AI youth in their program with the rest of the youth being white [30]. This 3-year program used the elements of family, peers, school, and community in their intervention and saw lowered alcohol use percentages among those in their program versus controls, although results were not stratified on ethnicity and no specific AI data was reported [30].

Protective Factors for Alcohol Initiation and Abstinence

Various research studies have found several protective factors for AI and alcoholism. A sense of belonging has been found to be important to AI Arizona urban youth in terms of decreasing alcohol use [31]. The People Awakening Project conducted with various age groups of adults over 21 years old found that family, parenthood, and community were factors important to Alaskan Natives in their abstaining from alcohol [32]. Maintaining a positive relationship with culture has also been found to decrease drinking in the adult AI South Dakota population [33]. In southwestern AI youth, this sentiment is also confirmed as Kulis et al. found those with greater pride in their culture had strong negative beliefs when it came to drinking alcohol [34].

Conclusion

Alcohol has been shown to produce serious adverse effects in the American Indian community. From alcohol-related health conditions to fetal alcohol syndrome and alcohol-related deaths, alcoholism is a major concern of the AI population. American Indians have various risk factors that make them susceptible to alcohol such as past historical trauma, discrimination, and biological factors. There are numerous tribal-specific or AI-specific organizations that are trying to combat alcoholism by using tradition and culture as well as other methods. Due to the large amount of federally recognized tribes in the United States, alcohol-related research has not been done on every tribe for biological processing, nutrition, prevention, and intervention. What is available is specific to that tribe and cannot be used to generalize all American Indians. In addition to a disparity in alcohol research being conducted with more tribes, the differences in urban and reservation American Indians need to be studied further as well as intervention/prevention research conducted on AI adults. For research conducted this far, researchers have found the value of using cultural influences in interventions of various tribes and various age groups.

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Chapter 12
Metabolism of Ethanol to Acetaldehyde in the Rat Mammary Tissue: Inhibitory Effects of Plant Polyphenols and Folic Acid

Gerardo Daniel Castro and José Alberto Castro

Key Points

1. NADPH- and oxygen-dependent metabolism of ethanol to acetaldehyde in mammary tissue microsomes
2. Xanthine oxidoreductase-mediated generation of acetaldehyde and hydroxyl radicals from ethanol in the mammary tissue cytosolic fraction
3. Preventive potential of plant polyphenols and folic acid by inhibition of in situ oxidation of ethanol to acetaldehyde in mammary tissue
4. Acetaldehyde and oxidative stress in the promotion of breast cancer by alcohol drinking. Its blockade by plant polyphenols and folic acid
5. Acetaldehyde accumulation in mammary tissue during alcohol drinking

Keywords Acetaldehyde • Breast cancer • Mammary • Plant polyphenols • Acetaldehyde accumulation • Folic acid • Oxidative stress and cancer

Introduction: Alcohol Drinking and Breast Cancer

Alcohol consumption is causally related to an increased risk of cancer of the upper aero-digestive tract, liver, colorectum, and female breast [1–5].

Of particular concern is the case of breast cancer promotion by chronic alcohol consumption in women, since according to estimates of the World Health Organization, about 3% of total breast cancer worldwide was attributable to alcohol consumption in 1990 [1]. Further, combined analysis of data from 53 studies around the world showed a clear dose–response relationship between alcohol consumption and increased risk of breast cancer [6]. The last study showed a 9% increase in risk per 10 g intake of alcohol per day. In fact, other recent epidemiological studies in a total of 1,280,296 middle-aged women in the UK reported that even drinking women consuming an average of only 10 g of alcohol (one drink) per day showed a 12% increased risk of breast cancer [4]. In addition, a detailed

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prospective epidemiological cohort study in 254,870 women, made in eight European countries, reported that 5% of the female breast cancer was attributable to alcohol consumption [7].

This points to the need to further reduce or avoid drinking by women since alcohol intake is one of the few modifiable risk factors for breast cancer and also to the relevance of learning about biological and molecular mechanisms of the marked susceptibility of mammary tissue to alcohol consumption.

Despite the significance of that need, there is limited information regarding possible mechanisms for this effect and about the positive modulatory effects of dietary factors if alcohol drinking is not avoided.

Several lines of evidence indicate that acetaldehyde, a product of alcohol metabolism, and alcohol-promoted oxidative stress might play an important role in alcohol-related liver or esophageal carcinogenesis [2, 3, 5, 8].

In the case of mammary tissue, it was evidenced that alcoholic beverage used in women causes an increase in the level of estrogen and/or androgen, which may promote development of breast cancer [9–12]. However, most workers in the field consider that hormone-mediated effects of ethanol on mammary epithelial cells play a promotional role in breast carcinogenesis, essentially by stimulating mitotic division of already initiated cells [12–18].

Notwithstanding alcohol consumption by postmenopausal women who are under estrogen replacement therapy may significantly increase blood estradiol levels, and this may increase the risk of breast cancer [19]. Other workers also pointed the potential existence of underlying hormonal basis for the association between alcohol use and breast cancer [20]. It is also relevant to take into account that some workers in the field of estrogen-induced breast cancer also consider estrogen as complete carcinogen able to lead to the formation of DNA adducts, be mutagenic and provoke cell transformation [21]. Whether those adducts are found under alcohol drinking conditions is something that remains to be established.

Other factors considered to play a promotional role in the case of ethanol-induced cancer in target organs other than breast, e.g., oxidative stress in the case of liver [3, 8], might also be involved in the case of mammary tissue. In past studies from our laboratory, it was shown that repetitive alcohol administration for 28 days evidenced the ability of ethanol to promote oxidative stress in that tissue [22]. More recently, we provided additional results related to the mechanism for the occurrence of that ethanol-promoted oxidative stress. In effect, under repetitive alcohol drinking for 28 days, significant decreases were found in the mammary tissue content of glutathione and alpha tocopherol and in glutathione-S-transferase or glutathione reductase activities and of lipid peroxidation process as detected by the xylenol orange procedure [23].

Concerning the nature of the mutational event responsible for the initiation step of the carcinogenic process in mammary tissue, previous studies from other laboratories suggested that acetaldehyde produced elsewhere (e.g., in the liver) or arriving at mammary tissue via blood could be a key putative initiating agent of the ethanol-promoted breast cancer [16, 24, 25]. However, later studies from our laboratory strongly suggested that acetaldehyde produced in the mammary tissue by metabolic transformation of ethanol in situ and the local lack of ability to detoxify further the acetaldehyde formed would be the major player in the highly significant and long-lasting acetaldehyde accumulation process observed in mammary tissue during alcohol drinking. In effect, the acetaldehyde accumulated in that tissue after giving to the rat three different doses of ethanol (low, medium, and high) was directly proportional to alcohol dose given. In contrast, blood levels of acetaldehyde at different times did not change markedly with alcohol dose [26].

Ethanol Metabolism in the Rat Mammary Tissue, Polyphenols and Folic Acid

Two different pathways of bioactivation of ethanol to acetaldehyde were reported by our laboratory to be present in the rat mammary tissue. One is in the cytosolic fraction and the other at microsomal level. Both were preliminarily characterized and both showed to be susceptible to inhibitory effects of plant polyphenols present in foods.

Notwithstanding, in the case of the cytosolic pathways of alcohol metabolism, the simultaneous high consumption of purine-rich food (e.g., red meat, seafood, some vegetables) or beverages (coffee, tea) or soft drinks (soda, energy dinks) containing high amounts of caffeine would lead to increased formation via this pathway not only of acetaldehyde but also of hydroxyl free radicals [27].

The enzyme involved in this cytosolic pathway was evidenced to be xanthine oxidoreductase (XOR) because of its susceptibility to inhibitory effects of allopurinol and by the ability of the process to occur only when the presence of NAD^+ was accompanied by substrates of the XO form of the enzyme such as hypoxanthine, xanthine, caffeine, theobromine, theophylline, or 1,7-dimethylxanthine [27]. Moreover, it is also known that during acute alcohol intoxication, there is an increased purine degradation and hyperuricemia [28]. The enhanced supply of purines resulting from this process would also provide an extra amount of cofactors for the XOR-mediated pathway of metabolism of ethanol to acetaldehyde and free radicals in the mammary tissue.

The presence of XO, XDh, and XOR in mammary tissue is well known [29, 30], and past studies from our laboratory evidenced their presence in high amounts in the rat mammary tissue epithelial cells [22]. Interestingly, the activity of this cytosolic metabolic pathway significantly increased after repetitive alcohol drinking of a Lieber and De Carli diet for 28 days [22].

Those increased levels of XOR present in mammary tissue might also lead to increased bioactivation of mammary tissue pro-carcinogens (e.g., nitroheterocyclic compounds present as contaminants in honey) to their ultimate reactive forms involved in their ability to initiate the carcinogenic process [31]. In fact, several nitrofurans and nitroimidazoles widely used in veterinary medicine appear as contaminants in food. Some of these compounds are breast carcinogens in rodents, and their mechanism of action is hypothesized to be related to reactive metabolites generated by nitroreduction and/or via oxygen-dependent redox cycling. In our work, the metabolism of nitrofurazone, nitrofurantoin, furazolidone, and metronidazole by the cytosolic and microsomal fractions of rat mammary tissue was studied. All the nitrofurans were nitroreduced by the XOR present in the cytosolic fraction. Furthermore, they were also reduced by the microsomal fraction in the presence of NADPH, with the exception of nitrofurazone, suggesting the participation of cytochrome P450 reductase. These results suggest that the nitroreductive metabolism of nitrofurans and the subsequent redox cycling might be involved in the associated mammary tissue carcinogenic effects.

In contrast, other food components, like some plant polyphenols and folic acid, were very potent inhibitors of this pathway of cytosolic XOR-mediated bioactivation of ethanol to acetaldehyde. Of particular significance was the inhibitory effect of folic acid, dihydrofolic acid, ellagic acid, myricetin, quercetin, luteolin, kaempferol, baicalein, hesperetin, silibinin, morin, enterodiol, and apigenin. In most cases, their inhibitory effect was of the same order of that of allopurinol, at concentrations as low as 10 μM [32].

These results might be of particular interest in light of previous reports that higher folate consumption was associated with decreased breast cancer risk among women drinking alcohol regularly but not among nondrinkers in three cohort studies [33–36]. Whether the preventive effect of folate on alcohol-promoted breast cancer is related to the inhibitory effects of folic acid on XOR-mediated cytosolic bioactivation of ethanol to metabolites like acetaldehyde and free radicals [27] is something that remains to be established. However, it is an attractive possibility. The preventive effects of folate were not observed when breast cancer risk was not associated with high alcohol intake, despite an increasing number of specific cancers having been linked to folate status [37]. In those cases, several alternative hypotheses were put forward to explain the beneficial effects of folate [37]. In alcoholism, there is decreased liver uptake of folate. Folate retention in the liver is reduced as well. Excess alcohol intake decreases the absorption of folate in the intestines [38]. Alcoholism may lead to lack of folate due to malnutrition. In addition, an alcoholic most likely does not consume adequate amounts of fruits and vegetables. Alcoholics also have increased loss of folate in the kidneys since excess alcohol use makes folate less available for use in the body.

In contrast to the case of folic acid, there are no reports available in the literature on the effect of diets rich in plant polyphenols on breast cancer risk among women consuming alcohol regularly. However, it is known that diets rich in vegetables and other plant products significantly reduce breast cancer risk [39, 40]. These diets are an important source of polyphenols [41–43] and of other cancer-preventive agents [40]. Because excess breast cancer risk related to alcohol consumption was observed, even in women drinking relatively modest amounts of alcohol [1, 6], the possibility exists that diets containing sufficient plant polyphenols are protective in those cases. Our studies and those available in literature on the preventive effects of plant polyphenols on cancer risk [41, 43, 44] suggest the need to evaluate the potential preventive contribution of diets rich in polyphenols on breast cancer risk in women consuming varying amounts of alcohol.

The contribution of enzymes present in cytosolic fraction of mammary tissue, other than XOR, to the activation of ethanol to acetaldehyde, e.g., alcohol dehydrogenase (ADh), may be more limited. On one hand, previous studies by Guerri and Sanchis [45] showed that no ADh activity was found in homogenates of rat mammary tissue. More recently, in cytosolic fractions of mammary tissue, our laboratory reported traces of ADh activity that was about 16 times smaller than in the liver [26]. On the other hand, Triano et al. [46] reported that human mammary tissue contains a Class I ADh, having a limited potential to biotransform alcohol to acetaldehyde.

In addition to the mammary tissue cytosolic pathway of ethanol metabolism to acetaldehyde described above, our laboratory reported the presence of other one occurring in the microsomal fraction of that tissue.

Concerning the microsomal pathway of oxidation of ethanol to acetaldehyde and its susceptibility to inhibitory effects by plant polyphenols, we considered convenient to analyze first the nature of the enzymatic process involved and its response to polyphenols afterwards.

In our earlier studies on this pathway, it was established that the enzymatic transformation involved was oxygen and NADPH dependent but that cytochrome P450 was not involved because it was not inhibited by either $CO:O_2$ (80:20 v/v) or by SKF525A [47].

Interestingly, this microsomal transformation of alcohol to acetaldehyde was strongly inhibited by diphenyleneiodonium (DPI), sodium diethyldithiocarbamate, sodium azide, and nordihydroguaiaretic acid but not by dapsone, aminotriazole, or indomethacin. Those results suggested us the potential participation in this biotransformation of an oxidase or a peroxidase but not of lactoperoxidase or cyclooxygenase [47]. We were unable to detect the formation of either hydroxyl or 1-hydroxyethyl radicals in those early studies. In the course of following studies performed at the opportunity in rats exposed to a standard Lieber and De Carli diet for 28 days, we observed the induction not only of the XOR cytosolic activation pathway but also of the microsomal one [22]. That was of particular significance, since we showed in the course of additional recent work that this enhancing effect is not due to a participation of CYP2E1 after chronic alcohol drinking as it is known for the liver microsomal fraction [23]. Further, acetone, another inducer of microsomal CYP2E1-mediated alcohol metabolism in liver microsomes, failed to enhance ethanol bioactivation and CYP2E1 enzymatic activity in the microsomal rat mammary tissue counterpart [23]. To ensure that CYP2E1 enzymatic activity was not present or was very low, we also included in those studies determinations of chlorzoxazone hydroxylase activity. This activity was considered in literature as having a significant response to the presence of CYP2E1 in a given tissue [48]. We were not able to detect CYP2E1-mediated metabolism of chlorzoxazone in the mammary tissue microsomal fraction despite the fact we employed a particularly sensitive procedure developed in our laboratory, where the formation of 6-hydroxychlorzoxazone metabolite could be determined by HPLC with coulometric detection [49].

That further excluded the participation of CYP2E1 in this microsomal pathway of alcohol metabolism in the mammary tissue and encouraged us to challenge the possibility that a peroxidase or a lipoxygenase was involved in that process instead. That hypothesis was originally coined because of the potent inhibitory effect of nordihydroguaiaretic acid. In fact, this polyphenol is a known inhibitor of lipoxygenases [50, 51]. We also envisaged the possibility that the potent inhibitory effect of DPI could be sug-

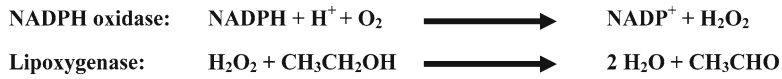

Fig. 12.1 Cooperative mechanism between NADPH oxidase and lipoxygenase in the microsomal oxidation of ethanol to acetaldehyde

gesting the additional participation of an NADPH oxidase enzyme as a supplier of hydrogen peroxide. Under this view, the role of NADPH oxidase would be the generation of the necessary co-substrate required by lipoxygenase to exert its activity against xenobiotics [52–55]. On behalf of this hypothesis is the fact that the specific inhibitory effect of DPI on NADPH oxidase is well established [56]. That hypothesis visualizes the overall process of microsomal ethanol oxidation to acetaldehyde in rat mammary tissue as a cooperative mechanism between NADPH oxidase and lipoxygenase (Fig. 12.1).

Working Hypothesis and Potential Applications

All the above discussed findings suggest that acetaldehyde produced "in situ" would be critical to explain acetaldehyde accumulation in that tissue after ethanol administration. However, other factors, such as poor handling in the accumulated acetaldehyde in that tissue, could be of significant relevance. In effect, we also detected a very low activity of aldehyde dehydrogenase in the cytosolic, mitochondrial, and microsomal fractions of mammary tissue [23], and consequently, its potential contribution to get rid of the acetaldehyde formed in situ or even to the smaller amount arriving via blood would be minimal. Further, in our hands, repetitive alcohol drinking during 28 days of a standard Lieber and De Carli diet was found to produce significant decreases in the content of glutathione, glutathione-S-transferase, and glutathione reductase in this tissue, indicating that also this glutathione-dependent metabolic pathway of handling acetaldehyde might be impaired during alcohol poisoning [23].

Other critical consequence of the ethanol metabolism in mammary tissue is related to the nature and properties of the metabolites formed in their subcellular fractions and of the putative enzymes involved in those processes. In effect, not only acetaldehyde was formed in both the cytosolic and the microsomal fractions [26, 27, 47] and accumulates but also, in the case of the former cellular fraction, hydroxyl radicals are formed [27]. These free radicals, the decreases in antioxidant defenses observed, and the alcohol-inductive effects on xanthine oxidase, lipoxygenase, and NADPH oxidase activities observed led to increased oxidative stress manifestations in mammary tissue after both acute and repetitive alcohol drinking [22, 23]. For example, increased formation of lipid hydroperoxides was detected; delay in the t-butyl hydroperoxide-induced chemiluminescence and a significant decrease in protein sulfhydryls [22].

However, we failed to detect lipid peroxidation occurrence via malondialdehyde production in mammary tissue from animals receiving the Lieber and De Carli diet for 28 days. We interpreted this result as suggesting that either the ethanol-promoted lipid peroxidation process is still in early course or that the sensitivity of the procedures employed was not adequate to reveal its occurrence. Additional different procedures or experimental conditions would be required to elucidate the reasons for these apparently contradictory observations.

The hypothesis that an oxidative stress process could be induced in mammary tissue and how it could be sparked was coined because of our previous experiments showing not only that during ethanol metabolism in the mammary tissue cytosolic fraction, hydroxyl radicals were produced [22, 27] but also because acetaldehyde generated in situ in that pathway and the additional one arisen during the ethanol metabolism reported to occur at the microsomal level significantly accumulate in mammary tissue [26, 47]. In effect, on one hand, it is known that hydroxyl radicals are potent inducers of

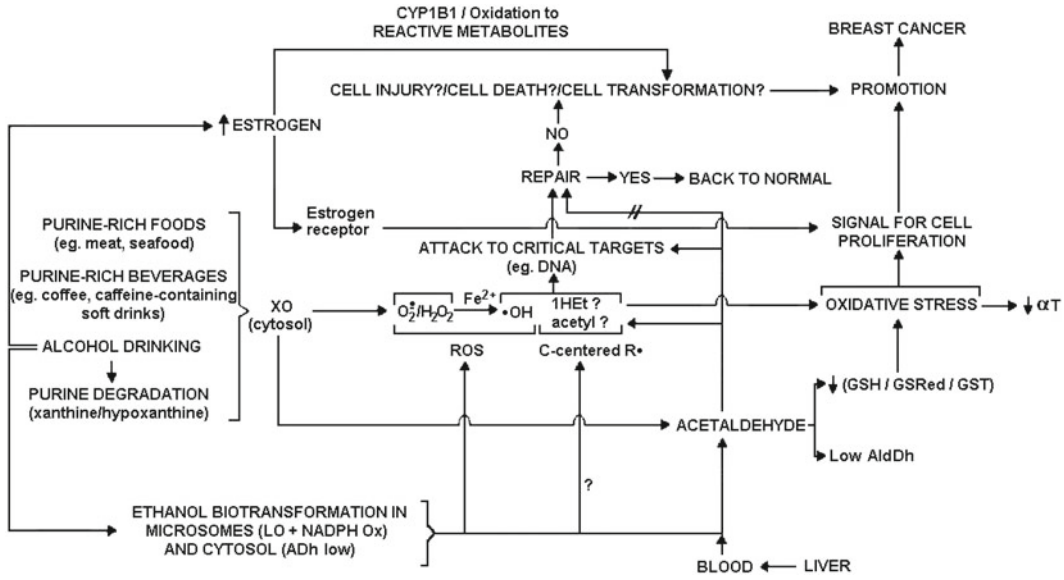

Fig. 12.2 Working hypothesis about the mechanism of the promotion of breast cancer by alcohol drinking

oxidative stress in biological systems, but on the other, it has been repetitively established that acetaldehyde besides interacting with DNA and proteins also significantly reacts with glutathione to decrease its content in organs such as the liver [57]. This molecule plays an essential role in protecting cells from oxidative damage and the toxicity of xenobiotic electrophiles (e.g., acetaldehyde). These roles were recently reviewed by Forman et al. [58]. Accordingly, in recent experiments, we found highly significant decrease in the mammary tissue glutathione in the animals receiving the standard Lieber and De Carli diet for 28 days [23]. Further, significant decreases were also observed in the activity of glutathione reductase, an enzyme needed to regenerate glutathione from its oxidized form, and in glutathione transferase, an enzyme very effective in catalyzing the reaction between acetaldehyde and other alkylating agents with glutathione. In contrast, glutathione peroxidase (which is able to catalyze the destruction of hydroperoxides and hydrogen peroxidase) remained basically unchanged [23].

Notwithstanding, not only the hydrosoluble glutathione antioxidant-related defenses against oxidative stress were decreased in mammary tissue from animals repetitively receiving an alcohol containing diet, the content of the key lipid soluble antioxidant alpha tocopherol (its role was reviewed by Blatt et al. [59]) was also significantly decreased under similar experimental conditions [23].

All the above discussed results clearly suggest that oxidative stress-prone conditions occur in mammary tissue from rats receiving that alcohol treatment.

Both accumulated acetaldehyde and oxidative stress promotion might play a significant role in alcohol drinking promotion of cancer [2, 3, 5, 8]. Acetaldehyde could be a major initiator of the ethanol-promoted breast cancer since it is well known that it is a potent mutagen and carcinogenic compound [5, 60–62].

The increased oxidative stress conditions provoked by the formation of hydroxyl radicals and lipid hydroperoxides and aggravated by the diminished defenses against oxidative insult described above might also be involved in the carcinogenic process. It has been previously demonstrated that oxidative stress could play a role in the initiation, promotion, and progression stages of cancer development [63, 64].

Both factors as well as the increased levels of estrogen promoted by alcohol drinking are part of our present "working hypothesis about the mechanism of the promotion of breast cancer by alcohol drinking" that is depicted in Fig. 12.2.

If that working hypothesis were even partially valid, the opportunities for positive modulation of the undesirable carcinogenic outcome might be envisaged, beyond avoiding or limiting alcohol drinking to prudently established levels.

On one hand, it might be conceivable to trap acetaldehyde formed in vivo via nontoxic dietary compounds. That possibility was previously explored by other authors to prevent damage induced by alcohol drinking in target organs like oral cavity or the gastrointestinal tract and administrating cysteine simultaneously [65, 66]. Thiol products, such as the amino acid cysteine, are known to be able to protect against acetaldehyde toxicity. Cysteine is able to bind acetaldehyde efficiently by forming a stable thiazolidine-carboxylic acid adduct. Special cysteine preparations (e.g., in the form of chewing gum) have already been developed to bind smoking- and alcohol drinking-derived acetaldehyde from the oral cavity [65, 66].

Avoidance of the simultaneous excessive consumption of other compounds present in our meals or beverages which might enhance acetaldehyde formation in vivo or generate additional oxidative stressful conditions still is a preventive strategy. Oppositely, the consumption of food components having inhibitory effects on pathways either of acetaldehyde formation or of antioxidant nature or both properties would be helpful.

For example, simultaneous presence of purine-rich foods or beverages could increase acetaldehyde production via the XOR-mediated cytosolic pathway [27]. Notwithstanding, simultaneous consumption of some polyphenols, already tested and perhaps many others as well, has evidenced to have potent inhibitory actions not only in the mammary tissue cytosolic pathway of acetaldehyde generation from ethanol but also on the accompanying microsomal counterpart [32, 67]. The tested polyphenols included representative members of chalcones, flavones, flavonols, flavanones, flavanols, anthocyanidines, isoflavones, phenolic acids, and their derivatives, stilbenes and lignans [32, 67].

It is of particular significance that flavonoids have inhibitory effects on prooxidant enzymes like lipoxygenase and xanthine oxidase and are potent antioxidants (reviewed in Maciel et al. [67]). Further, some of the polyphenols tested in our studies were evidenced by others to have antiestrogenic properties (e.g., daidzein and genistein), which might offer an additional preventive contribution against alcohol-induced mammary cancer [68]. In addition, several plant polyphenols tested by our laboratory against metabolic activation of ethanol in mammary tissue have additional beneficial effects such as antiproliferative actions or proapoptotic effects on cancer cells or by inhibiting tumor angiogenesis (reviewed in Maciel et al. [67]).

It is important to note, however, that the bioavailability of these compounds determines their in vivo ability to exert their beneficial effects [69]. For most of these chemicals, peak plasma concentrations were in the low micromolar level [41, 69–71]. Further, for some polyphenols, biphasic and synergistic effects were reported [42, 71]. In those cases, inhibitory properties were observable at low concentrations, and stimulatory properties were observable at higher concentrations [42]. In our case, no stimulation of acetaldehyde formation from ethanol was observed with the polyphenols tested at the 10-μM level employed for this initial screening study. Further detailed studies are required to determine whether or not these biphasic or synergistic effects might occur before designing appropriate in vivo studies with ethanol-treated animals.

Conclusions

There are suggestive results that metabolic activation of ethanol in the mammary tissue cytosolic and microsomal fractions to acetaldehyde and free radicals as well as the resulting promotion of oxidative stress coupled to a defective capacity of this tissue to cope with those deleterious actions and exposure to increased estrogen levels might be involved in the alcohol drinking promotion of breast cancer. Our previously reported modulatory effects of products of purine metabolism and of plant polyphenols might be useful tools able not only to further understand the harmful properties of ethanol on mammary

tissue but also to envisage preventive or therapeutic opportunities. Those possibilities based in this working hypothesis are, however, still far from being proved and must be considered only as a challenge for further research.

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Chapter 13
Dietary Zinc Supplementation and Prenatal Ethanol Exposure

Peter Coyle, Brooke Summers-Pearce, Carina J. Cowley, and Allan M. Rofe

Key Points

- Drinking alcohol during pregnancy is associated with increased risk of spontaneous abortion, growth retardation, congenital malformations and central nervous system dysfunction.
- While the mechanism(s) of alcohol-mediated teratogenicity remains unclear, there is emerging evidence that the maternal immune response is involved.
- Metallothionein is a zinc-binding protein arising during the acute phase response that is induced in the mother's liver by a range of stressors including infections, stress and various xenobiotics including alcohol.
- Induction of metallothionein causes a whole-body Zn redistribution, where Zn is sequestered into the mother's liver, causing a reduction in plasma Zn that in turn results in a transient fetal Zn deficiency.
- Zn is critical for growth and development, and as the fetus does not store Zn, a transient deficiency in supply can result in fetal malformations and neurodevelopmental anomalies.
- Dietary Zn supplementation throughout pregnancy ameliorates ethanol-mediated teratogenicity and neurodevelopmental anomalies associated with prenatal activation of the maternal immune response.
- This chapter discusses the benefits of maintaining a positive Zn status in pregnancy and furthermore describes the current knowledge of Zn supplementation in pregnancy.

Keywords Zinc • Pregnancy • Zinc deficiency • Birth defects • Fetus • Metallothionein • Ethanol/alcohol • Infection • Zinc supplementation

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Introduction

Exposure to alcohol during pregnancy is associated with an increased risk of spontaneous abortion, growth retardation, congenital malformations and central nervous system dysfunction [1, 2]. These negative birth outcomes, which are collectively referred to as fetal alcohol spectrum disorder (FASD), range in severity from full fetal alcohol syndrome (FAS) through milder although clinically significant forms which can affect physical and behavioural outcomes (i.e. alcohol-related birth defects (ARBD) and alcohol-related neurodevelopmental disorders (ARND)). These outcomes are associated not only with chronic consumption of alcohol at high intakes and frequency but also with a single episode of alcohol intake, which is commonly called 'binge drinking' (>4 drinks/occasion). Although abstinence from alcohol during pregnancy would prevent these disorders, the motivation for self-restraint from drinking alcohol is not uniformly accepted among women [3–6]. Moreover, up to two-thirds of pregnancies are reported to be unplanned, indicating that many women may be unaware of their pregnancy when consuming alcohol [3, 5, 6]. Thus, the consumption of alcohol during pregnancy will continue to negatively impact on birth outcomes well into the future.

In spite of extensive research into alcohol-related teratogenicity, little is known of the mechanisms that underpin the cellular damage and explain the spectrum of outcomes for these disorders. It is clear that alcohol interferes with numerous molecular, neurochemical and cellular events, leading to a wide variability in the type and severity of fetal outcome. It is widely believed that alcohol-related teratogenicity occurs by a range of mechanisms that work either independently or in combination to cause negative birth outcomes depending upon the dose, duration and timing of alcohol exposure during critical stages of fetal development. Acceptance of this dogma has partly constrained further studies to determine cellular events that lead to damage or identify factors that may contribute to co-morbidity. Studies on the origin of alcohol-mediated damage are fundamental to the discovery of intervention strategies that may potentially ameliorate the morbidity. Nonetheless, new insights into primary events that initiate the pathways leading to alcohol-related teratogenicity are now arising from animal models of FAS and other disorders that affect fetal development. In this regard, a mechanism of growing interest among researchers from various scientific disciplines is that the maternal immune response in pregnancy may play a role in the teratogenicity caused by a range of events including infections, stress and various xenobiotics such as alcohol. While pro-inflammatory cytokines and oxidative species have been hallmarked as potential mediators of this damage, the findings of studies to date have been inconclusive. A hypothesis that is gaining momentum is that the maternal inflammatory response can cause a whole-body maternal zinc (Zn) redistribution. This reapportioning of Zn within the mother is thought to cause fetal Zn deficiency, which underpins much of the teratogenicity and neurodevelopmental dysfunction. The focus of this chapter is to discuss the evidence supporting this mechanism, as well as the possible merits and risks associated with dietary Zn supplementation in pregnancy, which in animal models has been found to ameliorate negative birth outcomes from various activators of the maternal inflammatory response. However, it is necessary to first review those maternal and environmental factors that are known to alter the vulnerability of the fetus to alcohol and briefly highlight some of the other potential mechanisms that have been associated with alcohol teratogenicity.

Patterns of Alcohol Exposure During Pregnancy

The incidence of morbidity and mortality in offspring from prenatal alcohol exposure has long been recognised to increase in a dose-dependent manner [7–9]. As a result, the number of drinks per occasion or peak blood alcohol concentrations (pBAC), rather than presence of alcohol in utero, is the critical factor in producing defective embryos. The higher the concentration of alcohol and the quicker it is consumed, the higher the pBAC and the more likely that a teratogenic 'threshold' is reached [10].

While it is unclear what this threshold might be, a recent study showed mothers of FAS children had higher estimated pBACs than those with children where only partial FAS expression was observed [11]. Acute alcohol intake or binge drinking results in high pBAC in the mother and similar levels in the fetus and is a pattern of alcohol consumption that is particularly harmful, even if the overall alcohol amount consumed is less than those of chronic intake patterns [12, 13]. In animal studies, a single exposure to alcohol giving high pBACs early in pregnancy can result in fetal malformations, neurodevelopmental anomalies and increased risk of postnatal death [13–17]. Similarly, children of binge-drinking mothers are found to display especially severe cognitive and behavioural deficits as well as being 1.7 times more likely to have IQ scores in the mentally retarded range and 2.5 times more likely to have delinquent behaviour [18].

Timing of Exposure During Pregnancy

The timing of alcohol exposure in pregnancy is crucial to the form of fetal dysmorphology that is observed. This is because fetal development occurs in a very structured and regulated pattern, with specific differentiation and developmental processes occurring at precise periods during pregnancy (e.g. see review [19]). Moreover, these stages of development may not be equally vulnerable to alcohol or display distinctive physical phenotypes or measurable outcomes of neurodevelopmental dysfunction. The most vulnerable period for the embryo is probably the first few weeks after conception, including the time before the woman is aware of her pregnancy. During organogenesis (i.e. 3–8 weeks of gestation in humans), alcohol exposure can cause craniofacial and brain pathologies, the most common malformations of FAS [14, 20–22]. Another critical period is during the brain growth spurt [23–25], where alcohol-induced apoptosis during this time explains the reduced brain mass and neurobehavioural disturbances that are associated with FAS [25]. Despite these windows of vulnerability, brain development may be harmed by alcohol consumption anytime in pregnancy.

Maternal Factors: Socio-economic Status, Age, Genetic and Ethnic Susceptibility

In addition to the pattern of alcohol consumption and the timing of exposure, other maternal factors may alter the likelihood of the fetus being damaged by alcohol in utero. FAS children more likely come from families impoverished or from low socio-economic status (SES) areas [10, 26–28]. This is typically seen in South Africa where the highest reported incidence of FAS is observed [29–31]. In one such study, the frequency of FAS was significantly higher among offspring born to chronic alcoholic mothers who were in low SES areas (70.9%) than offspring of upper-middle-class mothers (4.5%). Poor nutrition is commonly associated with chronic alcohol abuse and FAS [10, 32] as alcoholics often replace other energy sources in their diet with alcohol. In pregnancy, this may contribute to the severity of FAS by reducing the availability of nutrients to the fetus which are required for optimal development [33–36]. It has been argued that the difference in the incidence of FAS between socio-economic groups may be due to an interaction of genetic factors, social factors, poor nutrition and the cumulative effect of intergenerational maternal alcohol abuse [27, 28].

FAS occurs in only 4.3% of all live births in women who drink ‘heavily’ during pregnancy [26]. This low incidence of disorders in a population of women apparently at the highest risk of FAS would suggest that alcohol-related damage is not equally manifested [26], nor can the severity of fetal outcomes be fully determined by the level of alcohol exposure. Older mothers appear to be at greater risk of bearing children with FAS [28, 37]. In this regard, infants born to mothers drinking five or more

drinks per occasion at least weekly were 2– 5 times more likely to be functionally impaired when the mother is 30 years of age or older, despite having equivalent alcohol intakes to their younger counterparts. This may be due to age-related increases in maternal body fat-to-water ratio and therefore a faster rate of alcohol metabolism in younger women [38, 39].

Studies on monozygotic and dizygotic twins report similar FAS outcomes in identical twins, but not in non-identical twins [40], indicating that genetics alters the susceptibility to the effects of alcohol and this has largely been confirmed in studies with mice [41]. Disparities in alcohol metabolism in the mother, possibly a result of genetic polymorphisms of genes encoding enzymes that metabolise alcohol, have been proposed to explain differences in the peak alcohol exposure of the fetus between individuals and have been implicated in the pathogenesis of FASD [42, 43]. However, genetic differences in other pathways may be involved, for example, modulation of nitric oxide synthase expression has also been linked with neuropathology caused by alcohol-induced oxidative stress [44].

The prevalence of FAS in populations characterised by African American or people of indigenous background has been reported to be higher than those with a Caucasian background [26]. Studies have shown that African Americans or Native Americans have higher rates of alcohol elimination compared with Caucasians, indicating that different susceptibility to alcohol toxicity may occur in different ethnic groups [45]. However, variability in FASD between individuals and different ethnic groups may result from a combination of genetic and environmental factors which influence alcohol metabolism [46]. The drinking behaviour between cultures and socio-economic status (which is highly associated with race/ethnicity) may also contribute to differences in FAS between groups. Studies on Black, Hispanic, American Indian/Alaskan native and Asian/Pacific Islander women have indicated an unwillingness to quit binge drinking while pregnant compared to Caucasian women [47].

Smoking and Illicit Drug Use

Women who consume alcohol during pregnancy are more likely to smoke (tobacco) and use illicit drugs [6, 48, 49], a combination that potentially increases the incidence of FAS. Tobacco smoking or cocaine or heroin usage during pregnancy is associated with women that deliver low-birth-weight offspring [50]. This would appear to be an additive effect as the incidence of bearing a small-for-gestational-age infant was found to be highest among women who combined drinking with smoking compared to those that consumed alcohol alone [51]. In one study, 80% of mothers with FAS children were reported to smoke during pregnancy [6] to the extent that, in some studies, smoking status can predict prenatal alcohol abuse [48, 49]. In a cohort of low-income women, those that were illicit drugs abusers were also more likely to frequently drink, binge-drink and consume alcohol during pregnancy [52]. It has been proposed that smoking and/or illicit drugs may enhance the teratogenic effects of alcohol by reducing the levels of placental nutrients or reducing fetal oxygenation by impeding uterine blood flow that results in hypoxia and increased free radical formation [53].

Mechanisms of Alcohol-Mediated Teratogenicity

There have been many mechanisms proposed to explain the fetal morbidity associated with alcohol. There is currently no sufficient information to identify the most likely, and indeed, it is plausible that they all may have some relevance and possibly interact to cause the spectrum of FAS disorders in a manner dependent upon the dose, duration and timing of alcohol exposure in pregnancy; some of these potential mechanisms are highlighted below.

Ethanol and Its Metabolic Intermediates

Ethanol has been found to block various metabolic pathways. Impaired DNA myelination [54], decreased synthesis of DNA [55], altered protein synthesis [56], RNA transport [57], cell membrane fluidity and composition [58, 59] and impaired growth signalling [58] have all been purported to result from the direct effect of ethanol on these processes. It is therefore plausible that inhibition of these pathways may underpin much of the damage in FASD. This is supported by evidence that (1) alcohol diffuses freely across the placenta, reaching concentrations in fetal blood equal to that of maternal blood in women [60] and in a variety of animal models [54, 61–65]; (2) embryos cultured in alcohol in vitro show dose-related damage similar to that observed in vivo, with the most prevalent abnormalities being growth retardation and the failure of the neural tube to close [66–70]; and (3) when the metabolism of ethanol to acetaldehyde is blocked in culture experiments by inhibiting alcohol dehydrogenase with 4-methyl-pyrazole, teratogenic effects are still observed [71, 72]. However, while the in vitro findings are indicative of a direct effect of ethanol, there remains doubt as to its relevance to the whole-body system where alcohol concentrations reaching the fetus are likely to be lower and more transient. In addition, although compelling evidence that ethanol itself is the active agent, the argument does not take into consideration that by inhibiting alcohol dehydrogenase activity, a compensatory increase in peroxisomal and microsomal metabolism may occur, resulting in the production of acetaldehyde and reactive oxygen species. In this regard, acetaldehyde is a highly labile substance that is rapidly metabolised to acetic acid by aldehyde dehydrogenase in the cytoplasm. Excess alcohol is also metabolised by the microsomal alcohol-oxidising system (MEOS) or within peroxisomes. Ethanol metabolism by the MEOS involves the P450 cytochromes CYP2E1, CYP1A2 and CYP3A4, with CYP2E1 being of specific interest since it is inducible in hepatocytes and Kupffer cells at high alcohol concentrations [73, 74] and is increased in both alcoholics [75] and after a single acute dose of alcohol [76]. The MEOS produces harmful intermediates as it generates superoxide radicals [77] which can be directly toxic to the fetus, or deplete the amount of glutathione, making the body vulnerable to oxidative stress [78]. The MEOS also produces acetaldehyde from ethanol which has its own level of toxicity; however, the question remains as to whether sufficient amounts can reach the fetus after ethanol metabolism. Studies on acetaldehyde transfer from mother to fetus are conflicting. Some return extremely low concentrations of acetaldehyde, 1,000-fold less than the corresponding ethanol concentration [79], while others when conducting in vitro studies on term placenta have shown that the acetaldehyde concentration in the fetus can reach 50% of maternal perfusate concentrations [80]. In addition, the concentration of acetaldehyde required to cause teratogenicity is at variance. While some studies have demonstrated teratogenic effects at low concentrations of acetaldehyde [68, 81, 82], others have reported no teratogenicity at all [83] or at extremely high concentrations that might be considered to be pharmacological [55, 71, 84, 85].

Peroxisomal metabolism of ethanol produces reactive oxygen species via the generation of hydrogen peroxide by catalase, which is also a source of other oxygen radicals [86]. Additionally, liberation of peripheral fatty acids through activation of an adrenergic response to ethanol provides added substrate that may further accelerate the peroxisomal metabolism of ethanol. The net effect is the local and, presumably, systemic release of short-lived reactive oxygen species including superoxide anions, hydroxyl radicals, singlet oxygen and hydrogen peroxide that destroy cellular integrity by oxidising membranes, lipids, proteins, receptors and chromosomes [87, 88]. Fetal cells appear at risk from oxidative stress as they possess lower levels of superoxide dismutase and antioxidants, such as selenium and vitamin E [89]. This heightened susceptibility has been linked to genetic polymorphism [42, 43], and recent attention has focused on the gene encoding neuronal nitric oxide synthase (nNOS), with brain cell cultures from nNOS (–/–) mice being more susceptible than wild-type cells to alcohol-induced cell death [90]. The findings in animal experiments showing that co-administration of antioxidants with alcohol provides protection against teratogenicity is compelling evidence that ROS

are causative agents in the cascade of events that lead to fetal cell toxicity [91, 92]. Presumably, antioxidants prevent ROS from reaching the fetus by quenching them as they are formed in the liver. Alternatively, they may prevent the initiation of an inflammatory response in the mother's liver, which may harm the fetus in other ways.

The rapid metabolism of excess ethanol may also affect the cellular redox state due to the marked change in the NAD+/NADH ratio that could influence a plethora of key regulatory steps in various metabolic pathways. Large quantities of ethanol may also upset the lining of the gastrointestinal tract and increase its permeability, allowing bacterial-derived endotoxin to permeate into the systemic circulation, thus activating a maternal inflammatory response in the mother [93]. This link with inflammatory mediators (cytokines and ROS) provides a new direction for understanding how ethanol may influence the maternal-fetal interrelationship including a potential effect on Zn redistribution and fetal Zn supply.

Ethanol Interfering with Retinoic Acid Synthesis

Ethanol is a competitive inhibitor of retinoic acid (RA) synthesis, the oxidised form of retinol (also known as vitamin A). RA acts as a transcriptional regulator by binding to specific receptors that signal the initiation of a cascade of events that affect gene expression and, ultimately, controls anterior and posterior patterning in early developmental stages [94, 95]. RA is particularly high in the developing embryo where RA signalling occurs through Hox genes that regulate a network of pathways important in organogenesis and the development of the CNS [96]. The conversion of retinol to RA occurs through the same two-step enzymatic process as the metabolism of ethanol; retinol is first oxidised to retinal in a reaction catalysed by alcohol dehydrogenase before being converted to RA by aldehyde dehydrogenase. Thus, it has been proposed that ethanol competes with retinol for alcohol dehydrogenase, resulting in lower amounts of RA being synthesised. This is supported by evidence from studies conducted in zebrafish, mice and frogs which showed that developmental defects in alcohol-treated embryos can be prevented by providing RA supplementation [97–99]. However, the findings from mutation studies indicate that aldehyde dehydrogenase-2 (RALDH2) is the key enzyme controlling the synthesis of RA in early embryogenesis [100, 101], and a more recent study has suggested that an alcohol-mediated reduction in its activity may be an alternative cause of alcohol-mediated decrease in RA signalling and teratogenicity. In that study, which was conducted on *Xenopus laevis*, developmental defects characteristic of high alcohol exposure were obtained when RALDH2 activity was partially inhibited in the presence of low concentrations of alcohol. Over-expression of RALDH2 activity, concurrent with high alcohol concentration, resulted in higher RA signalling and rescuing from developmental malformations. In RALDH2 knockdown studies, a similar reduction in RA signalling was found regardless of whether this was carried out alone or in combination with alcohol treatment, evidence that further supports RALDH2 being the main enzyme that is targeted by alcohol [102].

Excess RA can also cause teratogenicity and shares many of the phenotypic manifestations of FAS including heart defects, craniofacial malformations and CNS abnormalities [102–106]. Evidence that alcohol may mediate and increase RA signalling comes from studies in pregnant mice where the effects of prenatal alcohol on all-*trans*-retinoids were quantified in various fetal tissues. Acute alcohol administration was found to increase RA levels in the fetal hippocampus (1.6-fold), liver (2.4-fold) and testes (1.5-fold), whereas 20-fold and 50-fold increases were found in the fetal hippocampus and cortex, respectively, after chronic alcohol feeding [107]. This data would seem to indicate that at least in fetal brain, high levels of RA coexist at sites commonly associated with alcohol-mediated injury.

Clearly, more studies are required to determine whether prenatal alcohol consumption at levels used in animal models of FAS results in low or high RA levels and whether this in turn alters RA signalling that can be linked to fetal cell toxicity.

Ethanol Altering Prostanoid Metabolism

Based upon evidence that raised levels of prostanoids are found in maternal and fetal tissues after prenatal alcohol administration, and that non-steroidal anti-inflammatory drugs (NSAID) can protect against alcohol-mediated teratogenicity, it has been proposed that prostanoids are the active toxic agents underlying the fetal damage caused by alcohol. Prostanoids, including prostaglandins (PGE_2, $PGF_{2\alpha}$, PGD_2, PGJ_2), prostacyclin (PGI) and thromboxane (TX), are peroxidation products of membrane phospholipids that have hormone-like properties but are short-lived and act locally to mediate a diverse range of physiological functions [108]. In pregnancy, prostanoids are crucial for implantation, fetal growth, neurodevelopment and in the initiation of labour [109–112]. They have divergent vasoactive roles and regulate the contraction or relaxation of smooth muscle (e.g. TX is vasoconstrictive and promotes platelet aggregation, while PGI is a vasodilator and inhibits platelet aggregation). Differential regulation is maintained by controlling the expression of the enzymes that synthesise prostaglandins, COX-1 and COX-2. COX-1 is constitutively produced in all cell types, whereas COX-2 is induced by inflammatory stimuli including cytokines, bacterial endotoxin and in endothelial cells by shear stress [113, 114]. It is argued that oxidative stress, manifested by increased lipid peroxidation and decreased antioxidant protection, may be the cause of the altered prostanoid metabolism. In this regard, increased TX production and decreased PGI levels have been found in preeclampsia, where it has been proposed that placental vasoconstriction may occur unopposed, resulting in maternal hypertension and decreased utero-placental blood flow [115].

Early studies demonstrated that PGEs are teratogenic in various animals models [116, 117]. However, there is doubt as to the relevance of these studies since very high doses of prostanoids were used, well above those likely to be reached in the mother after alcohol consumption [10]. Nonetheless, a number of studies using various animal models have reported findings that implicate prostanoids in alcohol-mediated fetal damage. In a study using fertilised chicken eggs, alcohol was found to decrease chick brain weight by 19% but not when administered with indomethacin, an NSAID that non-selectively inhibits COX-1 and COX-2 [118]. Similarly, when alcohol was administered to pregnant mice on gestational day (GD) 10, high levels of TX and PGE were found in uterine/embryo tissue. This was associated with an increased incidence of prenatal mortality and teratogenicity, both of which were attenuated by pretreatment with indomethacin or aspirin (a selective COX-1 inhibitor) [7, 119, 120]. In studies on near-term pregnant ewes, PGE_2 and TX were increased in maternal and fetal plasma and CSF after alcohol administration, and this occurred concurrently with suppression of fetal breathing. When indomethacin was given shortly after the alcohol, the fetal breathing rate improved but was then reversed by administering PGE_2, indicating that alcohol-induced suppression of fetal breathing, and potentially hypoxia sufficient to cause fetal damage, was due to increased prostanoids [121, 122]. As further reports have not been forthcoming, several questions remain unanswered. Are the elevated levels of prostanoids found in mother and fetus after alcohol administration the cause of cytotoxicity or markers of the inflammatory process? Do prostanoids levels in blood, urine or in uterine/embryo systems reflect biological activity of prostanoids in tissue, where local rates of synthesis and degradation are more likely to dictate their paracrine or autocrine action? It has also been argued that the protection offered by NSAIDs against alcohol-mediated birth defects may be independent of their COX-enzyme inhibitory role. For example, NSAIDs are known to be chelators and may sequester free iron that would prevent Fenton reactions and thereby reduce the number of reactive oxygen species [10]. Advances in anti-inflammatory drugs that inhibit specific cytokines and non-prostanoid components, as well as knockdown studies on specific prostanoid-producing synthetases, may further help clarify the potential involvement of individual prostanoids in alcohol-related birth defects.

Impaired Placental Nutrient Delivery

It has been proposed that alcohol-mediated changes in placental function and/or umbilical cord blood flow may influence nutrient transfer to the fetus during development [123, 124]. This premise is supported by the findings from human and animal studies. In a study on 13 alcoholic women, placental weights at term were significantly lower (526 +/−116 g) compared to controls (653 +/−77 g) [125]. Similarly, when pregnant mice were administered various concentrations of alcohol in their drinking water from GD 11 to 18 (mouse gestation; 21 days), placental weight decreased with increasing alcohol consumption [126]. Abnormal placental histopathology appears to be consistent with chronic alcohol exposure. Abnormal placental membranes were shown to be more prevalent in a group of alcohol-exposed women from an alcohol treatment programme compared to control women [127]. Villus infarction and the presence of intervillous thrombi were also more common in alcohol-exposed pregnancy, with 22% of alcohol abuse cases displaying villitis, a condition associated with intrauterine growth restriction [128]. In rodents, alcohol exposure during pregnancy was found to cause advanced degenerative changes in the basal zone of the placenta [126] and, in another study, impair the conversion of uterine vessels required for expansion of maternal circulation into the placenta during the period of placentation [129]. Chronic alcohol exposure in pregnant rats has been shown to redistribute blood, decreasing the supply to the placenta [130]. Alcohol exposure in human placentas induced placental vasoconstriction in a dose-dependent manner and increased fetal-placental vascular resistances and perfusion pressure [131, 132]. A transient but marked collapse in umbilical vasculature in pregnant monkeys was observed within 15 min after intravenous injection of alcohol, producing severe hypoxia and acidosis in the fetus [133]. Furthermore, alcohol caused dose-dependent contractions in isolated human segments of umbilical cord veins and arteries, lasting as long as the alcohol was present [134, 135], suggesting that alcohol may increase umbilicoplacental resistance and thereby decreased maternal-fetal blood flow.

The fetus is dependent on efficient placental function and blood flow for the delivery of oxygen and essential nutrients from the mother. Thus, any interference in the delivery of these nutrients is likely to be detrimental to fetal growth and development. In this regard, prenatal alcohol exposure has been found to impair the placental transport of a number of important nutrients (reviewed by [123]) including amino acids [136, 137] particularly after chronic alcohol consumption [138, 139]; the vitamins pyridoxal (B6), biotin and folic acid [140–142]; n-3 polyunsaturated fatty acids [143]; and glucose [144, 145].

Zinc Deficiency During Pregnancy

There is considerable experimental evidence linking alterations in Zn homeostasis with the teratogenic effects of alcohol. In rodents, there are remarkable similarities in the adverse pregnancy outcomes associated with Zn-deficient and alcohol-exposed dams. These include increased fetal resorptions, low birth weight, anophthalmia, exencephaly, clefts of the lip and palate, major skeletal defects and impairments in neurodevelopment, resulting in cognitive anomalies in offspring [16, 17, 146–153]. Moreover, Zn deficiency and alcohol both cause programmed cell death in specific embryonic cell populations [154–156]. It has also been noted in rodents that concomitant short- or long-term Zn deficiency and alcohol consumption during pregnancy are synergistic with an increased incidence of fetal abnormalities compared to either insult alone [150, 157].

Zn has well-described roles in a plethora of biological processes, such that maintaining a positive maternal Zn status is paramount for a successful pregnancy outcome (see reviews [158, 159]). The unique size of the Zn^{2+} ion and its strong electrophilic nature allow Zn to cross-link with oxygen, nitrogen and sulphur species on amino acid side chains, thus forming a range of coordination

geometries in a multitude of proteins and enzymes. The functions of these protein interface Zn sites include catalysis or inhibition of enzymes or other activities, the stabilisation and induction of folding of protein sub-domains, including dimerisation of proteins and formation of protein/receptor complexes, and packaging of proteins for storage [160]. Consequently, Zn is pivotal to fundamental processes such as transcription, translation and cellular differentiation, which are critical for fetal growth and development. Zn metalloenzymes include DNA polymerase, reverse transcriptase, RNA polymerase, tRNA synthetase, protein chain elongation factor, thymidine kinase and ribonucleases [161, 162]. In addition, there are over 2,000 'Zn-finger' transcription factors that regulate the genetic code affecting a diversity of functions. Zn is essential for the epigenome, and there is now emerging evidence that Zn is involved in pathways for generating and controlling methylation equivalents (i.e. methionine cycle/transsulfuration pathway), as well as in the structures of enzymes that epigenetically modify DNA and histones (e.g. DNA methyltransferases and histone deacetylases). If these pathways or enzymes are affected by Zn deficiency, this could result in changes in heritable gene expression without alterations in DNA sequence, leading to similar adverse fetal outcomes (e.g. teratogenicity and cognitive impairments) to those associated with folate deficiency [163]. Zn is also required for membrane integrity. Free Zn ions play an important role in cellular signalling, and a growing list of molecular targets has now been identified. Zn acts as a neuromodulator and is selectively stored and released from neurons, specifically those that release glutamate in the cerebral cortex that affect both cognition and behaviour [164]. In summary, Zn participates in protein, nucleic acid, carbohydrate and lipid metabolism, as well as the control of gene transcription and the regulation of cell proliferation, differentiation and apoptosis [158, 159]. Thus, Zn has far-reaching roles that affect virtually every cell and process in the body, and this is most clearly demonstrated in reproduction.

Studies involving species as diverse as rodents, pigs, sheep and monkeys demonstrate the catastrophic effect of maternal Zn deficiency to the fetus during pregnancy [147, 165–168] (see review [169]). While virtually all organ systems can be affected, the degree of dysmorphology appears largely to depend upon the severity and duration of Zn depletion in utero. In rats, Zn deficiency throughout pregnancy has been shown to reduce fetal body weight, with 90% of the fetuses demonstrating gross malformations affecting every organ system [170]. Nonetheless, acute or short-term Zn deficiency can also be teratogenic, with altered incidences of abnormalities depending upon the timing of Zn depletion relative to the stage of development, similar to that observed with the specific timing of alcohol exposure. When Zn deficiency occurs early in pregnancy, it is associated with defects of the head region, including the eyes, facial structures and brain. Later in pregnancy, Zn deficiency results in a more frequent incidence of skeletal malformations [168] and with defects in the urogenital system and tail. The most vulnerable period for fetal malformations occurs during organogenesis (days 7–12 in rodents that have gestation period of 21 days). A number of studies have reported that Zn deprivation during pregnancy and lactation can result in poor fetal activity, newborn motor development, learning and long-term, short-term and working memory in adult offspring [167, 171–176].

Accumulating evidence from studies using animal models indicate that the maternal plasma Zn concentration is exquisitely sensitive to dietary Zn insufficiency and is the primary determinant of the amount of Zn that is exchanged between mother and fetus. The latter reflects the fact that plasma Zn is the main 'exchangeable' pool of Zn in blood for maternal-to-fetal Zn interchange. In rodents, maternal plasma Zn levels fall by 30% after a single day of feeding rodents a Zn-deficient diet [168, 177]. A striking demonstration of the disastrous consequences and rapid onset of Zn deficiency in humans is found in the genetic disorder acrodermatitis enteropathica (AE), a rare (1/500,000 children) autosomal recessive disorder that results in insufficient Zn uptake by the duodenum and jejunum [178]. Infants with AE present with severe symptoms of Zn deficiency, including acral dermatitis, alopecia, growth arrest, reduced immune function and neuropsychological disturbances [179]. These infants appear normal at birth but have rapid and progressive onset of symptoms when weaned from breast milk. The disorder is caused by a defective gene identified as SLC39A4, which encodes a Zn transporter protein, Zip4, responsible for the absorption of Zn in the upper small intestine. It is thought that

these infants are protected by breast-feeding because the bioavailability of Zn in human milk is greater than that from cow's milk or by dietary means. While the disorder can be fatal, early diagnosis and therapy with excess Zn in their diet returns normal function which can be maintained over a lifetime if the patient is compliant with the treatment [179, 180]. The disorder demonstrates how rapidly the exchangeable Zn pool in the human body is depleted and how vulnerable humans are to deficits in Zn supply. Although severe Zn deficiency in humans is rare, marginal Zn deficiency caused by suboptimal intake is common and may be prevalent in up to half of the world's population. This is because of the poor bioavailability of Zn from many plant-derived staples forming the basic diet of the third world population. Unlike red meat which is rich in highly available Zn, most plant staples also contain phytic acid, a strong binder of Zn in the gut that prevents its absorption. While it is difficult to assess the accumulative effect of a marginal Zn intake on pregnancy outcome in communities where poor Zn intake is endemic, many studies have reported a higher risk of pregnancy complications in women who have a low plasma Zn concentration (reviewed by [169]).

Adaptive Response to Zinc Utilisation in Pregnancy

The importance of Zn in reproduction is also reflected in the maternal compensatory mechanisms that occur throughout pregnancy to retain Zn. Daily Zn requirements increase from 2.0 mg at the beginning of gestation to 2.6 mg at the end [181]. Based upon the bioavailability of Zn being 25% from the diet, it is estimated that at least 10.5 mg/Zn per day is required to meet the Zn requirements late in gestation. As women do not increase their Zn intake during pregnancy, an adaptive response in Zn uptake and/or retention has been proposed to meet this increased need. Urinary Zn excretion has been shown to be less in pregnant women with equivalent Zn intake; however, this effect appears to diminish late in pregnancy [182]. In studies on intestinal loops from pregnant rodents, an 80% increase in the duodenal Zn uptake and transfer has been reported, independent of other nutrient absorption [183, 184]. An adaptive response in Zn absorption also occurs in humans when Zn requirements are increased during pregnancy and lactation [185, 186]. In a study using stable Zn isotopes that was conducted using women from northeast China, the fractional absorption of Zn was found to be 70% higher during the second month of lactation compared with non-lactating women on similar low Zn intakes. These women also increased their total food intake during lactation, thereby increasing their overall intake of Zn by 50%. Faecal endogenous Zn losses were also lower [186]. A similar finding was reported in a longitudinal study that showed that fractional Zn absorption was increased during lactation but was not significantly different in the period before conception and the end of the second trimester in women on normal Zn intakes [187]. However, a second study conducted on Brazilian women with low Zn intakes found that the fractional Zn absorption increased from 29% to 43% from the beginning to the end of pregnancy and remained at this level during lactation. Here, the increase in fractional Zn absorption was inversely proportional to plasma Zn concentrations [188].

Metallothionein: A Link Between Prenatal Alcohol Exposure and Fetal Zinc Deficiency

While it is clear that Zn is necessary for fetal development and that a deficiency of Zn during pregnancy leads to similar adverse fetal outcomes to those caused by alcohol, an important question that needs to be addressed is how does alcohol intake in pregnancy lead to a Zn deficiency in the fetus? It could be argued that reactive oxygen species formed by microsomal metabolism of alcohol might cause prostanoid release that could restrict maternal/fetal blood flow and nutrient uptake by the fetus.

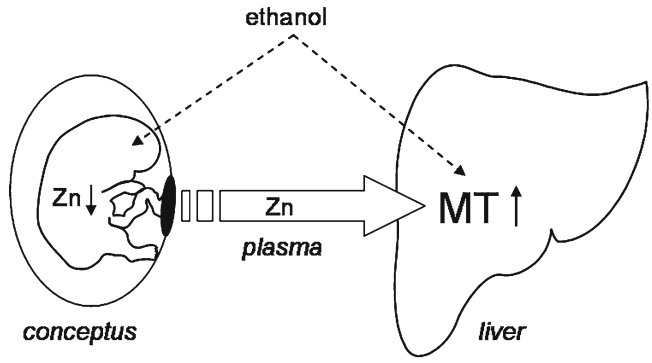

Fig. 13.1 The effect of ethanol-mediated induction of hepatic metallothionein (*MT*) on maternal-to-fetal Zn transfer. Ethanol induces the Zn-binding protein, MT, in the mother's liver. This causes a redistribution of whole-body Zn, as Zn is sequestered locally and systemically via the plasma to be incorporated into the tertiary structure of MT within the hepatocyte. The net result is a reduction in the Zn concentration in the mother's plasma which is the conduit of Zn to the fetus. Maternal-to-fetal transfer of Zn is bidirectional depending upon the Zn gradient; thus the fall in plasma Zn impairs fetal Zn uptake. As the fetus does not store Zn, any impediment to Zn transfer is either detrimental in itself (as Zn is an essential element in growth and development) or exacerbates the direct effects of alcohol on the fetus since Zn is an antioxidant and has roles in anti-apoptotic and repair processes

Such a mechanism, however, would be expected to cause a multi-nutrient deficiency in the fetus rather than one pertaining to a single element. Moreover, one might expect that long-term alcohol exposure would more likely potentiate nutrient deficiencies in the fetus than short-term or acute alcohol exposure. In this regard, alcohol was not found to inhibit the uptake of Zn in perfusion studies on human term placenta [189].

A mechanism that continues to receive growing support was proposed by Daston and colleagues [190] in their seminal work. In their studies, which were conducted on pregnant rats, they demonstrated that a range of xenobiotics (including ethanol), hormones and inflammatory mediators can alter Zn homeostasis by inducing a Zn-binding protein in the liver, called metallothionein (MT). Induction of this protein was found to result in Zn sequestration into the mother's liver primarily from the maternal circulation, consequently causing a reduction in plasma Zn that was found to impair the fetal uptake of Zn [190]. In the first study, urethane injection in pregnant rats on GD 11 was found to significantly induce maternal liver MT, decrease maternal plasma Zn concentrations by 30% and inhibit the transfer of ^{65}Zn into the fetus by 50% [190]. Fetuses exhibited decreased weight and delayed skeletal ossification when examined on GD 18. A range of other teratogenic compounds with different pharmacological actions, such as α-hederin, TNF-α, 2-ethylhexanoic acid, arsenic and alcohol, were later demonstrated to similarly induce maternal hepatic MT causing Zn redistribution and fetal dysmorphology [191–194]. A number of cytokines (IL-6, IL-1β and TNF-α), hormones (glucocorticoids, glucagon), metals (Cd, Zn) and exogenous compounds (endotoxins, turpentine, reactive oxygen species and xenobiotics including ethanol) have now been found to induce hepatic MT expression, independently and synergistically (for reviews see [161, 195, 196]) (Fig. 13.1).

MT is a well-characterised protein of low molecular weight (6–7KDa) where one-third of the amino acids are cysteine residues. This high sulphydryl content results in the binding of 7-gramme atoms of Zn per molecule when forming the tertiary structure of the protein. While a clear physiological role for MT has not been forthcoming, the fact that it is rapidly and highly inducible during an inflammatory response suggests that the ensuing whole-body Zn redistribution is an important component of the immune response. In this regard, MT could be considered to be an acute phase protein differing only in that it is not exported from the hepatocyte into the circulation. In the setting of maternal Zn

deficiency, MT provides a reproductive advantage as surviving embryos of MT-knockout mice show greater morphological abnormalities than wild type [197].

Our group confirmed and expanded on the studies of Daston and colleagues [190, 192] using a C57BL/6 mouse model. Firstly, we demonstrated that a single binge of alcohol on GD 8, which caused blood alcohol concentrations to reach 0.2–0.3% over 8 h, resulted in a 20-fold increase in maternal liver MT and a 65% reduction in maternal plasma Zn concentration within 16 h of the alcohol insult [198]. We found that the alcohol-mediated decrease in maternal plasma Zn markedly impaired the transfer of ^{65}Zn into GD 12 fetuses and caused a 20% reduction in total fetal Zn content, 3 h after an acute alcohol intake [199]. We then used a MT-knockout mouse to demonstrate the involvement of MT in teratogenicity. This genetically modified mouse was derived by Michalska and Choo [200] and lacks the genes that code for two inducible isoforms, MT-1 and MT-2. In studies where we injected alcohol in wild-type dams on GD 8, we observed a 27% increase in the incidence of fetal dysmorphology that was associated with a decreased maternal plasma Zn concentration. Abnormalities pertaining to the eye contributed 50% of the total abnormalities in the wild-type fetuses from alcohol-treated dams. However, when we administered the same dose of alcohol to pregnant MT-knockout mice, we found a very low frequency of abnormalities that was even lower than in our saline-treated controls (2.2%) [198]. This was compelling evidence that MT was associated with alcohol-mediated teratogenicity. We further found that following alcohol exposure in the MT-knockout mouse, the mother's plasma Zn levels increased rather than decreased as in wild-type mouse, a finding that results from a direct effect of alcohol on maternal muscle and skin that causes the release of Zn into the mother's blood [199]. We also confirmed that ^{65}Zn transfer into the fetus of MT-knockout dams was unaffected by alcohol administration, a finding that is in stark contrast to the impairment of Zn transfer we observed in wild-type fetuses [199].

We have also found that ethanol-mediated induction of MT accompanied by maternal hypozincaemia is not restricted to early pregnancy [201]. However, whether the impact of such induction is as devastating to the fetus in mid- and late pregnancy is unknown. It is plausible that the embryo would be at a higher risk from lower concentrations of maternal plasma Zn in early pregnancy when albumin-bound Zn is critical for phagocytotic processes that allow Zn absorption through the yolk sac and uterine glands [202]. Later in pregnancy when the placenta is functional and the fetus has developed its own homeostatic mechanisms for Zn, temporary reductions in maternal blood Zn might be expected to have less influence. While the transition from histotrophic nutrition to a functional placenta occurs earlier in humans than in rodents [203], it nonetheless is thought to be an important source of nutrients throughout organogenesis in the first trimester [204].

Considering that damage to the developing brain is arguably the most socially and economically disruptive problem related to prenatal alcohol exposure, we also investigated whether a single binge of alcohol in early pregnancy could result in cognitive impairments. Studies in rodents [20, 205] and monkeys [206, 207] have shown that prenatal alcohol exposure has a marked effect on brain growth and cognitive function. The deficits caused by alcohol appear mainly due to degenerative changes in the basal forebrain, neocortex and hippocampus that are characterised by reduced numbers of neurones, lower dendritic spine density on pyramidal neurons and changes in synaptic activity [208]. However, evidence suggests that cognition may be affected by alcohol very early in pregnancy and long before these brain structures have developed. In studies on macaque monkeys who were fed alcohol weekly during their pregnancies, the most developmentally delayed infants were born to mothers whose drinking began as early as week 1 of pregnancy compared to those starting on week 5 and regardless of whether higher doses of alcohol were commenced at week 5 [206, 207]. Our C57BL/6 mouse model shows the full range of birth defects and cognitive deficits in offspring caused by acute alcohol administration early in pregnancy [15–17, 146, 209]. In our studies, we have taken offspring with no visible abnormalities that were prenatally exposed to alcohol on GD 8 and demonstrated that they have significant cognitive and behavioural abnormalities. Adult offspring randomly selected from litters of alcohol-treated dams performed poorly in spatial memory in a water cross maze escape task and in object recognition memory [15, 16].

Zinc Supplementation Protects Against Alcohol-Mediated Birth Abnormalities

In more recent studies, we have been able to demonstrate that the nutritional Zn status of the mother is a major determinant of alcohol-related fetal dysmorphology, and in this regard, we now can prevent birth abnormalities caused by alcohol with prenatal Zn treatment. In studies in wild-type mice, where we elevated the mother's Zn status by injecting Zn subcutaneously concurrent with ethanol treatment on GD 8, we found that the incidence of physical birth abnormalities was no greater than that in controls [209]. In addition, we found that subcutaneous Zn treatment also prevented spatial memory impairments caused by prenatal alcohol exposure on GD 8 [16]. These findings support an earlier study which demonstrated that intraperitoneal Zn treatment has a protective influence against ethanol teratogenicity, as ethanol + Zn-treated fetuses had a higher number of somites, cardiac development was more advanced and embryonic protein content was higher than ethanol alone [210]. While these studies demonstrate that maternal plasma Zn levels can be altered to limit ethanol teratogenicity, the administration of Zn via injections is not a desirable method of delivery and subcutaneous Zn treatment transiently increases the maternal plasma Zn to levels that may be viewed as being non-physiological (5 times higher than normal) [209]. This raised the question of whether dietary Zn supplementation, a more generally accepted and less invasive form of Zn treatment, could also alter the effects of alcohol on maternal Zn homeostasis and significantly increase the maternal Zn status to protect against ethanol teratogenicity.

It has previously been discussed [209] that bypassing the gastrointestinal processing step may be necessary to obtain an increase in the resultant plasma Zn concentration. Indeed, in the gastrointestinal tract, MT is thought to play a role in restricting Zn absorption in times of excess [211]. While it is unlikely that oral Zn supplementation can increase the maternal plasma Zn to levels comparable to those achieved by subcutaneous Zn injection, several studies have indicated that high dietary Zn intakes during pregnancy can increase plasma Zn levels to some degree [212]. In addition, Mendeleson and Huber [213] found that the reduction in fetal Zn caused by long-term ethanol exposure (6% ethanol in drinking water) throughout pregnancy was prevented by diets fortified with Zn [213]. A number of studies have previously examined the influence of dietary Zn supplementation on ethanol-related birth defects. Tanaka and colleagues demonstrated that excess Zn in the diet in ethanol-treated pregnant rats resulted in an increased fetal body weight and increased protein content of the cerebrum and prevented the resorptions obtained with ethanol alone [214]. It also increased the metabolic activity in the hippocampus (evidence of prevention of the brain dysfunction by ethanol treatment) and increased cerebral weight and RNA compared to ethanol alone [215]. They later found no benefit of Zn supplementation [216, 217]. Keppen and colleagues [218] also found that supplemental Zn (four times the recommended daily allowance) was not protective against the effects of ethanol on fetal development and appeared to have an adverse effect on fetal weight and prenatal mortality [218]. These inconsistent findings, however, were all from studies which used a chronic alcohol model (i.e. ethanol is continuously consumed in the diet throughout pregnancy) rather than a 'binge' alcohol model in which we have shown that a transient MT-induced Zn deficiency is involved in the aetiology of teratology [198].

Using our 'binge'-alcohol mouse model, we have recently demonstrated that dietary Zn supplementation throughout pregnancy (200 mg Zn/kg vs. 35 mg Zn/g control diet) prevents physical birth abnormalities caused by ethanol exposure on GD 8 [17]. It also was beneficial in preventing postnatal mortality associated with GD 8 alcohol exposure. More stillbirths were born to dams given alcohol alone compared to those also given dietary Zn supplementation, and the cumulative postnatal mortality for the 60 days after birth was significantly higher in offspring from alcohol-treated dams (35% deaths) compared to those also treated with dietary Zn supplementation (12% deaths; saline controls, 10%) [17]. Furthermore, by supplementing the dams diet with excess Zn throughout pregnancy, we found that offspring exposed in utero to alcohol had normal cognitive scores (i.e. dietary Zn prevented spatial and object recognition memory impairments caused by alcohol) [15].

While dietary Zn supplementation did not affect liver MT concentrations or the MT response following alcohol exposure on GD 8, it did significantly increase maternal plasma Zn concentrations. Dams on the Zn-supplemented diet had higher plasma Zn concentrations prior to (20% higher) and following alcohol exposure on GD 8 (66–80% higher) than those on the control diet, with a significant increase rather than decrease in plasma Zn in response to alcohol induction of hepatic MT [17]. This response may be explained by dynamics of plasma Zn homeostasis. Plasma Zn is an exchangeable Zn pool that represents only 0.1% of total body Zn and hence at any given time reflects the equilibrium between tissue requirements, secretion and intestinal Zn absorption [161]. Thus, mice fed a Zn-supplemented diet presumably have a larger Zn reserve possibly bound to or internalised within the mucosa of the gut wall to be mobilised and replete the plasma compartment after liver MT sequestration than those fed normal Zn diets.

There are several possible mechanisms by which a positive Zn balance may be protective. Zn supplementation may prevent the fetal Zn deficiency arising from the fall in plasma Zn levels, which we have shown to be transiently decreased by up to 65%, due to the alcohol-mediated induction of MT in the mothers liver [198]. That Zn treatment overwhelms the MT response so that Zn can be accessed and utilised by the fetus is supported by our findings where plasma Zn concentrations did not decline but increased above baseline after alcohol exposure in dams given a Zn injection or supplemented with dietary Zn and prevented alcohol-related impairments [16, 209]. However, the possibility that Zn has MT-independent effects cannot be overlooked. Ethanol is known to generate free radicals which are key factors involved in the induction of apoptosis [219, 220]. Apoptosis has been well characterised in various fetal tissues following alcohol exposure during pregnancy and is suggested by other studies to be the cellular basis for alcohol-related birth defects [154]. Zn is involved in a number of anti-apoptotic pathways [221–223], and Zn treatment has been shown to promote cell survival after exposure to other teratogenic agents [222]. Thus, Zn treatment may influence repair mechanisms in the fetus by preventing apoptosis and protecting against alcohol-generated oxidative stress. Regardless of the mechanism of protection, these studies nevertheless demonstrate that the higher-than-normal plasma Zn levels following dietary Zn supplementation are sufficient to reduce teratogenicity, postnatal mortality and cognitive impairments associated with acute alcohol exposure in early pregnancy. They also provide further evidence that fetal Zn insufficiency caused by a low maternal plasma Zn is a key mediator of alcohol-related teratology.

Zinc Protects Against Infection-Mediated Birth Abnormalities

In studies paralleling those with ethanol, we administered bacterial endotoxin, lipopolysaccharide (LPS), to mice on GD 8 in order to activate a maternal immune response. These studies were performed because a growing body of evidence suggests that a maternal immune response in pregnancy may underpin fetal dysmorphology and neurodevelopmental anomalies associated with a wide range of infectious agents of both bacterial and viral origin [224–231]. Early in infection, inflammatory cytokines mediate a complex change in acute phase reactants in the host's liver, with the induction of MT being a component of this acute phase response. We found that similar to ethanol, LPS caused a marked induction of maternal hepatic MT and maternal hypozincaemia that was associated with an increased incidence of fetal malformations and cognitive impairments in offspring. In addition, LPS caused teratology in wild-type mice but not in MT-knockout mice [232]. We also found that the frequency of LPS-related abnormalities was inversely proportional to the amount of Zn in the mother's diet [233] and that Zn supplementation of wild-type mice throughout pregnancy prevented teratogenicity as well as cognitive and behaviour changes in their offspring [234]. These findings are

consistent with both LPS- and ethanol-related teratogenicity being mediated by a MT-mediated mechanism that results in Zn being redistributed in the mother and away from the fetus to its detriment. The findings of our studies further point to the maternal immune response being the likely mediator of both ethanol- and LPS-mediated teratogenicity. In studies on primary cultures of mouse hepatocytes using varying concentrations of ethanol in the culture medium, we found that ethanol was not a primary inducer of hepatic MT (unpublished data). More recently, in our prenatal ethanol mouse model, we have found that high blood alcohol concentrations are associated with an increase in pro-inflammatory cytokines TNF-α and IL-6 (unpublished data). Thus, it is plausible that hepatic MT is induced as part of an acute phase response to the pro-inflammatory cytokines that are released when alcohol levels are sufficiently high to cause inflammatory damage. This damage may be exacerbated by the microsomal metabolism of alcohol that releases reactive oxygen species into the hepatic milieu when the dehydrogenase pathway is overloaded. A response element in the promoter region of the MT gene has been identified which is sensitive to ROS, and their involvement in MT induction might in part explain why antioxidants are effective in protecting the embryo against alcohol-mediated birth defects [88, 235, 236].

Co-teratogenic Factors

It is clear from our findings that high blood alcohol concentrations are linked to the activation of a maternal acute phase response that causes the hypozincaemia and limitation in fetal Zn supply. While Zn limitation of less than 24 h is sufficient to cause fetal deformities in mice, this period can only be surmised in humans. In this regard, the half-life of MT is approximately 20 h, so the vulnerable period of Zn limitation could be up to 48 h in humans. These high blood alcohol concentrations are likely to be achieved after single or episodic consumption of large quantities of alcohol that could result from heavy social drinking or be a part of the binge-like behaviour associated with chronic alcoholism. It has long been reported that alcoholic mothers have significantly lower plasma Zn levels than non-alcoholic women and that an inverse relationship occurs between maternal plasma Zn levels and expression of FAS [237]. Nonetheless, the vagaries of an individual's tolerance to alcohol and the possibility that certain maternal conditions may make an individual more vulnerable to alcohol make it unlikely that any intake of alcohol is safe in pregnancy. If MT induction underpins ethanol-mediated teratogenicity and neurodevelopmental abnormalities in humans, then one might speculate that if another inducer of MT is raised at the same time, then this would compound the effect of alcohol. It is well described that the promoter region on the MT gene is activated by a range of factors including reactive oxygen species, inflammatory cytokines (IL-1β, TNF-α and IL-6), corticosteroids and certain xenobiotics [161, 195, 196]. Many of these effectors work in combination on the MT gene to give additive or synergistic responses on its transcription. Thus, it can be predicted that in a mother with a pre-existing condition that induces MT, even small amounts of ethanol might further amplify the hepatic MT pool sufficiently to cause hypozincaemia and impair fetal Zn uptake. In this regard, it is well recognised that infection, inflammation, severe stress and chronic disease cause hypozincaemia in humans through hepatic MT induction. Poor nutrition with inadequate Zn intake would also be likely to aggravate the detrimental effect on alcohol in pregnancy [169] and, as previously mentioned, have a synergistic effect on adverse outcomes when combined with alcohol exposure [150, 157]. All in all, it is perhaps not surprising that a much higher incidence of FAS and ARND has been reported among some Australian indigenous communities where poor nutrition, chronic infections and substance abuse coexist with added stress to women of physical abuse and family breakdown [238–240].

Could Dietary Zinc Supplementation Be Beneficial?

Although we found in mice that dietary Zn supplementation in pregnancy ameliorates the adverse effects of alcohol on birth outcomes, there are many questions that still need to be addressed before this could be applied to humans. It must be made clear that the authors do not imply that dietary Zn supplementation should be used as a prophylactic measure so that women can continue to imbibe during pregnancy or that we would recommend to manufacturers that they should enhance their alcoholic beverages with Zn in order to safeguard their product. In fact, our findings confirm that the only safe option is no alcohol consumption in pregnancy. Indeed, a MT-mediated mechanism of teratogenicity identifies a range of maternal factors that may heighten the risk to the fetus from alcohol and therefore supports a premise that even low-level drinking may still carry a risk. The current health guideline in Australia recommends that for women who are pregnant or planning a pregnancy, not drinking is the safest option since the relative risk has not been determined across a range of drinking levels, nor is there sufficient evidence of genetic and age variability to alcohol. We would now add to this list the other predisposing maternal factors that may amplify a MT-mediated response to alcohol. Nevertheless, there may be some communities that have a high incidence of FAS, where women could benefit from dietary Zn supplementation during pregnancy. Chronic alcoholic women of reproductive age, or women who drinking heavily and are likely to be recalcitrant to public health warnings on alcohol, as well as those that have pre-existing conditions, including chronic infections and/or severe stress, might be targeted. Before such recommendations can be considered, far more information is required on the safety and efficacy of Zn supplementation in pregnancy.

Zinc Supplementation in Pregnancy

The authors have been unable to find any human trials where Zn supplementation in pregnancy has been investigated specifically with the aim to reduce birth defects or cognitive abnormalities from teratogenic agents such as alcohol. Indeed, most randomised control trials where Zn has been supplemented in pregnancy have focused on the beneficial effects of Zn on fetal health and well-being in predominantly healthy women who are considered at risk of low Zn intake as a result of poor bioavailability from their staple diet. Women with chronic illness and/or presumably those with a history of substance abuse are deliberately excluded from these trials in order to reduce the number of confounding factors in these studies. Even in studies where Zn supplementation has been used to improve pregnancy outcomes in women, suspected low Zn intake, the findings have been conflicting possibly due to limitations of sample size and/or the lack of a uniform methodology. The Cochrane Pregnancy and Childbirth Group's Trials Register contains the largest review of studies involving Zn supplementation in pregnancy [241]. The review investigated the findings of 17 randomised control trials that were conducted over three decades on 9,000 healthy women from 10 countries. Thirteen of these trials contained subgroups of women of low-income status that were malnourished and suspected of being Zn-deficient. Across all trials, Zn supplementation was between 15 and 44 mg/day for a minimum duration of 26 weeks of pregnancy. The only pertinent finding of the review was a 14% reduction in the number of preterm births that was primarily due to a subset of studies conducted on undernourished women from low-income families that participated with Zn supplementation in Bangladesh, Nepal and Peru. The general consensus was that benefits would be gained by improving the overall micronutrient status of pregnant women, particularly those of low-income status. In this regard, UNICEF/WHO currently recommends the antenatal use of folic acid and iron after clear improvements in birth weight and mortality were found in studies on malnourished women from rural Nepal [242–244]. In those trials, Zn supplements did not provide a benefit above those of folic acid and iron.

However, in a follow-up study of the children when they reached school age, it was reported that Zn above folic acid and iron supplementation resulted in a modest increase in height and a reduction in peripheral adiposity [245].

The WHO recommends that children in developing countries take Zn supplements based upon trials that clearly show its efficacy in reducing the incidence and prevalence of diarrhoea [244, 246, 247]. The benefits may also be gained by the earlier intervention during pregnancy. In a study conducted in Bangladesh, infants of mothers who received 30 mg of Zn daily from 12 to 16 weeks of gestation until parturition were noted to have less acute diarrhoea, dysentery and impetigo; however, this benefit was restricted to a subset of low- rather than normal-birth-weight infants [248]. Similar protection against diarrhoea was found in a double-blinded randomised control trial of 421 infants born to women in Lima, Peru, who received 15 mg of Zn daily during their pregnancy [249].

There is a clear need for more randomised control trials on Zn supplementation in pregnancy not only focusing on the potential benefits for offspring born to mothers with low Zn intakes but also including a wider group that encompasses subsets of women that consume alcohol and/or suffer from chronic infections or severe stress during gestation. Such studies will require longitudinal assessment of offspring at critical times during development with the aim to investigate cognition and behaviour.

Conclusion and Perspectives

It is apparent that all of the mechanisms discussed in this chapter and those that have not been identified may have relevance in alcohol-mediated teratogenicity and interact depending upon dose, duration and timing of alcohol exposure in pregnancy. However, very few of the proposed mechanisms have undergone the rigour of scientific testing in animal models of FAS. It is well recognised that Zn nutrition is important for a successful pregnancy. However, even when the mother has an adequate Zn intake during gestation, much less is known about the effect on the fetus of a maternal Zn redistribution caused by activation of a maternal immune response. Accumulating evidence from animal studies suggests that the transfer of Zn from mother to fetus can be impeded by hypozincaemia caused by induction of MT during an acute phase response. Consumption of alcohol leading to a high blood alcohol concentration causes hepatic MT and hypozincaemia similar to that observed after severe stress or infection. In our rodent model of FAS, we now can demonstrate a clear link between maternal Zn redistribution, fetal Zn deficiency, teratogenicity and neurodevelopmental anomalies in offspring. However, the mechanism now needs to be validated in a higher-order species, more specifically one with a gestational time and neurodevelopmental traits that mimic more closely the hallmarks of human pregnancy.

That in the rodent, Zn supplementation throughout pregnancy ameliorates teratogenicity and neurodevelopmental anomalies associated with prenatal activation of the maternal immune response is most compelling, as it provides a potential treatment that might protect the fetus against the consequences of maternal Zn redistribution. Therefore, one might predict that pregnancy outcome and the general well-being of offspring would be improved by enhancing the Zn nutrition in the diets of women, in particular those living in communities where poor nutrition, alcohol abuse and infections are endemic. However, before embarking on such human trials, more needs to be known about the whole-body Zn redistribution that occurs during an acute phase response and particularly what role the hypozincaemia plays in the overall immune response. There is growing evidence that pro-inflammatory cytokines and other inflammatory mediators use intracellular Zn ions to signal between receptors and a diverse range of molecular targets that regulate the function of immune cells. However, the complex homeostatic mechanisms that regulate these signalling processes are unclear [250]. Consequently, it could be argued that if the MT-driven hypozincaemia is required for an

appropriate immune response, then Zn treatment might endanger the mother's health which in turn could compromise fetal well-being. The role of hepatic MT induction and hypozincaemia in the immune response therefore needs to be clarified. There is also the possibility that epigenetic programming might occur in utero as a result of hyperzincaemia after Zn supplementation. A study demonstrated that supplementation of the maternal diets with methyl donors (e.g. choline, folic acid) and Zn epigenetically altered agouti gene expression in offspring, suggesting that dietary supplementation which is presumed to be beneficial may actually have long-term deleterious effects on gene expression [251]. Although Zn supplementation between 15 and 45 mg/day appears to be safe during human pregnancy, this has not been adequately investigated. While these levels are tolerated and do not appear to interfere with the bioavailability of other micronutrients, an overall understanding of putative long-term effects of taking Zn supplements is warranted.

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Chapter 14
Tocotrienol and Cognitive Dysfunction Induced by Alcohol

Kanwaljit Chopra and Vinod Tiwari

Key Points

- Up to 50–75% of long-term alcoholics may show permanent cognitive impairment, making chronic alcoholism the second leading cause of dementia behind Alzheimer's disease.
- Both clinical observations and animal studies have shown a direct relationship between chronic alcohol and learning and memory deficits.
- The cellular, biochemical, and molecular mechanisms behind alcohol-induced cognitive deficit are not fully understood, but several explanations have been proposed including oxidative–nitrodative stress leading to free radical damage, alcohol-induced neuroinflammation, activation of nuclear factor-κβ (NF-κβ) and toll-like receptor 4 (TLR 4) signaling and neuronal apoptosis, NMDA receptor supersensitivity, suppression of growth factors, disruption of the hypothalamus–pituitary–thyroid axis, and inhibition of neurogenesis.
- Tocotrienols possess more potent neuroprotective and antioxidant activities than α-tocopherol due to their better distribution in the fatty layers of the cell membrane.
- Findings from our laboratory demonstrated neuroprotective potential of tocotrienol against alcohol-induced cognitive deficits not only in adults but also in neonatal rats by inhibiting oxido-nitrodative stress-mediated inflammatory signaling and cell death cascade.

Keywords Alcohol • Apoptosis • Cognitive deficits • Fetal alcohol spectrum disorder • Oxidative–nitrodative stress • Tocotrienol • Vitamin E

Introduction

Alcoholism, the chronic and excessive consumption of alcohol, is a syndrome characterized by severe peripheral as well as central nervous system toxicity. However, the neurobehavioral deficits induced by alcohol and their impact on quality of life of an individual, are often unrecognized. Over 17 million Americans, that is, 8.5% of the population, meet the DSM-IV diagnostic criteria for alcohol dependence or alcohol abuse, more commonly referred to as chronic alcoholism [1]. Up to 50–75% of

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long-term alcoholics may show permanent cognitive impairment, making chronic alcoholism the second leading cause of dementia behind Alzheimer's disease [2]. A significant number of alcoholics have clinically relevant cognitive deficits, even when the most severe alcohol-related dementias are excluded [3] (e.g., Wernicke–Korsakoff syndrome or hepatic encephalopathy). Alcoholics consistently show deficits in executive function, declarative memory, and short-term memory and frequently show impairments in spatial learning and memory and impulsivity, effects which indicate hippocampal dysfunction [3, 4]. Parallel to the behavioral and cognitive impairments are observations of "brain shrinkage" or neurodegeneration in alcoholics [5, 6]. Human imaging studies, animal models, and postmortem analysis of brain structure support that chronic alcoholism is closely associated with brain damage or neurodegeneration. Alcoholics show significant volume loss in cortical and subcortical brain structures that includes both gray and white matter shrinkage. These widespread deficits occur in the absence of major nutritional deficiencies, although nutritional deficiencies can cause neurodegeneration and could contribute to alcoholic degeneration. Both postmortem and in vivo imaging studies of brain morphology reveal abnormally reduced brain volumes of gray and white matter across multiple regions. The frontal lobes are the most insulted region in the alcoholic brain with the superior frontal cortex showing significant neuronal loss [6, 7]. The frontal lobes regulate complex cognitive skills such as working memory, temporal ordering, discrimination, and reversal learning that underlie judgment, attention, risk taking, and motivation. Disorders in these behaviors are central if not causal to the consumption of dangerous amounts of alcohol despite the knowledge of negative consequences. Accordingly, chronic alcoholics demonstrate impaired judgment, blunted affect, poor insight, social withdrawal, reduced motivation, distractibility, attention, and impulse-control deficits [3, 4, 6]. Both clinical observations [3, 4] and animal studies have shown a direct relationship between chronic alcohol and learning and memory deficits [8–11].

O'Leary [12] recently summarized the epidemiological research on fetal alcohol syndrome (FAS) concluding that its estimated worldwide prevalence is around 1/100, making it the most common cause of learning difficulties. The cost of caring for children with FAS has been estimated at approximately US$ 74.6 million per year, with three quarters of this cost associated with the care of FASD cases with mental retardation [13]. Therefore, understanding how chronic alcohol consumption produces behavioral and cognitive deficits in adults as well as neonates with prenatal alcohol exposure is of great medical and economic importance.

Etiopathogenesis of Alcohol-Induced Cognitive Deficits

The cellular, biochemical, and molecular mechanisms behind alcohol-induced cognitive deficit are not fully understood, but several explanations have been proposed including oxidative–nitrodative stress leading to free radical damage [14], alcohol-induced neuroinflammation, activation of NF-κβ, and toll-like receptor 4 (TLR 4) signaling and neuronal apoptosis, NMDA receptor supersensitivity, suppression of growth factors [15], disruption of the hypothalamus–pituitary–thyroid axis [16], and inhibition of neurogenesis [17] (Fig. 14.1). Thus, although the occurrence of alcoholic dementia and neurodegeneration is well supported by multiple studies, the mechanisms of neurotoxicity are still poorly understood. Multiple pathways involved in alcohol-induced cognitive deficits are summarized below.

Role of Alcohol-Induced Neuronal Oxidative–Nitrodative Stress

Oxidative–nitrodative stress has been implicated in a variety of neurodegenerative disorders, including sclerosis, Parkinson's disease, and Alzheimer's disease and may also play an important role in the behavioral deficits (such as dementia) produced by ethanol [18]. Oxidative stress results from an

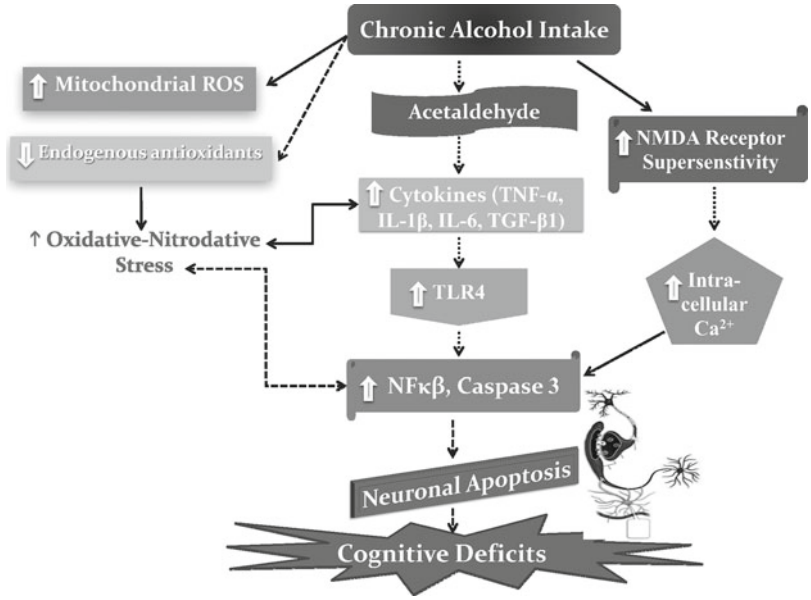

Fig. 14.1 Pathway representing the role of acetaldehyde in alcohol-induced cognitive deficits

imbalance between the endogenous antioxidant defense system and free radical generation. Excessive oxidative challenges impair the brain antioxidant defense systems and can activate secondary events leading to apoptosis by affecting DNA integrity, protein function, and membrane lipids [19] and ultimately producing neuronal death [18]. Ethanol enhances oxidative stress directly through generation of oxy free radicals and lipid peroxidation [20] and depletion of endogenous antioxidants such as α-tocopherol, glutathione, ascorbate, and vitamin E. Ethanol is converted into acetaldehyde via intracellular oxidation, eventually generating ROS such as superoxide anion, hydrogen peroxide, and hydroxyl radical [21] (Fig. 14.1). Neurons are highly dependent on glucose for ATP generation necessary for many biochemical processes and produce ROS as by-products of the oxidative phosphorylation within the mitochondria. The CNS is particularly susceptible to ROS-induced damage because (1) it has a high consumption of oxygen, (2) it contains high levels of membrane polyunsaturated fatty acids susceptible to free radical attack, (3) it is relatively deficient in oxidative defenses (poor catalase activity and moderate superoxide dismutase, SOD, and glutathione peroxidase activities), and (iv) a high content in iron and ascorbate can be found in some regions of the CNS, enabling the generation of more ROS through the Fenton/Haber Weiss reaction [22]. In addition, ethanol suppresses antioxidant enzymes such as glutathione peroxidase/glutathione reductase [23]. In addition, certain regions of the CNS, such as the hippocampus and cerebellum, may be particularly sensitive to oxidative stress because of their low endogenous levels of vitamin E, an important biochemical antioxidant, relative to other brain regions [24]. Such a depressed defense system may be adequate under normal circumstances. However, in pro-oxidative conditions, such as during alcohol exposure, these low antioxidant defenses can predispose the brain to oxidative damage. High dose or chronic exposure to alcohol (even at low dose) induces iNOS in the CNS, and an excess amount of nitric oxide (NO) suppresses various physiological functions. The relevance of these data is supported by the findings that NOS induction was detected in cerebellar cortical neurons of alcoholics [25]. Peroxynitrite, a harmful oxidant formed by reaction between superoxide and NO, reacts with protein and nonprotein thiols, unsaturated fatty acids, and DNA, thus affecting energy conservation mechanisms and oxidative posttranslation modification of protein and ultimately causing neuronal cell death [26].

Oxidative Stress Mediated Proinflammatory Signaling in Brain

Many findings suggest that ethanol-induced brain damage is related to oxidative stress from proinflammatory enzymes activated during ethanol intoxication. During the presence of ethanol, there are changes in protein transcription with increased DNA binding of NF-κβ and reduced DNA binding of CREB. CREB family transcription factors are activated by phosphorylation and promote neuronal survival, protecting neurons from excitotoxicity and apoptosis through regulating the transcription of prosurvival factors [27]. Conversely, NF-κβ is a transcriptional factor that is widely known for its ubiquitous roles in inflammatory and immune responses [28]. The balance in expression and activation of these transcription factors, and thus the balance of prosurvival versus proinflammatory states, suggests a mechanism by which alcohol induces brain damage in alcoholic neuropathology.

Activation of NF-κβ transcription is associated with increases in proinflammatory cytokines with tumor necrosis factor-α (TNF-α) being the prototype (Fig. 14.1). A role for cytokines in alcoholic neuropathology is suggested by several studies [29]. Acute ethanol increases cytokine induction by TLR2 and TLR4 ligands [30]. Both in vivo and in vitro evidences support the involvement of a proinflammatory cascade including increased NF-κβ-driven induction of oxidative stress enzymes as a key factor in alcohol-induced brain damage. TNF-α can directly potentiate glutamate neurotoxicity by inhibiting glutamate uptake through NF-κβ mechanisms [31]. In human astroglial cells, which normally regulate extracellular glutamate concentrations, ethanol enhances NF-κβ-DNA binding and the induction of iNOS [32]. Similarly, we found that ethanol induces COX2, iNOS, and NADPH oxidase gp91 and increases reactive oxygen species, producing enzymes that are downstream of NF-κB. NADPH oxidase is a multimeric enzyme composed of multiple subunits that in the active form catalyze the transfer of one electron from NADPH to oxygen, giving rise to superoxide [33]. Ethanol significantly increases the brain expression of NADPH oxidase subunits, gp91phox and p67phox, that persists for at least 8 days of abstinence [34]. Thus, ethanol promotes a proinflammatory and anti-survival environment through the activation of proinflammatory transcription factors and the inhibition of prosurvival transcription factors.

Activation of NF-kB Signaling

Reactive oxygen species producing enzymes including NOS, COX2, and NADPH oxidase are all induced by NF-kB activation suggesting that ethanol-induced ROS in brain may be related to NF-κβ activation [29]. There is indirect connection between ethanol and NF-κβ, as large acute doses or chronic administration of ethanol alters the fluidity of mitochondrial membranes and produces acetaldehyde, which generates oxidative species [35], including free radicals, hydrogen peroxide, and hydroxyl radicals, which are all known to rapidly and significantly activate NF-κβ [36] (Fig. 14.1). Crews et al. suggested that alcohol-induced neurodegeneration involves NF-κβ activation, microglial activation, and increased COX2 immunoreactivity, all of which are indicative of an enhanced neuroinflammatory response [29]. Valles et al. also found that 5 months of ethanol liquid diet induces inflammatory mediators IL-1β, COX2, and iNOS in brain via NF-κβ induction [37]. Izumi et al. also demonstrated that a single day of ethanol exposure in rats on postnatal day 7 results in significant apoptotic neuronal damage throughout the forebrain after 24 h of ethanol administration [38].

Jung et al. suggested a cascade of events in which oxidative insults induced by chronic ethanol lead to activation of protein kinase C, which subsequently phosphorylates Iκβ (the NF-κβ inhibitor) of NF-κβ-Iκβ complex [39]. On phosphorylation, a cell death signal NF-κβ is released to its active form and translocates to the nucleus. The NF-κβ then binds to DNA, induces the expression of target genes, and results in DNA fragmentation and apoptosis through activation of caspases [40]. Numerous factors can induce apoptosis of CNS cells, including insufficient blood supply to the brain; dysfunction of the

cell's energy-generating organelles, called the mitochondria; disruption of the normal calcium levels in the cells; and oxidative stress. Alcohol can also induce apoptosis, and this has been demonstrated both in animal models of alcohol exposure [41] and in isolated CNS cells grown in culture, including cells from the hypothalamus [42]. Heavy, binge-like alcohol exposure during the period of brain development that is comparable to that of the human third trimester has been shown to produce death of postmitotic neurons in the hypothalamus [42], cerebral cortex [43], cerebellum [44], and associated brain-stem structures [45]. It has been reported that administration of ethanol to immature mice during the synaptogenesis period induces widespread apoptotic cell death in the developing brain [43], and caspase-3 activation is believed to be responsible for generating the cytological changes that characterize neuronal apoptosis [46] (Fig. 14.1).

Toll-Like Receptor 4-Induced Neuroinflammation and Brain Damage

TLRs are a family of pattern-recognition receptors that enable the recognition of conserved structural motifs in a wide array of pathogens. Activation of TLRs triggers the downstream stimulation of nuclear factor-κβ (NF-κβ) and the induction of genes that encode inflammation-associated molecules and cytokines [47, 48] (Fig. 14.1). Most TLRs are expressed in the CNS, mainly in glial cells [49]. Recent evidence demonstrates that these receptors respond to pathogens and host tissue injury [50, 51], and they not only play a role in the innate immunity in response to infections but also participate in CNS neurodegeneration and neural injury [52, 53]. Activation of the TLR response significantly contributes to neuroinflammation [54], and TLR4-deficient mice are protected against ischemic brain damage and injury [55, 56]. The role of TLR4 in brain injury has been indicated in a number of recent studies demonstrating that elimination of TLR4 protects against oxidative stress in Alzheimer's disease [57], focal cerebral ischemia [58], human immunodeficiency virus-associated neurodegeneration [59], and ischemic brain injury [56]. Chronic ethanol consumption increases cytokines and inflammatory mediators in the rat brain, activating signaling pathways associated with neuroinflammation and triggering cell damage [37]. It was also found that ethanol activates TLR4 signaling in astrocytes [60], microglia, and macrophages [61], suggesting that activation of the TLR4 response by ethanol could be an important mechanism of ethanol-induced neuroinflammation (Fig. 14.1). Although chronic ethanol treatment increased the expression of iNOS and COX-2 in the cerebral cortices of the ethanol-treated WT mice, the induction of these proteins did not take place in the cortices of the TLR4-knockout mice. Previous findings demonstrate that ethanol at low/moderate concentrations activates the TLR4 receptors in astrocytes, triggers NFκβ activation, and leads to the induction of an inflammatory response [60], suggesting that TLR4 activation in glial cells is a critical event in the ethanol-induced inflammatory processes. In vivo findings also support the pivotal role of the TLR4 receptors in the activation of both microglia and astroglia induced by ethanol, since the deficiency of TLR4 function markedly reduces astroglia hypertrophy and completely abolishes microglia activation. A deficient TLR4 function prevents both glial activation and the inflammatory reaction, thus supporting the role played by the TLR4 function in these processes. Elimination of the TLR4 receptor function prevents ethanol-induced NF-κβ activation and cytokine upregulation, suggesting the critical role of TLR4/NF-κβ in the ethanol-induced inflammatory process in the brain.

NMDA Receptor Supersensitivity

Neuronal death can also be induced by excess activity of certain neurotransmitters, including glutamate. Early studies, mostly in vitro culture models, suggested that chronic ethanol inhibited glutamatergic N-methyl-D-aspartate (NMDA) receptors that in time resulted in NMDA supersensitivity, an effect

only revealed upon the removal of alcohol [62]. These in vitro studies and others suggested that during withdrawal, neurotoxicity occurs through the NMDA receptor [63]. Under certain conditions, when glutamate interacts with the NMDA receptor, it causes calcium to flow into the signal-receiving neuron. Calcium influx is a powerful regulator of the activity and function of a neuron. Excessive activation of the NMDA glutamate receptor, however, can lead to dangerously high calcium accumulation inside the neuron [64]. If sufficiently severe or prolonged, the rise in intracellular calcium can lead to cell death by either apoptosis or necrosis [64, 65] (Fig. 14.1).

Conditions of excitotoxicity can also occur during withdrawal from high levels of alcohol and may thereby contribute to alcohol-induced damage to the fetal brain, particularly when the mother binge drinks [66]. In these cases, the fetus experiences periods of heavy alcohol exposure, followed by withdrawal episodes. High levels of alcohol acutely inhibit NMDA receptor function. During withdrawal after a binge-drinking episode, however, glutamate stimulation of NMDA receptor activity increases temporarily and may lead to excitotoxicity [67]. Although some experimental support exists for the potential contribution of withdrawal-related events to alcohol-induced fetal brain damage [67], including the potential role of excitotoxicity, this hypothesis requires more research.

Glia and Alcoholic Neurodegeneration

Normal brain development and function require not only neurons but also non-neuronal cells, called glia, that support the growth and development of the neurons. Glia may also contribute to alcoholic neurodegeneration. Alcohol causes astroglia to degenerate, leaving a void in trophic and metabolic support, and then neurons degenerate [68]. The loss of astroglia results in reduced ability to take up excess glutamate, buffer K+(ion homeostasis), and eliminate free radicals [69]. Glia may be more sensitive than neurons to the effects of alcohol. Careful studies in postmortem human hippocampus found a statistically significant loss of 37% of the glial cells in alcoholic hippocampus that included a reduction of astrocytes and oligodendrocytes but no loss of neurons [70]. Long-term alcohol exposure in vivo decreases an intermediate neurofilament that is a characteristic of astrocytes, glial fibrillary acidic protein (GFAP), in the cerebellum of male and female rats [71]. The loss of GFAP expression suggests a loss of astrocytes [71] consistent with the finding that the number of astrocytes identified by Giemsa staining in human hippocampus is reduced in alcoholics [70, 72].

Alcohol Intoxication Inhibits Neurogenesis

Neurogenesis is the net result of four components: cell proliferation, cell differentiation, cell migration, and cell survival. Alcohol could potentially affect neurogenesis at any of these stages of cell development. Indeed, over 30 years of research on the effects of alcohol on fetal neurogenesis has shown that alcohol affects each of these components in the developing brain [73]. Longer alcohol exposure durations, specifically a 4-day binge, affects both cell proliferation and newborn cell survival. Reduced cell survival in this binge exposure model is consistent with both evidence of cell death in the DG following binge alcohol exposure [74, 75] and also the seminal finding of DG granule cell loss following chronic alcohol exposure [76]. Thus, alcohol inhibition of adult neurogenesis should be considered as a new mechanism underlying alcohol-induced neurodegeneration. Inhibiting neurogenesis has shown detrimental effects on hippocampus-based learning [77]. These findings imply that events that inhibit neurogenesis would have downstream effects on learning and memory. The learning and memory performance was examined at 3 weeks following binge exposure, and

deficits in hippocampus-dependent task were observed at the same time point where neurogenesis was inhibited [17]. Several groups have consistently shown that progenitor cell survival is also reduced, which suggests another mechanism by which alcohol reduces neurogenesis in rats [17, 78, 79]. Further, ethanol treatment during adult neurogenesis blunts the growth of the progenitor's dendritic arbor [79]. Taken together, these studies indicate that ethanol reduces neurogenesis during intoxication, contributing to neurodegeneration through loss of cell generation. Intriguingly, inflammatory processes may inhibit neurogenesis [80]. Thus, ethanol activation of proinflammatory cytokine-induced oxido-nitrodative stress cascades likely inhibits neurogenesis as well as mediates the other necrotic degenerative processes.

Involvement of Hypothalamic–Pituitary–Adrenal (HPA) Axis

Ethanol-exposed male and female rats show increased corticosterone, adrenocorticotropin (ACTH) hormone, and/or corticotropin-releasing hormone (CRH) responses to stressors such as repeated restraint, foot shock, and lipopolysaccharide (LPS) challenges or to morphine administration [81]. The mechanisms that underlie HPA axis hyperresponsiveness in ethanol-exposed offspring are not well understood. However, several reports suggest an abnormal production, and/or release of CRH after a stress challenge may be one of the causes for the altered stress regulation process in the ethanol-exposed offspring [82].

Disruption of Growth Factor Signaling

Alcohol can also interfere with the activity of growth factors that regulate cell proliferation and survival. Numerous growth factors are needed for cell division to proceed normally, including two factors called insulin-like growth factors (IGF) I and II. Alcohol can interfere with the activity of the IGF-I receptor. As a result, IGF-I still binds to its receptor, but the receptors signaling function is blocked, and IGF-I-mediated cell division cannot proceed [83]. Thus, alcohol can prevent the normal production of CNS cells by interfering with the growth factors that regulate cell division. Alcohol also may induce cell death by inhibiting several growth factors that support cells that have attained their final function (i.e., that are differentiated) and no longer divide [84].

Functional Uniqueness of Tocotrienol over Other Isoforms of Vitamin E

Tocotrienols are fat-soluble vitamins belonging to the family of tocopherols, that is, tocochromanols. Tocochromanols are group of amphipathic, lipid-soluble organic molecules composed of a polar moiety derived from tyrosine and a hydrophobic polyprenyl side chain originating from the isoprenoid pathway. Tocochromanols with a saturated phytyl-derived side chain are termed tocopherols, whereas those with unsaturated geranylgeranyl-derived side chain are termed tocotrienols. Structurally, tocopherols and tocotrienols share some resemblance consisting of a common chromanol head and a side chain at the C-2 position (Fig. 14.2). Tocopherols and tocotrienols are further separated into individual compounds assigned by the Greek letter prefixes (α, β, γ, and δ) depending on the number and position of methyl substitution on the chromanol ring. The alpha form has three methyl groups, the beta and gamma forms have two methyl groups, and the delta form has only one methyl group [85] (Table 14.1). Each of these forms of vitamin E has a different biopotency. While tocopherols are generally

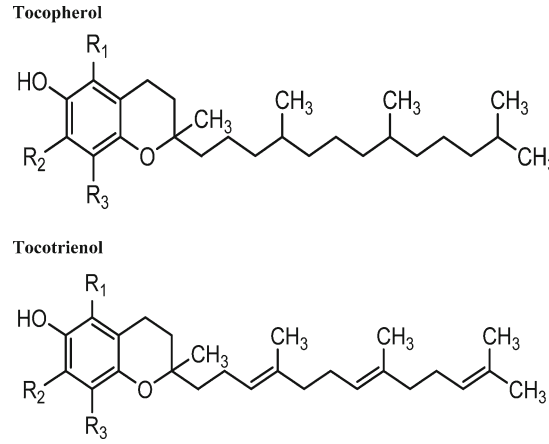

Fig. 14.2 Chemical structure of α-tocopherol and tocotrienol

Table 14.1 Structural differences between different isoforms of vitamin E

R-Groups for vitamin E			
Form	R1	R2	R3
Alpha	CH_3	CH_3	CH_3
Beta	CH_3	H	CH_3
Gamma	H	CH_3	CH_3
Delta	H	H	CH_3

present in nuts (i.e., almonds) and common vegetable oils (i.e., wheat germ, sunflower), tocotrienols are minor plant constituents especially abundant in palm oil, cereal grains, and rice bran [86].

Structurally, tocotrienols differ from tocopherols by the presence of three trans double bonds in the hydrocarbon tail. Because of these unsaturations in the isoprenoid side chain, tocotrienols are thought to assume a unique conformation [87] (Fig. 14.2). Indeed, α-tocotrienol possesses numerous functions that are not shared by α-tocopherol [88]. For example, nanomolar concentrations of α-tocotrienol uniquely prevent inducible neurodegeneration by regulating specific mediators of cell death [89–91]. Oral supplementation of tocotrienol protects against stroke [92]. Micromolar amounts of tocotrienol suppress the activity of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the hepatic enzyme responsible for cholesterol synthesis [93, 94]. Tocopherols do not share the cholesterol-lowering properties of tocotrienol [95, 96]. Experimental research examining the antioxidant, free radical scavenging effects of tocopherol and tocotrienols revealed that tocotrienols appear superior due to their better distribution in the fatty layers of the cell membrane [97]. Furthermore, tocotrienol but not tocopherol, suppresses growth of human breast cancer cells [98]. Further evidence supporting the unique biological significance of vitamin E family members is provided by current results derived from α-tocotrienol research. Tocotrienols possess more potent neuroprotective and antioxidant activities against hydrogen peroxide than α-tocopherol [99]. Likewise, the tocotrienol-rich fraction from palm oil was significantly more effective than α-tocopherol in protecting rat brain mitochondria and rat liver microsomes against oxidative damage [100]. A number of mechanisms may contribute to the strong antioxidant activity of α-tocotrienol compared to α-tocopherol, including: (a) a more uniform distribution in the membrane lipid bilayer, (b) a more efficient interaction of the chromanol ring with lipid radicals, and (c) a higher recycling efficiency from chromanoxyl radicals [101].

Neuroprotective Effects of Tocotrienol

Numerous reports indicate that tocotrienols exhibit neuroprotective effects under a wide variety of conditions [102–106]. Sen and his group have examined extensively the prevention of glutamate-induced neurodegeneration by tocotrienols [89–91, 107]. They found that modulation of c-Src, 12-lipoxygenase, and PLA2 is involved in the neuroprotective effects of tocotrienols. Khanna et al. showed that a subattomole quantity of α-tocotrienol, but not g-tocopherol, protected neurons from glutamate challenge [92]. Rats given α-tocotrienol supplement showed more protection against stroke-induced injury through downregulation of c-Src activation and 12-lipoxygenase phosphorylation at the stroke site. On a concentration basis, the neuroprotective effects of nM tocotrienol represent the most potent biological function of all natural forms of vitamin E. Glutamate toxicity is a major contributor to neurodegeneration. It includes excitotoxicity and an oxidative stress component also known as oxytosis [108, 109]. α-Tocotrienol was the most potent neuroprotective form of vitamin E in glutamate-induced degeneration of HT4 hippocampal neurons [91]. The neuroprotective property of tocotrienol holds good not only in response to glutamate challenge but also in response to other insults such as homocysteic acid-, glutathione deficiency-, and linoleic acid-induced oxidative stress [90, 91]. It is now evident that at micromolar concentrations, tocotrienol protects neural cells by virtue of its antioxidant property. At nanomolar concentrations, however, tocotrienol regulates specific neurodegenerative signaling processes. Results from our laboratory also demonstrated potent neuroprotective effects of tocotrienol in experimental model of diabetic neuropathy [103], in the rat model of alcoholic neuropathy [105], in chronic alcohol-induced cognitive dysfunction in rats [106], in intracerebroventricular streptozotocin-induced cognitive impairment and oxidative–nitrodative stress in rats [104], and in diabetes-associated cognitive deficits [102], all through suppression of proinflammatory pathways.

Tocotrienol and Alcohol-Induced Cognitive Deficits

Suppression of Neuroinflammatory Signaling Cascade by Tocotrienol Prevents Chronic Alcohol-Induced Cognitive Dysfunction in Rats

Chronic alcohol administration is known to cause memory deficits associated with enhanced oxidative stress and is well supported by numerous studies. Khalil et al. found that ethanol administration significantly increased the time to find the platform (latency period), indicating that ethanol induces deficit in spatial reference memory which was associated with increased levels of β-EN in the cerebral cortex and hippocampus of ethanol-treated rats [110]. Iliev et al. also suggested that galanthamine improves the speed of learning, short-term memory, and spatial orientation of rats in conditions of prolonged alcohol intake, indicating the deficits in cholinergic neurotransmission in chronic ethanol-administered rats [111]. Kasdallah et al. administered 35% ethanol at 3 g/kg body weight to male Wistar rats for 6 weeks and got significantly increased MDA levels by 51.5%, 53.7%, 72.7%, and 40.5% in the liver, heart, brain, and testis, respectively. This further demonstrates the vulnerability of alcoholic brain to oxidative stress [112].

Findings from our laboratory also showed that chronic ethanol administration for 10 weeks produced significant memory impairment in rats as evident from increased latency time in both Morris water maze (Fig. 14.3a) and elevated maze task (Fig. 14.3c). In probe trial of water maze also, the time spent in target quadrant is significantly decreased in ethanol-treated rats as compared to control group, which was significantly and dose-dependently reversed on treatment with both α-tocopherol and tocotrienol (Fig. 14.3b). However, in both the memory assessment paradigms, tocotrienol showed

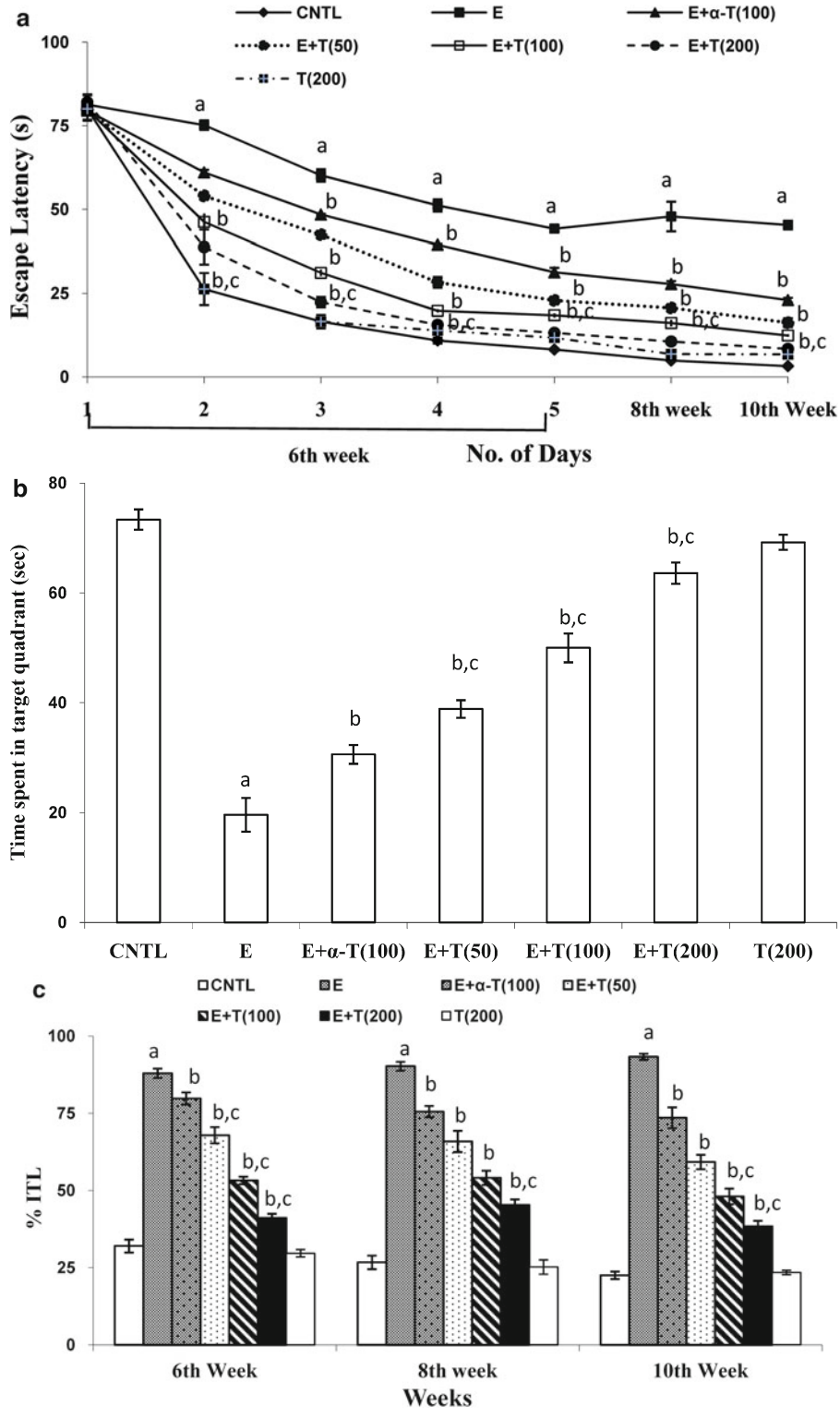

Fig. 14.3 Effect of chronic treatment with α-tocopherol and tocotrienol on the performance of spatial memory acquisition phase in Morris water maze (**a**), time spent in target quadrant in probe trial (**b**) and on percent initial transfer latency in elevated plus maze test (**c**), in ethanol-administered rats. *a.* Different from control group ($P<0.05$); *b.* different from ethanol-administered group ($P<0.05$); *c*. different from one another ($P<0.05$). *CNTL* control, *E* ethanol, *α-T* (100) tocopherol (100 mg/kg), *T* (50) tocotrienol (50 mg/kg), *T* (100) tocotrienol (100 mg/kg), *T* (200) tocotrienol (200 mg/kg)

Table 14.2 Effect of α-tocopherol and tocotrienol treatment on lipid peroxide, reduced glutathione, superoxide dismutase, and catalase levels (mean ± S.E.M.) in different brain regions of ethanol-administered rats. (**a**) Different from control group ($P<0.05$), (**b**) different from ethanol-administered group ($P<0.05$), (**c**) different from one another ($P<0.05$)

Treatment	LPO (nmol/mg protein)	GSH (μmol/mg protein)	SOD (units/mg protein)	Catalase (k/min)	
CNTL	Cerebral cortex	1.86 ± 0.12	0.17 ± 0.014	5.56 ± 0.285	4.81 ± 0.33
	Hippocampus	1.41 ± 0.14	0.11 ± 0.004	3.14 ± 0.518	3.30 ± 0.09
E	Cerebral cortex	6.04 ± 0.33[a]	0.05 ± 0.003[a]	0.45 ± 0.037[a]	0.82 ± 0.065[a]
	Hippocampus	3.29 ± 0.17[a]	0.038 ± 0.002[a]	0.44 ± 0.025[a]	0.71 ± 0.035[a]
E + α-T (100)	Cerebral cortex	4.96 ± 0.30[b]	0.08 ± 0.003[b]	0.88 ± 0.048[b]	1.05 ± 0.03[b]
	Hippocampus	2.66 ± 0.11[b]	0.056 ± 0.001	0.78 ± 0.039[b]	1.12 ± 0.05[b]
E + T (50)	Cerebral cortex	4.42 ± 0.10[b]	0.09 ± 0.003[b]	1.18 ± 0.049[b]	1.54 ± 0.09[b]
	Hippocampus	2.34 ± 0.10[b]	0.066 ± 0.004[b]	0.94 ± 0.042[b]	1.54 ± 0.09[b]
E + T (100)	Cerebral cortex	4.04 ± 0.10[b]	0.10 ± 0.003[b]	2.04 ± 0.086[b,c]	2.47 ± 0.10[b,c]
	Hippocampus	2.06 ± 0.09[b]	0.086 ± 0.002[b]	1.48 ± 0.124[b]	2.01 ± 0.08[b,c]
E + T (200)	Cerebral cortex	3.06 ± 0.20[b,c]	0.12 ± 0.005[b]	4.08 ± 0.102[b,c]	3.55 ± 0.09[b,c]
	Hippocampus	1.66 ± 0.08[b]	0.093 ± 0.003[b]	2.06 ± 0.100[b]	2.63 ± 0.09[b,c]
T (200)	Cerebral cortex	1.42 ± 0.10	0.14 ± 0.006	4.75 ± 0.154	4.59 ± 0.10
	Hippocampus	1.36 ± 0.07	0.097 ± 0.002	2.9 ± 0.177	3.16 ± 0.19

[a]Different from control group ($P<0.05$)
[b]Different from ethanol-administered group ($P<0.05$)
[c]Different from one another ($P<0.05$). α-*T*(100) tocopherol (100 mg/kg), *T*(50) tocotrienol (50 mg/kg), *T*(100) tocotrienol (100 mg/kg), *T*(200) tocotrienol (200 mg/kg)

more potent activity as compared to tocopherol. The biochemical estimations indicated a significant increase in MDA levels and marked decrease in the activity of reduced glutathione, superoxide dismutase, and catalase levels in the cerebral cortex and hippocampus of ethanol-treated rats. Treatment with α-tocopherol and tocotrienol returned the levels of lipid peroxides, reduced glutathione, superoxide dismutase, and catalase toward their control values (Table 14.2). The effect was again more pronounced with tocotrienol treatment. Besides the enhanced level of reactive oxygen species, acetylcholinesterase activity and nitrite levels were also markedly increased in both the brain regions of ethanol-treated rats (Fig. 14.4). Our previous results showed an increase in acetylcholinesterase activity and nitrite levels in the cortex and hippocampus of diabetic rats having cognitive deficits [102]. Chronic treatment with both the isoforms of vitamin E significantly decreased acetylcholinesterase activity and nitrite levels in both the brain regions in a dose-dependent manner; this observation is supported by the findings from Osakada et al. that tocotrienols provided significant protection against the cytotoxicity of a superoxide donor, paraquat, and nitric oxide donors, S-nitrosocysteine and 3-morpholinosydnonimine [99].

In addition to oxidative–nitrosative stress, chronic alcohol administration is also associated with enhanced inflammatory response. Qin et al. found that ten daily doses of ethanol exposure results in persistent alterations of cytokines and significantly increases the magnitude and duration of central and peripheral proinflammatory cytokines and microglial activation suggesting the role of cytokines in alcohol-induced neuroinflammation [34]. In our study, we observed a significant elevation in the levels of TNF-α and IL-1β in the cerebral cortex and hippocampus of ethanol-treated rats which is indicative of enhanced neuroinflammation in the two main regions of brain involved in learning and memory. Chronic treatment with tocopherol and tocotrienol significantly and dose-dependently reduced both the cytokines (TNF-α and IL-1β) in different brain regions of ethanol-administered rats (Fig. 14.5). Thus, findings from our study also point toward more potent effects (behavioral, biochemical, and molecular) of tocotrienol and are in agreement with the previous findings from other

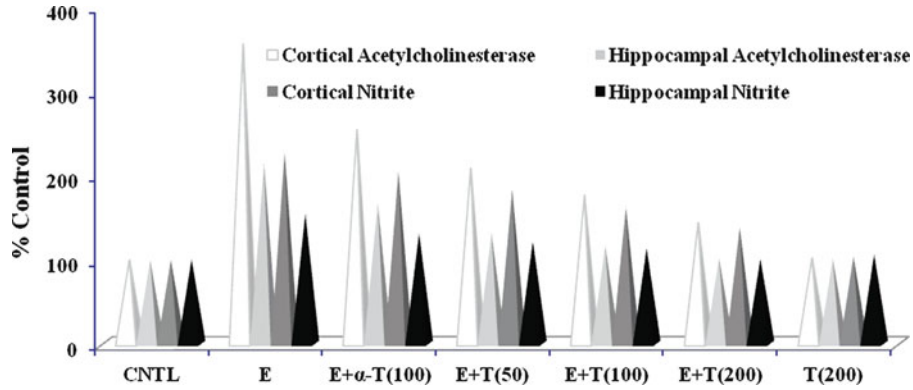

Fig. 14.4 Effect of α-tocopherol and tocotrienol treatment on acetylcholinesterase activity and nitrite levels (% control) in cerebral cortex and hippocampus of ethanol-administered rats. *a.* Different from control group ($P<0.05$); *b*. different from ethanol-administered group ($P<0.05$); *c*. different from one another ($P<0.05$). *CNTL* control, *E* ethanol, *α-T* (100) tocopherol (100 mg/kg), *T* (50) tocotrienol (50 mg/kg), *T* (100) tocotrienol (100 mg/kg), *T* (200) tocotrienol (200 mg/kg)

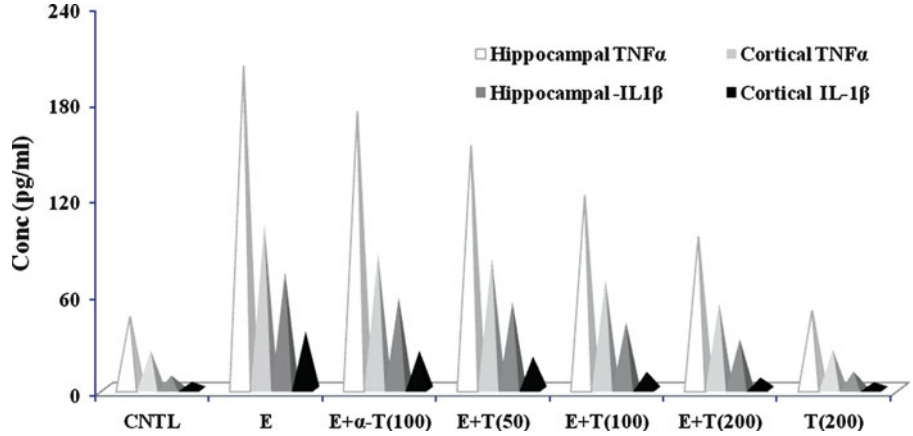

Fig. 14.5 Effect of α-tocopherol and tocotrienol treatment on TNF-α and IL-1β levels in cerebral cortex and hippocampus of ethanol-administered rats. Values were expressed as mean ± S.E.M. *a.* Different from control group ($P<0.05$); *b*. different from ethanol-administered group ($P<0.05$); *c*. different from one another ($P<0.05$). *CNTL* control, *E* ethanol, *α-T* (100) tocopherol (100 mg/kg), *T* (50) tocotrienol (50 mg/kg), *T* (100) tocotrienol (100 mg/kg), *T* (200) tocotrienol (200 mg/kg)

research groups [91, 101, 113]. This suggests that antioxidant property of tocotrienol may be responsible for protecting against the oxidative stress mediated activation of neuroinflammatory cascade, possibly by increasing the endogenous defensive capacity to combat oxidative stress induced by chronic alcohol administration. In addition to potent antioxidant activity, the suppression of nitrosative stress and elevated cytokine (TNF-α and IL-1β) levels in both the brain regions also contributes significantly in preventing the chronic alcohol-induced cognitive deficits in rats.

Protective Effects of Tocotrienol Against Alcohol-Induced Cognitive Dysfunctions and Neuronal Apoptosis in the Neonatal Rat Brain

Although human alcohol consumption during pregnancy leads to severe physical, mental, and behavioral deficits in children, there are no therapeutic options available to prevent the ethanol-associated damage to the developing central nervous system [114]. Recent findings from our laboratory (unpublished data) suggest that both the escape latency (Fig. 14.6a) and total distance traveled (Fig. 14.6b)

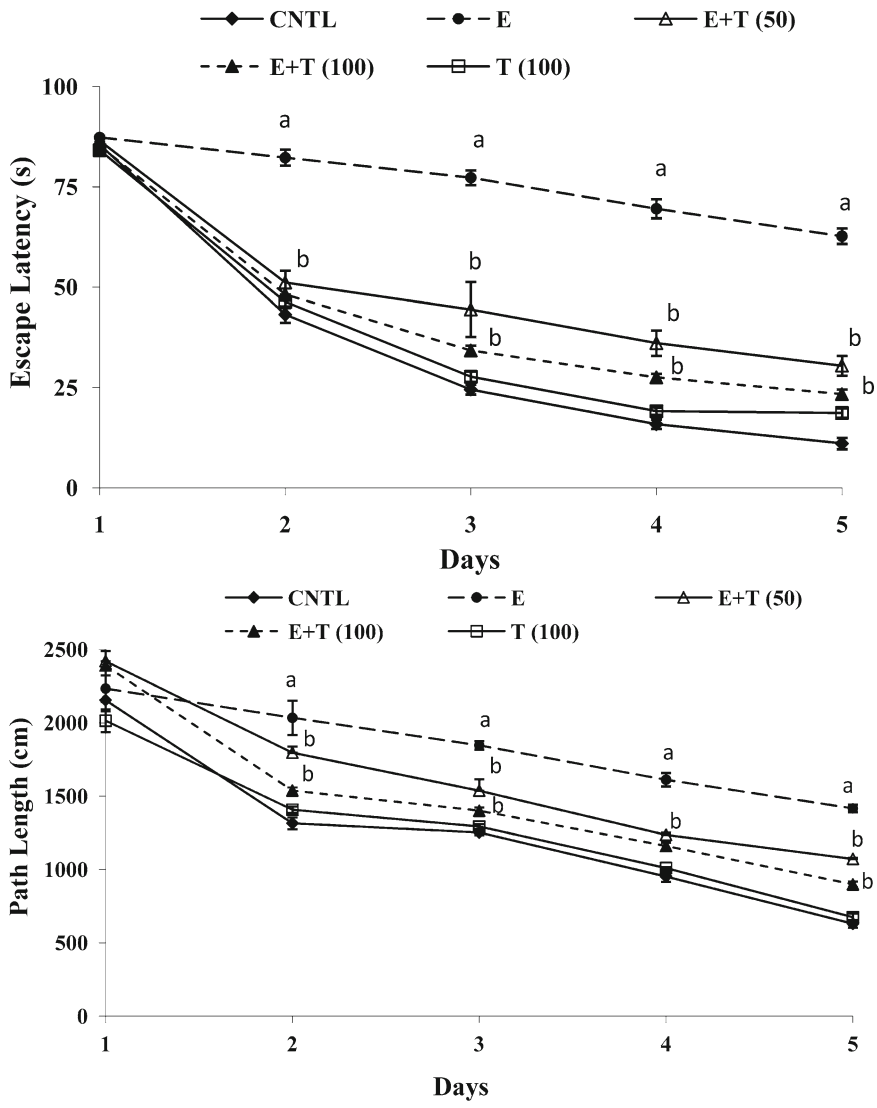

Fig. 14.6 Effect of chronic treatment with tocotrienol on escape latency (**a**) and path length (**b**) in Morris water maze and time spent in target quadrant (**c**) and frequency of appearance in target quadrant (**d**) in probe trial in ethanol-administered pups. Values were expressed as mean ± S.E.M. *a*. Different from control group ($P < 0.05$); *b*. different from ethanol-administered group ($P < 0.05$); (n = 5–8 per group). *CNTL* control, *E* ethanol, *T* (50) tocotrienol (50 mg/kg), *T* (100) tocotrienol (100 mg/kg)

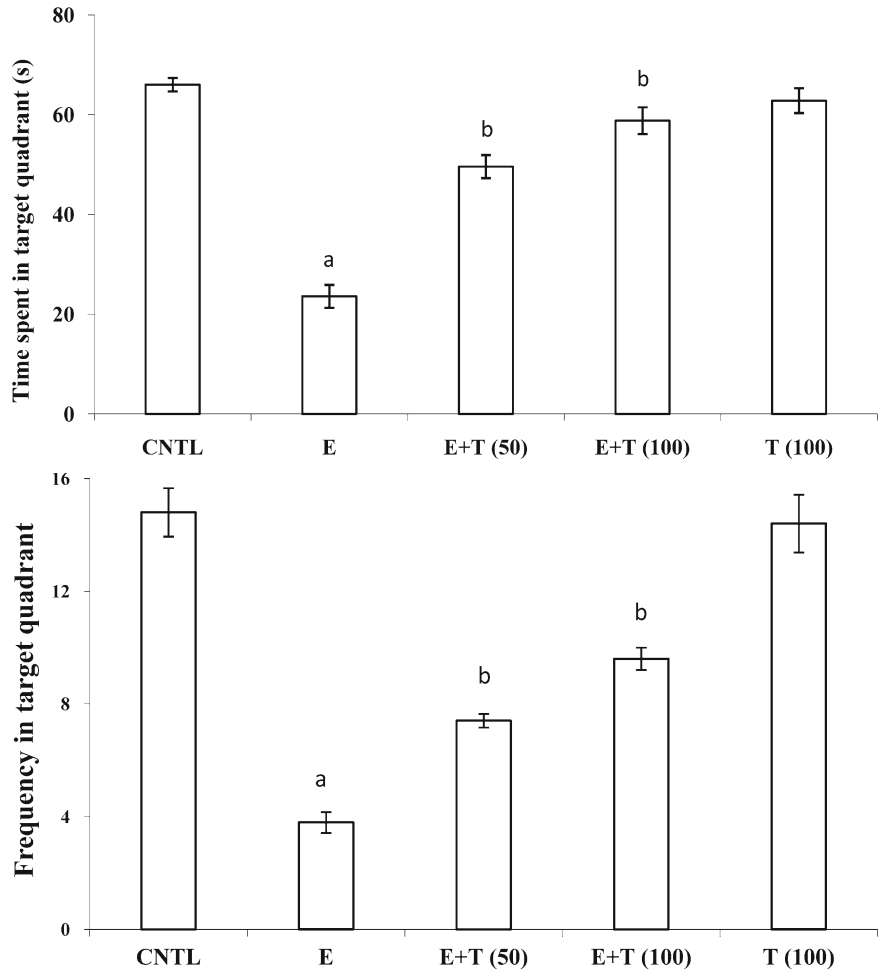

Fig. 14.6 (continued)

to reach the hidden platform in Morris water maze task were significantly increased in ethanol-exposed pups as compared to control group. In probe trial also, the time spent in target quadrant (Fig. 14.6c) and frequency of appearance in target quadrant (Fig. 14.6d) were significantly decreased in ethanol-administered group as compared to control group. Chronic treatment with tocotrienol significantly improved the cognitive deficits in ethanol-exposed pups in both Morris water maze (Fig. 14.6) and elevated plus maze (Fig. 14.7). It is known that maintaining blood ethanol concentration above 200 mg/dl for four consecutive hours is the minimum condition for triggering apoptotic neurodegeneration [43]. We therefore examined BAC to confirm that the apoptotic cell death and resulting cognitive deficits were due to ethanol. Administration of ethanol to 7-day-old rat pups resulted in blood ethanol concentration of 169.60 and 308.80 mg/dl at 2 and 4 h of ethanol administration, respectively. The blood levels of alcohol remained unaffected on treatment with tocotrienol at different time points, suggesting that tocotrienol does not interfere with the absorption of ethanol (Fig. 14.8).

We also observed a significant increase in lipid peroxide and marked decrease in the activity of reduced glutathione, superoxide dismutase, and catalase in the cerebral cortex and hippocampal region of ethanol-treated pups (Table 14.3). Besides the enhanced level of reactive oxygen species, nitrite levels were also markedly increased in both the brain regions of ethanol-treated rats (Fig. 14.9).

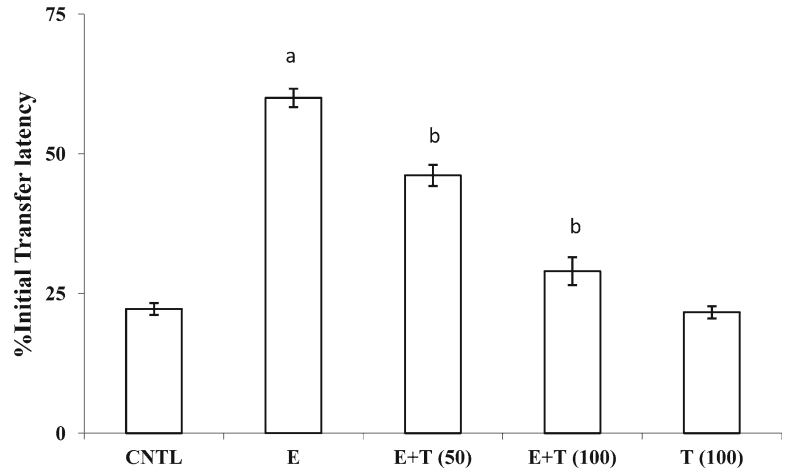

Fig. 14.7 Effect of chronic treatment with tocotrienol on percent initial transfer latency in elevated plus maze test in ethanol-administered pups. Values were expressed as mean ± S.E.M. *a*. Different from control group ($P < 0.05$); b. different from ethanol-administered group ($P < 0.05$); (n = 5–8 per group). *CNTL* control, *E* ethanol, *T* (50) tocotrienol (50 mg/kg), *T* (100) tocotrienol (100 mg/kg)

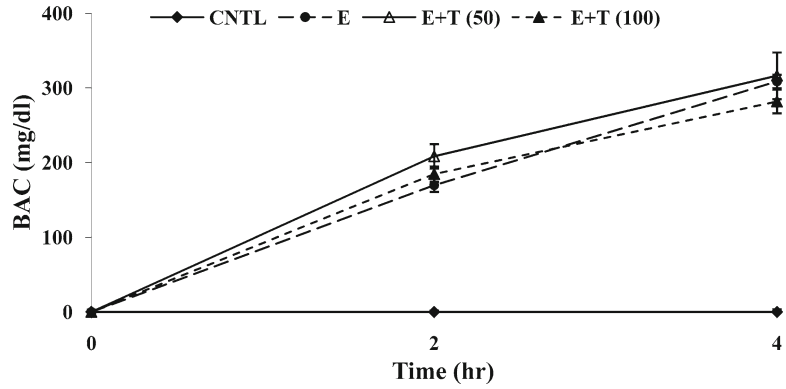

Fig. 14.8 Effect of tocotrienol on blood alcohol concentration (BAC) of ethanol-administered pups at different time points. Values were expressed as mean ± S.E.M. *a.* Different from control group ($P < 0.05$); *b.* different from ethanol-administered group ($P < 0.05$); (n = 5–8 per group). *CNTL* control, *E* ethanol, *T* (50) tocotrienol (50 mg/kg), *T* (100) tocotrienol (100 mg/kg)

Chronic treatment with tocotrienol significantly mitigated ethanol-mediated alterations in the levels of lipid peroxides and antioxidants enzymes along with attenuation of enhanced nitrite levels in both the brain regions of ethanol-exposed pups. Apart from this, we also observed a significantly enhanced levels of acetylcholinesterase in different brain regions of ethanol-treated pups which was significantly inhibited on treatment with tocotrienol (Fig. 14.9). In addition to oxidative– nitrodative stress, chronic alcohol administration is also associated with enhanced neuroinflammatory response. We found a significant elevation in the levels of TNF-α and IL-1β in the cerebral cortex and hippocampus of ethanol-treated pups which is indicative of enhanced neuroinflammation in the two main regions of brain involved in learning and memory. Treatment with tocotrienol significantly reduced the cytokines (TNF-α and IL-1β) in different brain regions of ethanol-administered pups (Fig. 14.10). Apart from increased cytokine levels, we have also found the significant enhancement in levels of NF-κβ (Fig. 14.11) and caspase-3 (Fig. 14.12) in the cerebral cortex and hippocampus of ethanol-treated

Table 14.3 Effect of tocotrienol treatment on lipid peroxide (**a**), reduced glutathione (**b**), superoxide dismutase (**c**), and catalase (**d**), levels in cerebral cortex of ethanol-administered pups. Values were expressed as mean ± S.E.M

Treatment		LPO (nmol/mg protein)	GSH (μmol/mg protein)	SOD (units/mg protein)	Catalase (k/min)
CNTL	Cerebral cortex	1.31 ± 0.06	0.281 ± 0.010	6.17 ± 0.44	5.27 ± 0.37
	Hippocampus	1.29 ± 0.06	0.195 ± 0.009	4.49 ± 0.40	3.01 ± 0.13
E	Cerebral cortex	6.25 ± 0.48[a]	0.05 ± 0.002[a]	0.41 ± 0.02[a]	0.52 ± 0.04[a]
	Hippocampus	4.43 ± 0.08[a]	0.034 ± 0.003[a]	0.21 ± 0.02[a]	0.40 ± 0.07[a]
E + T (50)	Cerebral cortex	4.94 ± 0.15[b]	0.086 ± 0.003[b]	1.29 ± 0.08[b]	1.34 ± 0.10[b]
	Hippocampus	3.97 ± 0.08[b]	0.077 ± 0.002[b]	0.93 ± 0.03[b]	0.94 ± 0.08[b]
E + T (100)	Cerebral cortex	4.63 ± 0.16[b]	0.124 ± 0.003[b]	1.68 ± 0.09[b]	1.67 ± 0.11[b]
	Hippocampus	3.39 ± 0.12[b]	0.115 ± 0.004[b]	1.35 ± 0.08[b]	1.39 ± 0.08[b]
T (100)	Cerebral cortex	1.18 ± 0.07	0.229 ± 0.016	6.38 ± 0.76	4.62 ± 0.25
	Hippocampus	1.15 ± 0.12	0.168 ± 0.019	3.8 ± 0..44	3.28 ± 0.25

[a]Different from control group ($P < 0.05$)

[b]Different from ethanol-administered group ($P < 0.05$); (n = 5–8 per group). *CNTL* control, *E* ethanol, *T* (50) tocotrienol (50 mg/kg), *T* (100) tocotrienol (100 mg/kg)

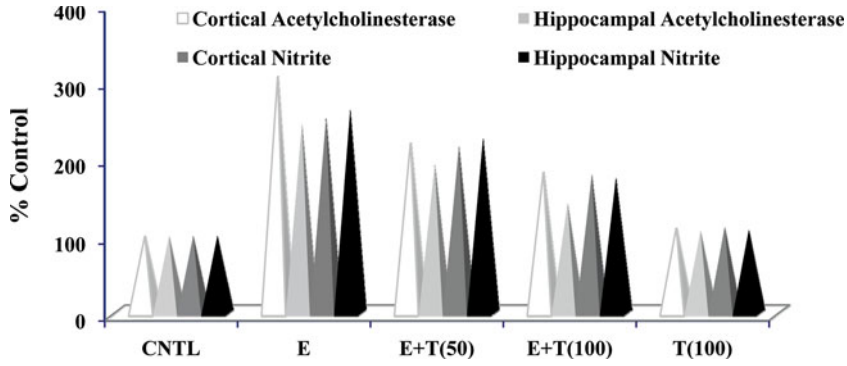

Fig. 14.9 Effect of tocotrienol treatment on acetylcholinesterase activity and nitrite levels in cerebral cortex and hippocampus of ethanol-administered neonatal rats. Values were expressed as % control. *a.* Different from control group ($P < 0.05$); *b.* different from ethanol-administered group ($P < 0.05$); (n = 5–8 per group). *CNTL* control, *E* ethanol, *T* (50) tocotrienol (50 mg/kg), *T* (100) tocotrienol (100 mg/kg)

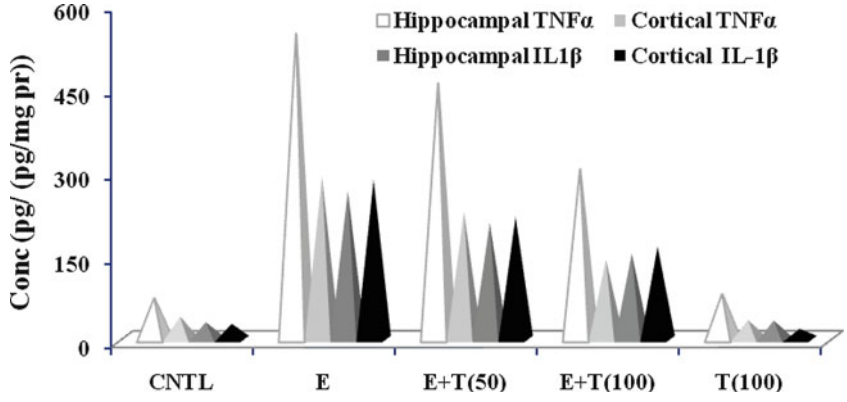

Fig. 14.10 Effect of chronic treatment with tocotrienol on TNF-α and IL-1β levels in cerebral cortex and hippocampus of ethanol-administered pups. Values were expressed as mean ± S.E.M. (**a**) Different from control group ($P < 0.05$); (**b**) different from ethanol-administered group ($P < 0.05$); (n = 5–8 per group). CNTL control, E ethanol, T (50) tocotrienol (50 mg/kg), T (100) tocotrienol (100 mg/kg)

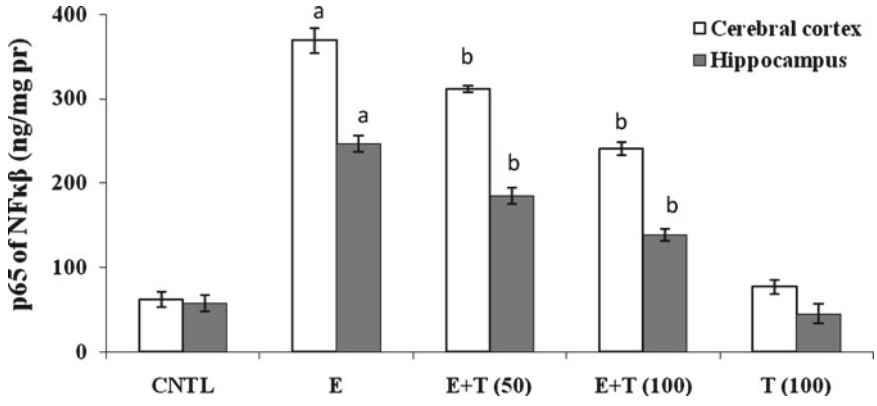

Fig. 14.11 Effect of chronic treatment with tocotrienol on NF-κβ level in cerebral cortex and hippocampus of ethanol-administered pups. Values were expressed as mean ± S.E.M. *a*. Different from control group ($P < 0.05$); *b*. different from ethanol-administered group ($P < 0.05$); (n = 5–8 per group). *CNTL* control, *E* ethanol, *T* (50) tocotrienol (50 mg/kg), *T* (100) tocotrienol (100 mg/kg)

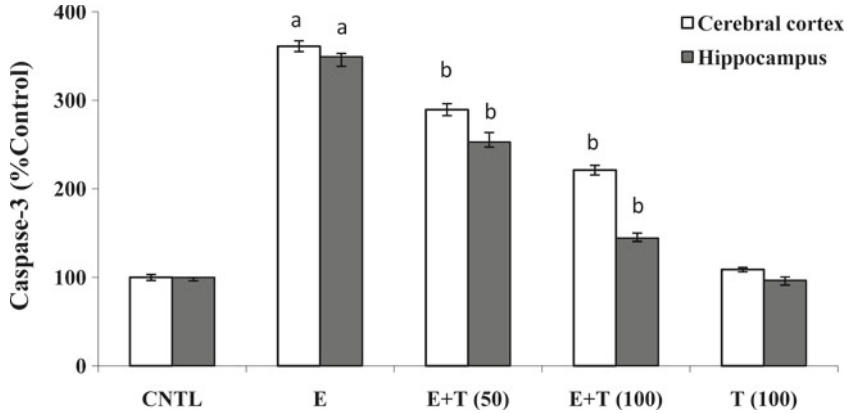

Fig. 14.12 Effect of chronic treatment with tocotrienol on caspase-3 level in cerebral cortex and hippocampus of ethanol-administered pups. Values were expressed as mean ± S.E.M. *a.* Different from control group ($P < 0.05$); *b.* different from ethanol-administered group ($P < 0.05$); (n = 5–8 per group). *CNTL* control, *E* ethanol, *T* (50) tocotrienol (50 mg/kg), *T* (100) tocotrienol (100 mg/kg)

pups, suggesting the role of apoptotic pathway in alcohol-induced cognitive deficits. Our findings are supported by observation from Jung et al. who found that chronic exposure to ethanol results in increased amounts of oxidative damage; activation of protein kinase C and NF-κβ, which results in DNA fragmentation; and ultimately increased neuronal death through apoptosis or other mechanisms that are responsible for the behavioral deficits including dementia [39]. In our study, treatment with tocotrienol significantly inhibited both NF-κβ and caspase-3 in cerebral cortex and hippocampus of pups administered ethanol (Figs. 14.11 and 14.12).

Thus, tocotrienol prevents cognitive dysfunction associated with postnatal alcohol exposure by attenuating oxido-nitrodative stress-mediated activation of apoptotic signaling pathway and thus has a potential to be a useful therapeutic option against cognitive deficits in children with FASDs.

Conclusion and Future Direction

Alcohol consumption leads to severe physical, mental, and behavioral deficits, and there are no therapeutic options available to prevent the ethanol-associated damage to central nervous system. Heavy prenatal alcohol exposure has been associated with widespread neuropsychological deficits across several domains including general intelligence, memory, language, attention, learning, visuospatial abilities, executive functioning, motor skills, and social and adaptive functioning. Therefore, understanding how alcohol exposure produces behavioral and cognitive deficits is of great medical and economic importance.

Tocotrienol, an isoform of vitamin E, is one of the most potent natural antioxidants and possesses numerous functions that are not shared by α-tocopherol. A review of the NIH CRISP database shows that funding for tocotrienol research represents less than 1% of all vitamin E research during the last 30+ years. Approximately only 1% of the entire literature on vitamin E addresses tocotrienols. This represents a major void in vitamin E research. During the last 5 years, tocotrienol research has gained substantial momentum. More than two-thirds (210/301) of the entire PubMed literature on tocotrienols has been published on or after 2000. This represents a major swing in the overall direction of vitamin E research. Evidence has started building up regarding potent neuroprotective properties of tocotrienol. Moreover, findings from our laboratory demonstrated neuroprotective potential of tocotrienol against alcohol-induced cognitive deficits not only in adults but also in neonatal rats by inhibiting oxido-nitrodative stress mediated inflammatory signaling and cell death cascade.

Thus, tocotrienol may find a place in the clinical armamentarium for treating patients with alcohol-induced cognitive deficits. However, the clinical relevance of tocotrienol for the treatment of alcohol-induced cognitive deficits warrants further investigations. The current state of knowledge warrants strategic investment into the lesser known forms of vitamin E with emphasis on uncovering the specific conditions that govern the function of vitamin E molecules in vivo.

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Chapter 15
Soy Products Affecting Alcohol Absorption and Metabolism

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Key Points

- Soy bean isoflavones have preventive effect for chronic diseases such as breast cancer, prostate cancer, hyperlipidemia, and so on.
- The isoflavones consisted of glycoside type and aglycone type; the latter is absorbed more quickly in rat and human study.
- Soy product intake inhibited the absorption of ethanol through gastrointestinal tract.
- Isoflavone aglycones in fermented soymilk have decreased the ethanol and acetaldehyde levels in serum. Those results indicated isoflavone-enhanced ethanol metabolism and antioxidative system.

Keywords Isoflavone • Soymilk • Fermented soymilk • Aglycone • Glycoside

Introduction

Ethanol absorption in humans is controlled mainly by gastric emptying because the primary region of ethanol absorption is the small intestine [1]. Vegetable oils such as soybean oil and coconut oil delay the elimination rate of gastric ethanol and lessen the resultant increase in plasma ethanol concentrations [2]. The clearance of ethanol and toxic acetaldehyde is achieved by ethanol-metabolizing enzymes such as alcohol dehydrogenase (ADH), acetaldehyde dehydrogenase (ALDH), and microsomal ethanol oxidizing system (MEOS) [3]. Therefore, components such as sesamin and garlic that stimulate the activity of these enzymes are expected to ameliorate alcohol toxicity [4, 5].

Soy Products and Isoflavones

Five traditional crops in Japan (rice, soybean, barnyard grass, foxtail millet, and wheat) are nutritionally important foodstuffs known collectively as "Go-Koku" ("Go" meaning five and "Koku" meaning cereals and beans in Japanese). Of these crops, soybean is particularly rich in protein, fat, and carbohydrate

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Fig. 15.1 Familiar soy products served in Japan

and contributes nutritionally to health as a so-called field meat. Various types of soy products are available (Fig. 15.1), including soymilk (soybean extract), tofu (soybean curd from soymilk), soy sauce, and natto (fermented soybeans with a slimy consistency).

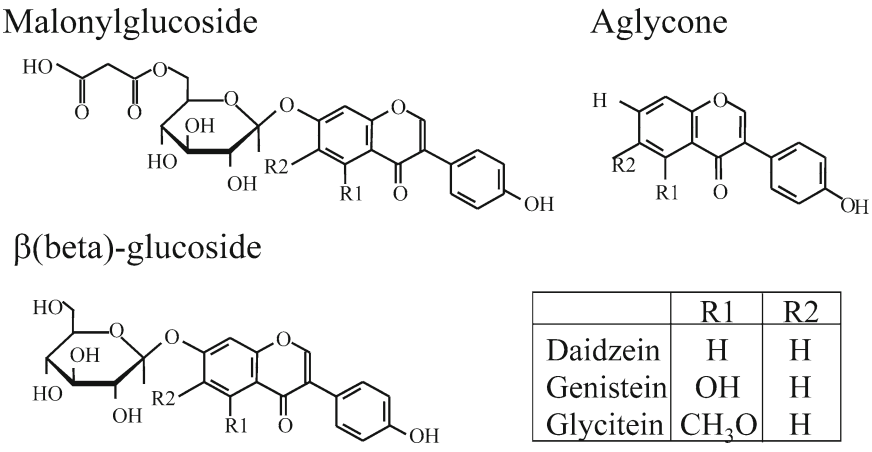
Recently, soybean and soy protein have attracted considerable attention for their preventive effect on chronic diseases such as breast cancer and prostate cancer, hyperlipidemia, atherosclerosis, cardiovascular disease, osteoporosis, and menopausal symptoms [6, 7]. Many of these benefits derive from soybean isoflavones (Fig. 15.2), which are nonsteroidal phytoestrogenic and antioxidative polyphenolic molecules [8–10].

Previous reports on the effects of isoflavones derived from a non-soybean source on ethanol consumption showed that isoflavones prepared from a crude extract of *Pueraria lobata* (Kudzu root) are used as a traditional medicine for anti-inebriation and suppress alcohol intake in alcohol-preferring rats [11, 12]. The major components of the extract, daidzin and daidzein, are inhibitors of in vitro mitochondrial low-Km ALDH and ADH, although these enzymes are not affected by intragastric or intraperitoneal injection of daidzin [13, 14]. Thus, the relationship of isoflavones with alcohol-suppressing performance remains to be fully clarified. Furthermore, only a few reports have been published on the effect of soy on ethanol consumption.

Bioavailability of Isoflavones After Ingestion of Soy Beverages

The natural isoflavones in soybeans and unfermented soyfoods are present in glucose-conjugated forms [15]. Intestinal microflora affects the metabolism or absorption of isoflavones; for example, isoflavones are hydrolyzed to absorbable aglycones or transformed into metabolites such as equol or *O*-desmethylangolensin from daidzein [16–18] (Fig. 15.3). The intestinal absorption of most isoflavones is thought to require the release of aglycone forms from glucoside conjugates.

Soymilk is a central material for soy products as well as a beverage in itself. We have previously developed fermented soymilk (FSM) using the probiotic *Bifidobacterium breve* strain Yakult [19–22] and investigated its physiological functions. Soymilk mostly contains the glucoside form of isoflavones



	R1	R2
Daidzein	H	H
Genistein	OH	H
Glycitein	CH_3O	H

Fig. 15.2 Structure of isoflavones

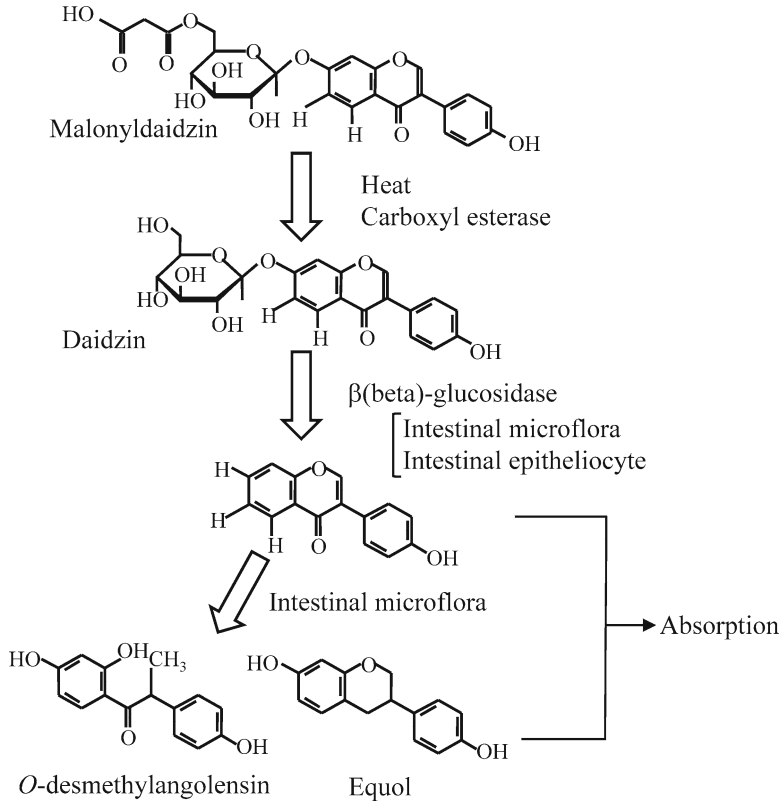


Fig. 15.3 Degradation and absorption of isoflavone

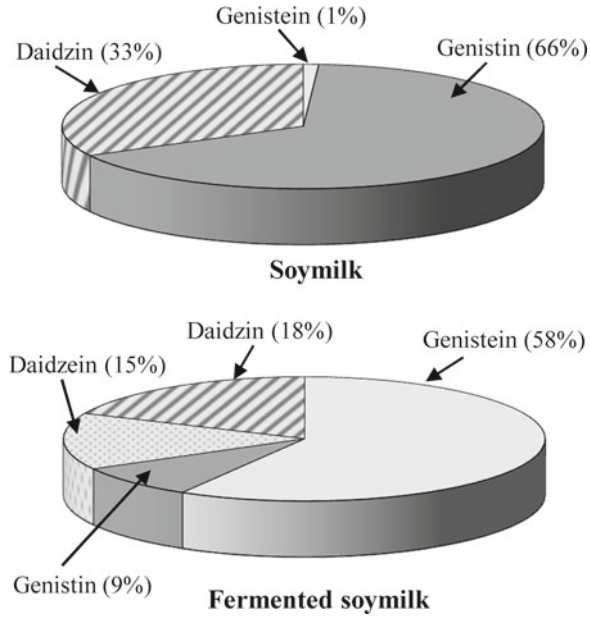


Fig. 15.4 Isoflavone concentrations in soymilk and fermented soymilk

(>99%), but the aglycone form of isoflavones is dominant in FSM (>90%) (Fig. 15.4). We investigated the absorption of isoflavones after ingestion of soymilk and FSM in two separate studies based on rats and humans.

Rat Study

We investigated the absorption of isoflavones after the ingestion of soymilk or FSM in male SD rats. Rats that had fasted overnight were intragastrically administered sample beverages, and blood isoflavone concentrations were then measured. These were found to be significantly higher after the ingestion of FSM compared with soymilk (Fig. 15.5) [23], suggesting that isoflavone aglycones are absorbed more rapidly and efficiently into rat blood than glucosides.

Human Study

Twelve healthy volunteers ingested soymilk and FSM. Soymilk was shown to elevate total serum isoflavone concentrations slowly, while FSM increased the isoflavone concentrations more quickly (Fig. 15.6) [24, 25]. This revealed that isoflavones converted to aglycones are absorbed more quickly

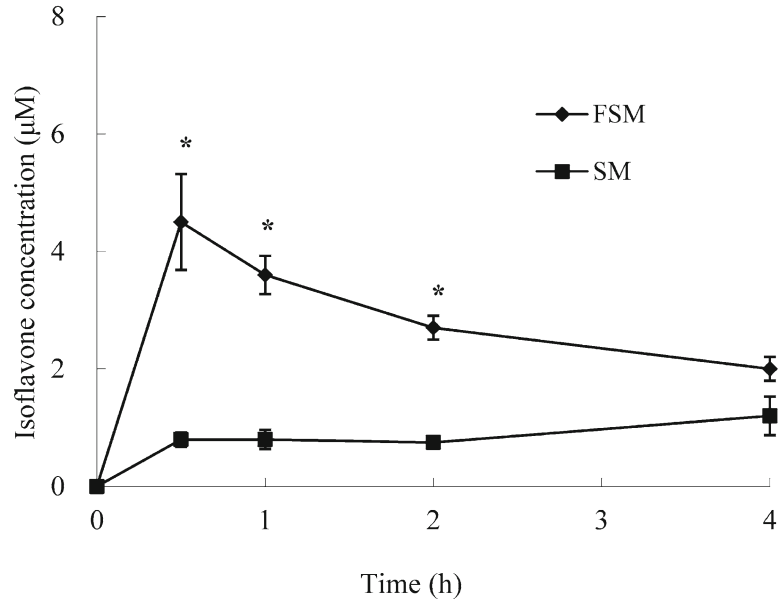


Fig. 15.5 Time-course change of concentration in plasma after oral administration of soymilk (SM) and fermented soymilk (FSM) (7.5 mL/kg of body weight). Values are means ± SEM of six rats. Asterisk indicates significant difference ($p < 0.05$) from the soymilk value by unpaired *t*-test

and in larger amounts in humans. This difference could reflect the effect of FSM on lipid metabolism [26–28] and on mammary carcinogenesis [29]. Therefore, ethanol consumption is potentially relevant to the isoflavone form and probably also to its absorbability or availability.

Soymilk Products and Ethanol Absorption

To determine whether soymilk products or differences in the isoflavone form affect ethanol absorption, we investigated the effect of soymilk and FSM in male SD rats [30, 31]. Overnight-fasted rats were intragastrically administered sample beverages in which 20% ethanol was added to the casein-based control, soymilk, or FSM solutions. At early stages after ethanol injection, the ethanol concentration in the stomach was greater in the FSM group than in the control group. However, portal ethanol levels differed between these groups, with the control group having the highest level, followed by the soymilk group, and lastly the FSM group (Fig. 15.7). Taking into consideration the fact that portal ethanol levels directly reflect ethanol absorption through the gastrointestinal tract, these findings suggest that FSM components other than those common to soymilk strongly contribute to ethanol absorption.

Similarly, the aortal blood flow through the liver reflects the hepatic ethanol metabolism in addition to absorption. After ethanol administration, aortal ethanol and acetaldehyde levels were lower in the FSM group than in the control group. From these results, the FSM effect appears to be dependent on lowering ethanol absorption or enhancing ethanol metabolism following acute ethanol administration.

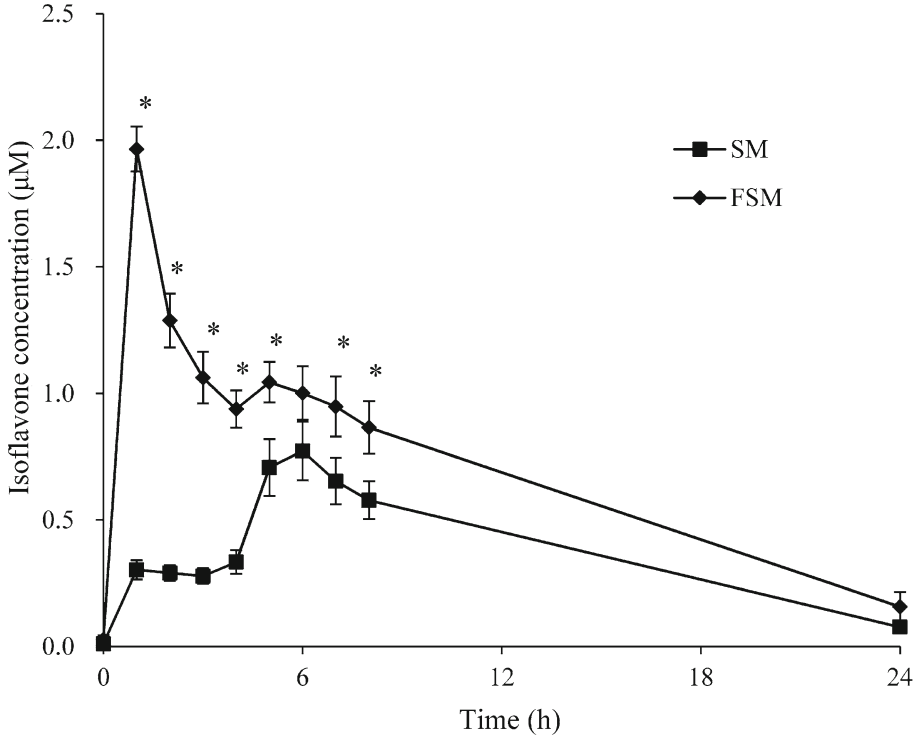


Fig. 15.6 Serum concentration of isoflavones (daidzein + genistein) in healthy adult subjects after ingestion of soymilk (SM) and fermented soymilk (FSM) (100 mL). Values are means ±SEM, n=11. Asterisk indicates significant difference ($p < 0.05$) from the soymilk value by paired *t*-test

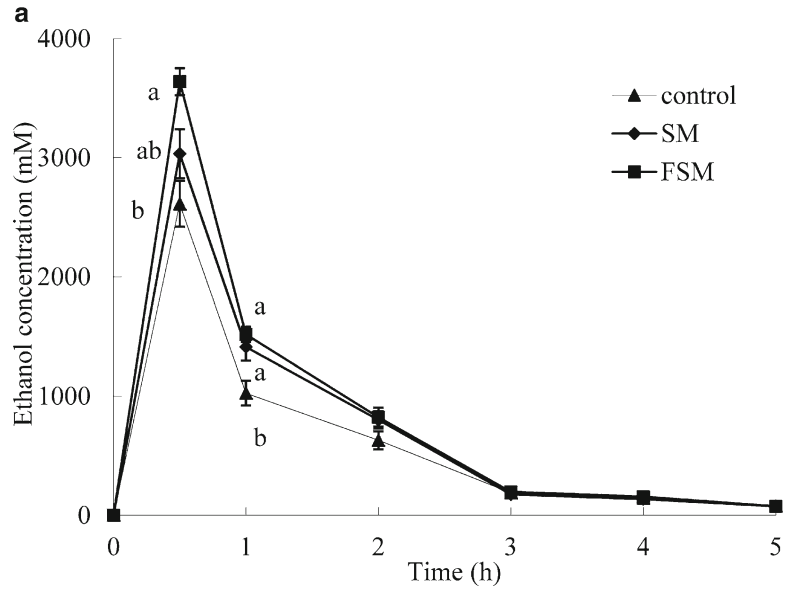


Fig. 15.7 Concentrations of ethanol in the gastric content (**a**), in the portal blood (**b**), and in the aortal blood (**c**) and concentration of acetaldehyde in the aortal blood (**d**) of rats after oral administration of control, soymilk (SM), or fermented soymilk (FSM) solutions containing 20% ethanol. The data represent mean ± SEM of eight rats. [ab]Mean values not sharing the same letter are significantly different at $p < 0.05$ by Tukey's test

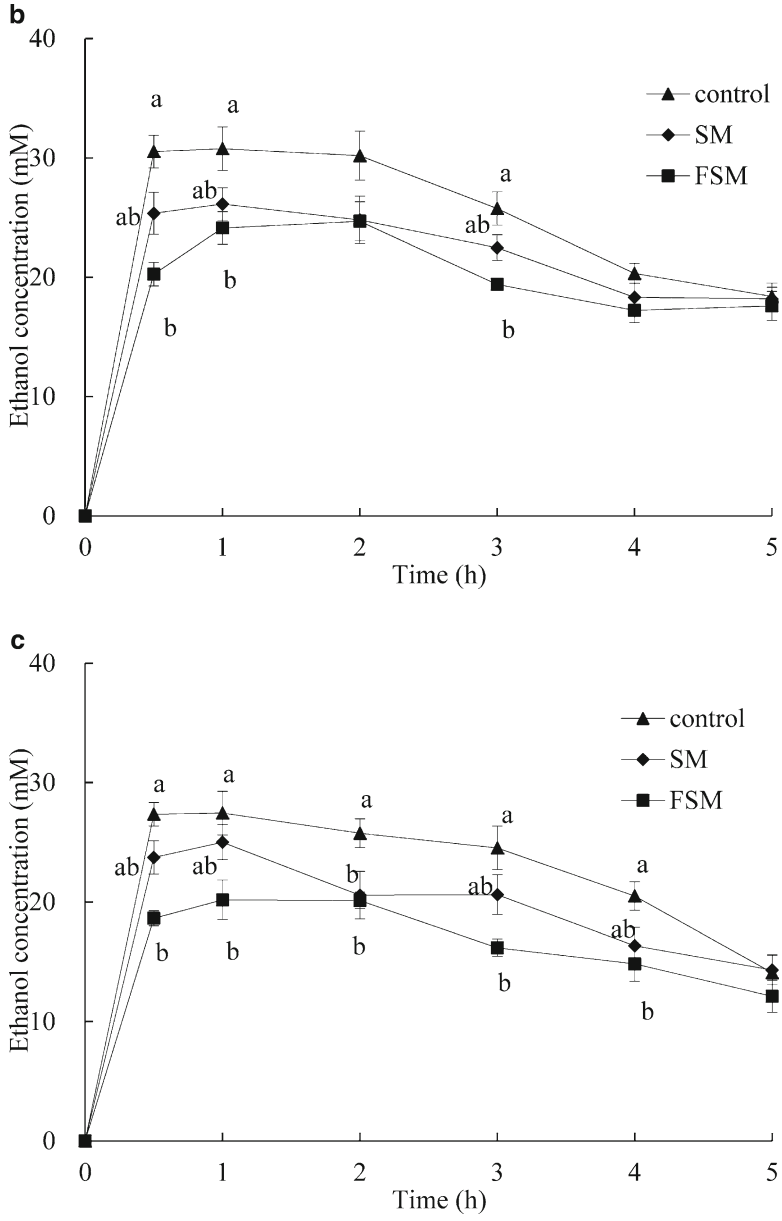


Fig. 15.7 (continued)

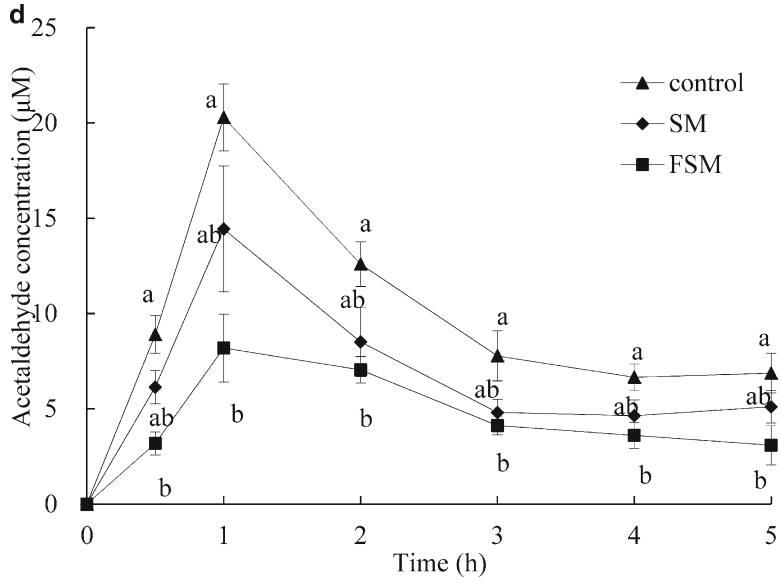


Fig. 15.7 (continued)

Soy Isoflavones and Ethanol Metabolism

Ethanol entering the liver through the portal vein is oxidized into acetaldehyde and further to acetate in the hepatocytes. Soy components, especially isoflavones, also flow into the liver through the portal vein. The previous experiment on rat hepatocytes cultured with 65 mM ethanol [30] shows that physiological doses of isoflavones (−5 μM) affect ethanol and acetaldehyde metabolism (Fig. 15.8), indicating that the in vivo decrease in aortal ethanol and acetaldehyde caused by FSM is closely related to the direct effect of soy isoflavones on liver function. Our observations differ slightly from those of previous reports. One report showed that the isoflavone glucosides, daidzin and genistin, inhibit human ALDH in vitro, whereas the corresponding aglycones, daidzein and genistein, do not [32]. Another study showed that daidzin suppresses ethanol intake without affecting acetaldehyde metabolism in hamsters [13].

Soymilk Products and Ethanol Metabolism

Induction of microsomal ethanol oxidizing system (MEOS) activity and restriction of ALDH activity by ethanol [33] are associated with an accumulation of acetaldehyde and reactive oxygen species following chronic or high consumption of ethanol. These toxic molecules derived from ethanol are considered to cause cell injury through lipid peroxidation, enzyme inactivation, and DNA damage

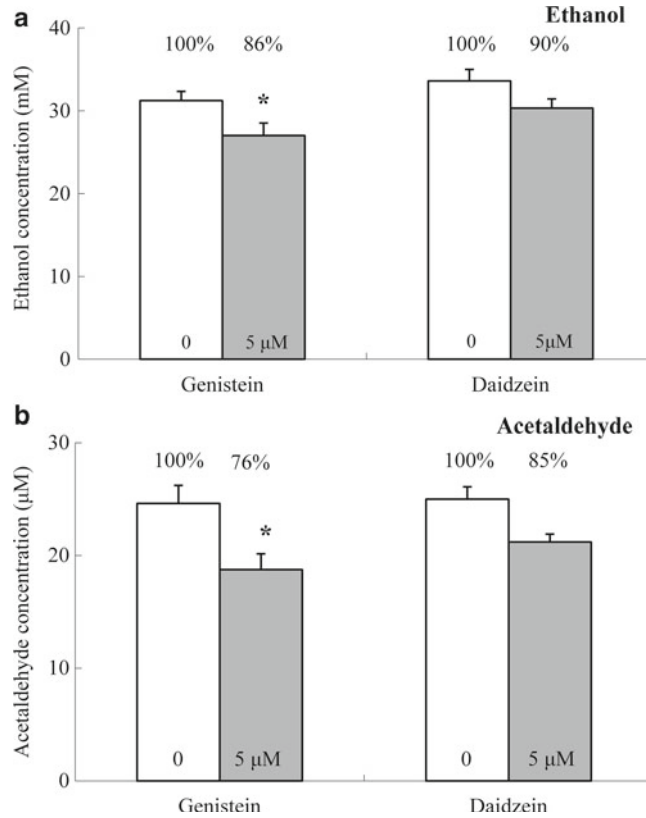


Fig. 15.8 Concentration of ethanol (**a**) and acetaldehyde (**b**) in the culture filtrates from isolated rat hepatocyte cultures (10^7 cells/10 mL medium) on addition of genistein or daidzein (0 or 5 μM). The data represent the mean ± SEM of six animals. Asterisk indicates significant difference ($p < 0.05$) by unpaired *t*-test

[34–36]. Glutathione S-transferase (GST) participates in the detoxification of acetaldehyde through glutathione conjugation [37] as well as the antioxidation of active xenobiotic metabolites and reduction of lipid peroxides [38]. The relationship between soy components and the P450 system is not well understood, but genistein appears to act as a potent inhibitor of CYP1A1 and/or CYP1A2 induced by β (beta)-naphthoflavone [39], and soy protein acts as an enhancer of the dexamethasone-induced mRNA expression of hepatic CYP3A2 [40].

In SD rats chronically exposed to ethanol (5%) [26], FSM feeding decreased MEOS activity, probably through its effect on CYP2E1, but did not affect cytosolic ADH activity (Fig. 15.9). Soymilk products were shown not only to enhance cytosolic GST and mitochondrial low-Km ALDH activities but also to restrict hepatic thiobarbituric acid-reactive substances, putative markers of lipid peroxidation [41], which were induced by chronic ethanol exposure. These facts suggest that the consumption of soymilk products contribute to the prevention of ethanol-induced liver injury through enhancement of ethanol metabolism and the antioxidation system. Furthermore, it should be noted that soymilk and FSM differ in their efficacy against ethanol metabolism, as shown by aortal ethanol and acetaldehyde levels after the oral administration of ethanol and in MEOS and GST activities following chronic

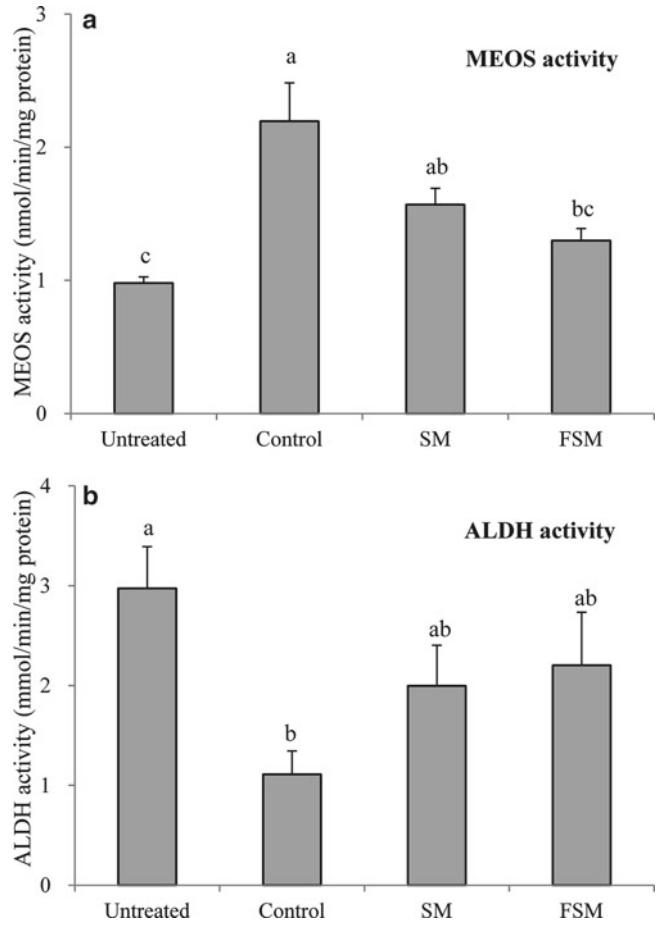


Fig. 15.9 Microsomal ethanol oxidizing system (MEOS) activity in the liver (**a**) and low-Km acetaldehyde dehydrogenase (ALDH) activity in the liver mitochondrial fraction (**b**) of rats consuming control diet + 5% ethanol (control group), soymilk diet + 5% ethanol (SM group), fermented soymilk (FSM group), and control diet + water (untreated group) for 24 days. The data represent the mean ± SEM of eight rats. [abc]Mean values not sharing the same letter above the bars are significantly different at $p < 0.05$ by Tukey's test

exposure. FSM contains organic acids (lactic and acetic acids) and probiotic bacteria that accumulate during the fermentation process as well as isoflavone aglycones [42], but it is not yet clear whether these are directly associated with ethanol consumption.

Anthocyanin has recently been studied as a physiologically functional food factor. The intake of purple sweet potato beverages, rich in anthocyanin, was found to significantly decrease serum levels of hepatic biomarkers, particularly γ (gamma)-GTP, in healthy men with borderline hepatitis [43]. γ (gamma)-GTP is a known parameter of alcoholic liver diseases, indicating that anthocyanins contribute to the suppression of alcohol-induced liver diseases.

Conclusion

Soymilk products inhibit ethanol absorption and enhance ethanol metabolism. Reactive metabolites generated during ethanol metabolism trigger ethanol-induced cell injury, which is suppressed by the antioxidation system. The antioxidative activity of soy isoflavones may also assist in reinforcing the system. Future studies should further investigate the physiological functions of isoflavones and anthocyanins in ethanol metabolism.

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Chapter 16
Oats Supplementation and Alcohol-Induced Oxidative Tissue Damage

Christopher B. Forsyth, Yueming Tang, Robin M. Voigt, Turan Rai, and Ali Keshavarzian

Key Points

- Alcohol and alcohol metabolism result in the generation of free radicals and ROS/RNS which are detrimental to cellular function.
- One potential mechanism which appears to be particularly important in alcohol-induced organ damage such as liver disease is increased circulating endotoxin occurring secondary to intestinal hyperpermeability and/or a dysbiotic intestinal microbiota.
- One potential way to reduce the oxidative burden and prevent alcohol-induced damage may be via dietary oats supplementation. Indeed, oats supplementation reduces endotoxemia and prevents alcohol-induced gut leakiness, dysbiosis, and liver damage in a rodent model.
- In humans, restoration of intestinal integrity by oats has not been studied; however, in light of the discussed animal studies, oats supplementation as a therapeutic strategy to prevent and/or treat alcohol-induced gut leakiness, endotoxemia, tissue oxidative injury, and organ damage like ALD as well as other disorders associated with gut leakiness and oxidative tissue injury (e.g. inflammatory bowel disease) is warranted.

Keywords Intestinal permeability • Oats • Endotoxin • Dysbiosis • Prebiotic • Antioxidant

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Introduction

Alcohol has been a widely used and abused substance throughout human civilization, with use reported as early as the Neolithic period circa 10,000 B.C. [1]. Alcohol remains a highly popular substance today with approximately 51% of Americans over the age of 21 reporting alcohol use. Of these individuals, 11% meet the criteria for alcohol abuse with approximately 18 million alcoholics in the United States. The consequences of alcohol abuse are numerous including liver cirrhosis, liver transplantation [2], and death occurring secondary to traffic accidents [3]. Thus, it is clear that chronic alcohol use/abuse is a significant public health problem.

Tissue/organ damage resulting from acute and chronic alcohol abuse typically are multi-systemic with the most commonly affected organs being liver, heart, and brain [4, 5]. A major factor accounting for the deleterious effects are alcohol metabolism products including both oxidative and non-oxidative mechanisms. For example, alcohol metabolism results in tissue injury through oxygen consumption, resulting in hypoxia, interaction between alcohol metabolism products and proteins or other macromolecules (adduct formation), and the formation of highly reactive oxygen (ROS) and reactive nitrogen (RNS) species [4, 6, 7]. Thus, alcohol-induced damage is multifactorial and can be the consequence of a number of different mechanisms.

Alcohol Metabolism Promotes Oxidative Stress-Mediated Tissue Damage

The mechanisms through which alcohol acts to damage tissues are not completely understood. While there are several mechanisms that contribute to alcohol-induced cellular damage, there is little doubt that oxidative stress is a major contributor [5]. Several pathways are thought to contribute to mechanisms through which alcohol induces cellular and tissue oxidative stress. The classically described oxidative pathway converts ethanol to acetaldehyde which is subsequently converted to acetate (Fig. 16.1) [4, 6, 7]. The initial conversion of ethanol to acetaldehyde is mediated through several enzymatic pathways: alcohol dehydrogenase (ADH) in the cytosol, cytochrome P-450 isoform 2E1 (i.e. CYP2E1) and lesser P-450 isoforms within microsomes, and catalase located in the peroxisome.

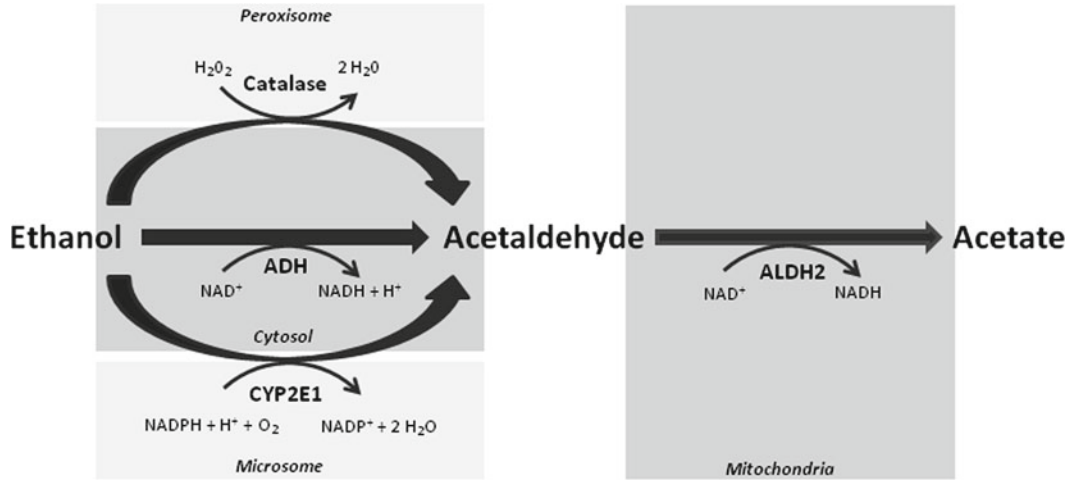

Fig. 16.1 Alcohol metabolism by ADH and ALDH2

Acetaldehyde is subsequently oxidized to form acetate by aldehyde dehydrogenase (ALDH2) in the mitochondria. These metabolic processes predominantly occur in the liver but also occur in the intestine, kidney, and brain [6, 7]. The consequence of these metabolic processes is the formation of nicotinamide adenine dinucleotide (NADH) resulting in an increased NADH/NAD^+ ratio. Increased availability of NADH enhances the activity of the respiratory chain including increased O_2 consumption (resulting in tissue hypoxia) and reactive oxygen species (ROS) formation damaging fats, proteins, and DNA [6, 8, 9]. Thus, alcohol metabolism via several mechanisms contributes to cellular and tissue oxidative damage.

In addition to these mechanisms, alcohol induces a 'feed-forward' process in which alcohol alters normal cellular function to engage processes which further promote ROS generation. For example, alcohol also increases CYP2E1 protein levels by stabilizing the enzyme and preventing proteasome-mediated degradation to shift the NADH/NAD+ratio toward NADH to favour the generation of ROS [10].

ROS have important roles in normal cellular function [11], and under normal conditions, the activity of ROS are kept in check by enzymes such as superoxide dismutase, catalases, and glutathione peroxidise as well as small molecule antioxidants such as ascorbic acid (vitamin C), tocopherol (vitamin E), and glutathione [11]. However, chronic increased production of ROS such as that which occurs after chronic alcohol consumption can overwhelm these natural defences, resulting in cellular/tissue damage and organ dysfunction [5, 7].

Another essential and key mechanism of alcohol-induced tissue injury and organ damage is inflammation. Indeed, it appears that a combination of alcohol-induced metabolic changes and inflammation is required for alcohol to cause clinically relevant organ damage. Alcohol-induced liver damage is a clear example of such a multifactorial mechanism [1]. Thus, in this chapter, we will focus on alcoholic liver disease as an example of the important interaction between altered metabolic homeostasis, tissue oxidative stress, and inflammation-associated cellular injury. These elements act as a potential trigger for an activated inflammatory cascade and oxidative stress that can lead to clinically significant organ damage, and we will address how oats supplementation can prevent this process and protect the intestine and liver against deleterious and injurious effects of alcohol abuse.

Alcoholic Liver Disease (ALD)

The liver is one of the most common organs to be affected by chronic consumption of alcohol [1, 5, 12]. Compelling evidence from animal and human studies indicates that alcohol-induced liver injury is the consequence of increased oxidative stress burden and increased release of injurious factors such as cytokines and proteases from activated neutrophils and resident macrophages (Kupffer cells) in the liver [12–15].

Liver lipid peroxidation resulting from excessive ROS/RNS generation appears to be critical for alcohol-induced liver damage. Ethanol-fed rats develop liver damage with a concomitant increase in hepatic lipid peroxidation products (e.g. malondialdehyde, MDA) [16]. This outcome suggests that ethanol-induced liver toxicity may be the consequence of liver lipid peroxidation. Specifically, replacement of readily oxidizable oil (e.g. fish oil) with poorly oxidizable oil (e.g. palm oil) reduces liver lipid peroxidation and ameliorates previously established liver damage [17, 18]. Likewise, humans with ALD also demonstrate markers of augmented liver lipid peroxidation (conjugated dienes, MDA, 4-hydroxynonenal, and F2-isoprostanes) [6, 7, 19]. Thus, it is clear that ROS-mediated lipid peroxidation may play a critical role in alcohol-induced liver damage.

One of the most intriguing observations in alcoholics is that not all alcoholics develop ALD. Although the quantity of alcohol consumed is correlated with the development of ALD, only about

30% of alcoholics develop liver disease [1, 20]. This discrepancy prompted a search for additional factors contributing to individual susceptibility to ALD, and one factor which has emerged is alcohol-induced endotoxemia (lipopolysaccharide (LPS), associated with gram-negative bacteria) [5, 21, 22].

Endotoxins and Alcoholic Liver Disease

Endotoxemia as an essential cofactor for ALD was initially proposed in the late 1980s [23–25]. Not only does alcohol consumption exacerbate endotoxin-mediated liver necrosis, inflammation, and fibrosis [26, 27] but alcohol also increases circulating endotoxin prior to the onset of overt liver damage in a rat model of ALD [28]. Furthermore, high endotoxin levels are reported in the serum of alcohol-fed animals [28–30] and alcoholics with liver disease [23, 24, 31]. Furthermore, there is a positive correlation between endotoxin levels and the severity of alcohol-induced liver damage in animals. Further supporting the notion that endotoxin contributes to ALD, antibiotics, which lower blood endotoxin levels by decreasing gut flora, reduce the severity of alcohol-induced liver damage in animal models [32]. Finally, it should be noted that gut-derived endotoxin promotes systemic and neural inflammation resulting from chronic alcohol use [5, 33, 34]. Taken together, these studies show that chronic and excessive consumption of alcohol promotes endotoxemia which appears to be a critical contributing factor in combination with alcohol-induced metabolic and cellular dysfunction that leads to tissue injury and organ failure like ALD.

In fact, endotoxin enhances alcohol-induced liver free radical production such as hydroxyethyl adducts [25, 35] and further exaggerates tissue oxidative injury by alcohol. Furthermore, gut-derived endotoxin promotes the production of pro-inflammatory cytokines including TNF-α, eicosanoids, ROS, and nitric oxide (NO) via activation of hepatic Kupffer cells which express receptors for endotoxin (i.e. toll-like receptors (TLR) and the TLR co-receptor CD14) [25, 35–38] which exaggerates tissue oxidative injury by alcohol. The Kupffer cell response is intended to be protective by removing circulating endotoxin; however, high levels of endotoxin stimulate Kupffer cells to release large amounts of cytokines and ROS/RNS. Indeed, plasma TNF-α concentrations are elevated in patients with ALD, and the values correlate with disease severity and mortality [39]. There is substantial evidence that Kupffer cells play a critical role in alcohol-induced liver injury. For example, inhibition of Kupffer cells decreases free radical formation and liver injury in chronic alcohol-fed mice [40], and mice deficient in TNF-α receptors are resistant to alcohol-induced liver injury [41]. Thus, identifying factors responsible for increased circulating endotoxin levels and finding ways to prevent alcohol-induced endotoxemia are critical. The primary source of endotoxin is the gut, and chronic alcohol ingestion increases the translocation of gut-derived endotoxin into the systemic circulation. One of the well-established mechanisms of endotoxemia in alcoholics is this alcohol-induced gut leakiness (hyperpermeability).

Mechanisms of Alcohol-Induced Endotoxemia

The primary source of endotoxin is the intestine, and levels of circulating endotoxin are dictated by intestinal permeability as well as by the rate of endotoxin production by intestinal bacteria. Thus, gut leakiness and/or excess production due to dysbiosis of colonic microbiota and small bowel bacterial overgrowth can result in endotoxemia. Indeed, increased intestinal permeability (leaky gut), small bowel bacterial overgrowth, and dysbiosis of colonic microbiota have each been reported in alcoholics and alcohol-fed rodents [22, 31, 42–45].

Thus, there are several potential causes of endotoxemia in alcoholics. First, shunting of blood away from the liver as a consequence of portal hypertension and/or defective Kupffer cell function due to liver disease will hamper the ability of the liver to clear gut-derived endotoxin, resulting in increased levels of endotoxin in the systemic circulation (i.e. endotoxemia). Although this mechanism undoubtedly contributes to endotoxemia in advanced liver disease, where portal hypertension and shunting of the blood as well as defective Kupffer cell function are present, it has no role for initiation of liver injury or in early stage hepatic inflammation (steatohepatitis) where portal hypertension and significant Kupffer cell dysfunction are not present. A second potential mechanism is increased production of endotoxin by the abnormal gut microbiota (dysbiosis) and/or small bowel bacterial overgrowth. Recent studies demonstrate that at least a subset of alcoholics have altered intestinal microbiota [46] and that gut flora are also altered in alcohol-fed animals [29, 42, 45]. In fact, daily alcohol consumption affects microbiome composition concurrent with an elevation in endotoxin levels and liver pathology [42, 45]. The probiotic *Lactobacillus GG* and prebiotic dietary oats supplementation support healthy intestinal microbiota and prevent alcohol-induced dysbiosis [45] and alcoholic liver pathology [47] in a rat model of ALD. Abnormal gut microbiota composition not only can cause endotoxemia by increased production of endotoxin in the gut lumen, it can also promote disruption of intestinal barrier function, resulting in increased translocation of luminal endotoxin into the circulation. Indeed, several recent studies have demonstrated the importance of a crosstalk between intestinal microbiota and intestinal epithelial cells in regulation of intestinal barrier function and the potential role of intestinal microbiota composition in gut leakiness [47–52]. This brings us to the third potential mechanism of alcohol-induced endotoxemia which is gut leakiness.

Alcohol-Induced Gut Leakiness and Disruption of Intestinal Barrier Integrity

Ethanol-mediated changes in intestinal permeability have been reported as early as the 1980s [24, 43]. More importantly, gut leakiness has been noted in only a subset of alcoholics and more specifically in those with liver disease and thus provides intriguing evidence of gut leakiness as the key cofactor for ALD [31, 53]. It should also be noted that even if increased production of endotoxin and decreased removal of endotoxin by the liver are involved in endotoxemia in alcoholics, it is intestinal permeability that is still regulating exposure of the luminal gut contents to the systemic circulation, and thus, intestinal permeability still plays a critical role in alcohol-induced endotoxemia [5]. Thus, an intervention that protects intestinal barrier integrity against injurious effects of alcohol could effectively be used to prevent alcohol-induced tissue damage and organ failure like ALD. In order to identify the optimal therapeutic target to protect the intestinal barrier, one needs to better understand the molecular mechanisms of alcohol-induced gut leakiness.

Molecular Mechanisms of Alcohol-Induced Increase in Gut Permeability

The intestinal epithelial lining is a dynamic and selective barrier allowing passage of nutrients from the lumen into the circulation but limiting translocation of potential injurious and pro-inflammatory factors such as bacteria and bacterial products (e.g. endotoxins) into the systemic circulation [54]. This function depends on intact inter-epithelial cell junctions. The health and integrity of inter-epithelial junctional pathways are dependent on normal tight junctions and adherens junctions – together called the apical junctional complex (AJC) that forms the intestinal epithelial barrier [55]. The AJC is regulated by a series of tight junctional proteins (occludin, claudins), adaptor proteins (e.g. ZO-1) that connect junctional proteins to cytoskeletal actin proteins, and adherens junctional proteins (e.g. E-cadherin) [56].

Disruption of any of these proteins can result in disruption of intestinal barrier function and result in increased gut leakiness. Indeed, multiple in vitro, ex vivo, and human and animal in vivo studies have shown that alcohol causes disruption of actin proteins and other AJC proteins [57–62].

Recent studies have revealed several contributing mechanisms of alcohol-induced disruption of tight junctional and adherens junctional proteins that are essential for intestinal barrier integrity. The first of these is disruption of the intestinal barrier by the acetaldehyde resulting from alcohol metabolism by intestinal ADH or possibly by intestinal bacteria [29]. Several studies have shown that acetaldehyde directly disrupts both tight junctions as well as adherens junctions forming the intestinal barrier in the epithelium [21, 63]. Studies by others have also shown a role for zinc in preventing alcohol-induced intestinal hyperpermeability both in vitro (i.e. Caco-2 cells) and mouse models of ALD [57, 59]. In other in vitro mechanistic studies, alcohol has been shown to induce production of nitric oxide by inducible nitric oxide synthase (iNOS) through an NF-kB-mediated mechanism [60]. Induction of iNOS results in the production of nitric oxide(NO) and resulting oxidative stress which leads to intestinal monolayer hyperpermeability via alterations in the intestinal epithelial cell cytoskeleton and APC proteins [61, 62]. An additional pathway mediated by iNOS activation is signalling through the transcription factor Snail, and Snail activation and intestinal hyperpermeability are prevented in iNOS KO mice [64]. Demonstrating the critical importance of NO, chemical inhibition of iNOS prevents chronic alcohol-induced oxidative stress, intestinal hyperpermeability, endotoxemia, and liver disease in a rat model of ALD [30]. Significantly, alcohol-mediated changes in the intestinal microbiota may be a key element in promoting the molecular alcohol-induced iNOS-mediated permeability pathways in vivo. Studies have shown that in the same chronic alcohol rat model noted above, dysbiosis occurs [45] and that amelioration of this dysbiosis with dietary supplementation of *Lactobacillus GG* [47] [65, 66] prevents not only the increase in alcohol-induced intestinal hyperpermeability but also prevents alcohol-induced increases in iNOS and nitrotyrosine markers of oxidative stress in the gut and liver [45].

These data support, therefore, that the ideal therapeutic agents for alcohol-induced oxidative stress and ALD are agents with prebiotic and antioxidant properties. These agents have a potential ability to prevent alcohol-induced endotoxemia by minimizing endotoxin production (prebiotic effects normalizing dysbiotic microbiota composition) and also by limiting translocation of luminal endotoxins into the circulation (by protecting intestinal barrier integrity through their antioxidant and prebiotic effects). Below, we present data to show that oats could be such an ideal therapeutic agent.

Oats and Alcohol-Induced Oxidative Tissue Damage

Oats could be an effective ‘natural’ remedy for alcohol-induced oxidative tissue injury because it has multiple effects on several distinct pathways that are involved in alcohol-mediated tissue injury as detailed in the above sections and depicted in Fig. 16.2. Although further studies are needed to rigorously establish the mechanisms of action of oats supplementation in alcohol-induced organ damage, the in vitro and in vivo data are clear and provide evidence for several excellent candidate mechanisms. First, oats have potent antioxidant properties [67–69] that may directly inhibit the oxidative stress associated with alcohol metabolism. Oats contain unique antioxidant polyphenols called avenanthramides (Avns) not found in other cereal grains [69, 70]. The beneficial health effect and especially the antioxidant effect of oats on intestinal barrier integrity [65] and prevention of liver damage in alcohol-fed rats [65] are likely, in part, due to these Avns. Avns are unique low-molecular-weight, alcohol-soluble phenolic antioxidants. These are conjugates of a phenylpropanoid with anthranilic acid or 5-hydroxy anthranilic acid. There are more than 20 different forms of Avns, but A, B, and C are the major three forms with AV-C being the most bioavailable and AV-A the most potent in a hamster model [68]. The antioxidant and anti-inflammatory properties of Avns have been extensively

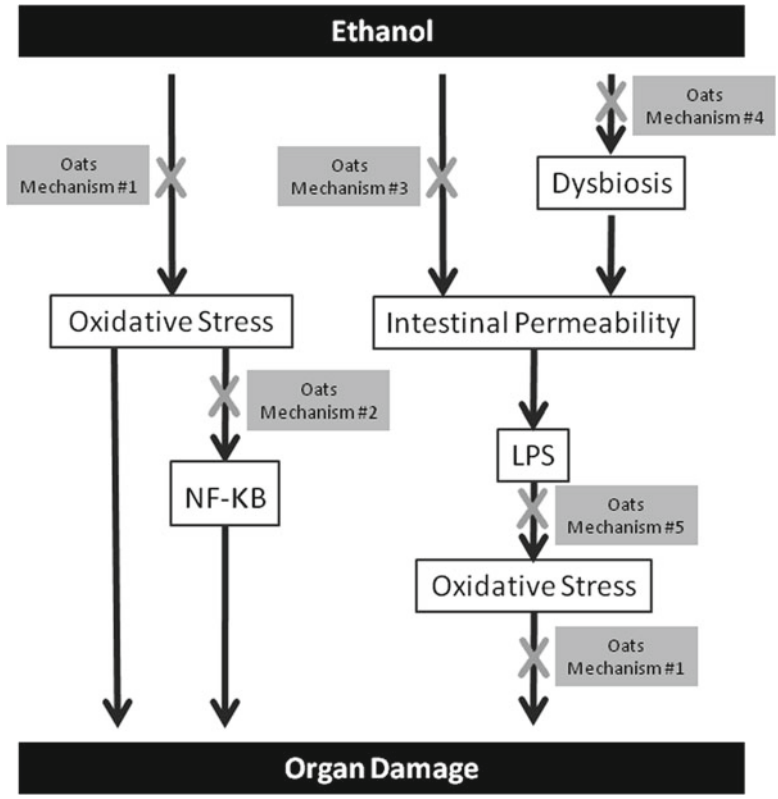

Fig. 16.2 Proposed mechanisms for oats protection against alcohol-induced oxidative stress

studied in vitro, and Avns inhibit LDL oxidation as well as the generation of ROS-peroxyl radicals [71]. In a mouse model of D-galactose-induced oxidative stress, oats reduced systemic markers of oxidative stress such as MDA and increased mRNA expression of superoxide dismutase (SOD) and other antioxidant enzymes [72]. Indeed, in rats, Avn extract supplementation increases the production of SOD and thus reduces ROS burden. Furthermore, Avns have been shown to accumulate in heart, muscle, and liver and are bioavailable after oral administration [73].

Second, oats appear to directly inhibit NF-kB activation, which may or may not be related to Avns antioxidant properties [69]. As noted above, NF-kB is a key transcription factor regulating both inflammation and immunity and associated with oxidative stress and production of inflammatory cytokines such as TNF-α in alcoholics and alcohol-fed rodents [13, 30, 74]. NF-kB is also involved in alcohol-induced disruption of the intestinal barrier resulting in endotoxemia and ALD [60]. Thus, systemic inhibition of alcohol-induced NF-kB activation by oats might significantly contribute to the effects observed in animal models of chronic alcohol use in which oats restored normal intestinal barrier function and prevented hepatic inflammation (alcoholic steatohepatitis) [65, 66]. Recent data on oat polyphenols has demonstrated their ability to inhibit NF-kB activation [75–77]. Polyphenols from oats have been shown to directly inhibit NF-kB as well as the production of inflammatory cytokines [78, 79] and to inhibit inflammatory pathways and proliferation of colon cancer cells [80]. Recently, another polyphenol resveratrol was shown to inhibit NF-kB activation in the brains of rat pups chronically fed with alcohol [75]. The authors concluded that this inhibition of alcohol-induced NF-kB activation resulted in elimination of brain oxidative-nitrosative stress as well as dramatic reductions of NF-kB target inflammatory cytokines such as TNF-α and IL-1β. These data support a similar

mechanism for oats Avn inhibition of alcohol activation of NF-kB-mediated inflammation and cytokine production in the intestine and liver.

Third, oats can prevent alcohol-induced intestinal hyperpermeability which is the primary source for systemic endotoxin associated with chronic alcohol use and so important in the pathogenesis of alcoholic liver disease as well as other systemic inflammatory effects of chronic alcohol use. Studies in a rat model of chronic alcohol consumption show that dietary oat supplementation prevents alcohol-induced intestinal hyperpermeability as well as the associated endotoxemia and liver disease [65, 66]. Furthermore, oats treatment was found to prevent changes in intestinal epithelial tight junction proteins and the cell cytoskeleton associated with alcohol-induced intestinal hyperpermeability. In addition, oats prevented alcohol-induced increases in markers of oxidative stress (carbonylation, nitrotyrosine) also associated with alcohol-induced intestinal hyperpermeability in both the intestine and the livers of alcohol-treated rats [65]. A recent study that measured portal vein endotoxin levels in rats without alcohol treatment showed that oats gavage significantly reduced portal vein endotoxemia [81]. Thus, oats appears to have significant effects on preventing gut leakiness and endotoxemia associated with chronic alcohol use in rodents, and this may play a key role in the reduction in systemic markers of alcohol-induced oxidative stress observed with oats gavage.

A fourth key mechanism through which oats may exert systemic protection against alcohol-induced oxidative stress is through their ability to act as a so-called prebiotic and promote a beneficial profile of intestinal bacteria [82, 83]. A large number of studies now support the function of oats as an effective prebiotic [83, 84] that promotes a more healthy gut microbiota, resulting in reductions in systemic markers of oxidative stress and markers of metabolic syndrome [48]. As noted above, evidence exists that both alcoholics [42, 46] and rodents chronically fed with alcohol [29, 45] exhibit an altered profile of intestinal microbiota composition (also known as dysbiosis). Modulation of this alcohol-associated dysbiosis with probiotics such as *Lactobacillus* species results in amelioration of hepatic markers of inflammation and oxidative stress in both alcoholics [46] and alcohol-fed rats [45, 47] as well as restoration of normal intestinal permeability and reduction of endotoxemia in both humans and rats. These data support the model that therapeutic modulation of alcohol-induced dysbiosis results in protection against alcohol-induced intestinal and hepatic oxidative stress and inflammation. Consistent with these data, studies have also shown that oats gavage also shifts the intestinal dysbiosis induced by alcohol back to a more normal microbiota profile associated with reductions in oxidative stress, normalized intestinal permeability, and reduced endotoxemia [42, 45, 65]. These data also agree with numerous studies showing amelioration of leaky gut and hepatic inflammation in non-alcoholic fatty liver disease using probiotics and prebiotics [48].

Finally, as a fifth potential mechanism, it has been suggested that protein or carbohydrate elements found in oats may directly bind to and sequester intestinal or serum endotoxin/LPS and prevent its pro-inflammatory biological effects, although this was not specifically tested in an alcohol-related model [81].

Taken together, these five mechanisms represent the potential mechanisms through which oats may be exerting the observed effects of reducing and even preventing the oxidative stress-related disease markers associated with chronic alcohol consumption. These compelling in vitro and animal studies provide a strong scientific rationale for conducting a large randomized double blind placebo controlled trial in human alcoholics to determine whether oats supplementation can prevent initiation and/or progression of oxidative tissue injury and organ damage in alcoholics.

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Chapter 17
Fish Oil n-3 Fatty Acids to Prevent Hippocampus and Cognitive Dysfunction in Experimental Alcoholism

Nataliya A. Babenko

Key Points

- Sphingomyelin and phosphatidylserine deficiency and ceramide accumulation in the hippocampus observed during chronic ethanol consumption lead to cognitive dysfunction.
- Ethanol increases sphingolipid turnover in the hippocampus mainly via oxidative stress- and cytokine-dependent activation of ceramide synthesis de novo and SMases activities, and inhibition of PS synthesis and content.
- Ethanol-induced disturbances of sphingolipid turnover in hippocampus and cognitive deficit are reversible.
- Enrichment of the diet with n-3 fatty acids of the fish oil increases the PS synthesis and thereby normalizes the sphingolipid turnover in ethanol-treated hippocampus and improves cognitive function.

Keywords Ethanol • Fish oil n-3 fatty acids • Hippocampus • Brain cortex • Cognitive function • Ceramide • Sphingomyelin • Phosphatidylserine

Introduction

Recently, a substantial body of evidence has evolved in literature indicating that n-3 polyunsaturated fatty acids (n-3 PUFA) are critical contributors to cell structure and function of the nervous system [1–4]. n-3 PUFA deficiency causes memory deficit [5], learning disability [6, 7], and visual activity loss [8]. Various neurological disease states in humans are associated with a deficient n-3 PUFA status [9, 10]. Epidemiological studies have shown interrelationship between n-3 long-chain PUFA intake, low plasma n-3 PUFA concentrations, and risk of cognitive impairment [11]. Such neurodegenerative diseases as generalized peroxisomal disorders and Alzheimer's disease are associated with low levels of docosahexaenoic acid (22:6n-3), the major n-3 fatty acid found in brain [12, 13]. Dietary supply of 22:6n-3 has been shown to reduce neuronal injury in experimental brain ischemia [14, 15] and Alzheimer's disease [16] and to improve some symptoms in patients with peroxisomal disorders [17]. It is worth noting that reference memory or working memory can be enhanced in normal animals or

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improved in 22:6n-3-deficient animals by fish oil supplementation [18]. Hippocampus and olfactory bulbs which accumulate greater 22:6n-3 showed stronger resistance to dietary 22:6n-3 deprivation and better 22:6n-3 recovery than the visual cortex, frontal cortex, and cerebellum. Results obtained suggest a critical role of 22:6n-3 in the development and maintenance of learning memory performance. Important trophic control by 22:6n-3 of hippocampus-dependent neuronal function such as learning and memory has been suggested [19, 20]. 22:6n-3 supplementation for 6 days increased the dendritic length and number of dendritic branches, which in turn would affect the number and quality of synaptic connections during organism development and in adulthood. It is conceivable that the derivatives of 22:6n-3 rather than 22:6n-3 by itself may mediate the observed effect of n-3 PUFA on the neurite growth [21, 22]. The trophic action of n-3 PUFA on the neuronal differentiation may be derived from the facilitated membrane interaction and activation of Raf-1 or Akt due to phosphatidylserine (PS) increase, as observed for neuronal survival.

22:6n-3 is required for the survival of retinal photoreceptors and exerts a protective effect on apoptosis of retinal photoreceptors during development [23, 24]. 22:6n-3 prevents oxidative stress-induced apoptosis of photoreceptor and amacrine neurons by enhancing the Bcl-2 expression and reducing simultaneously the pro-apoptotic lipid ceramide levels [24]. It has been demonstrated that 22:6n-3 supplementation prevented hippocampal cell death induced by ischemia-reperfusion in an animal model [25]. Dietary n-3 PUFA supplementation normalized an age-related decreased cognitive function and ceramide content and gave rise to a production of new polyunsaturated phosphatidylserine (PS) species in the brain cortex and hippocampus [26, 27].

Under trophic factor withdrawal condition, a dramatic increase in cell death was observed in n-3 fatty acid-deficient embryonic hippocampal cultures, whereas 22:6n-3 addition to the culture media significantly reduced apoptotic cell death [28]. Using neuroblastoma Neuro2A cells and embryonic hippocampal cultures, the antiapoptotic effect of 22:6n-3 has been found to depend on its ability to increase the PS content in neuronal membranes, to induce the PS-dependent acceleration of Akt translocation to membranes, and to suppress the caspase-3 activation [29, 30]. However, it has been determined that long-term exposure to ethanol could change the n-3 PUFA status. Ethanol lowered the 22:6n-3 fatty acyl content in brain, particularly from PS [31–33], reduced induction by n-3 PUFA PS accumulation and Akt phosphorylation in neurons and antiapoptotic potency of 22:6n-3 [29].

The central nervous system (CNS) is the target of alcohol toxicity and degeneration. Chronic ethanol consumption causes cognitive impairment and permanent structural brain damage. White matter degeneration (leukoencephalopathy), ventriculomegaly, cerebellar degeneration, and neuronal loss in hippocampus, cortex, and hypothalamus, which contribute to cognitive and motor deficits, are common alcohol-related brain lesions. Ethanol-inducing oxidative stress, DNA damage, mitochondrial dysfunction, and perturbing membrane lipid composition can directly cause the CNS injury and degeneration. It was well documented that the ethanol-induced oxidative stress increased production and content of pro-apoptotic sphingolipid ceramide. In human alcoholics, white matter atrophy and degeneration were found to be associated with oxidative stress and increased expression of pro-ceramide genes: ceramide synthase 2 and serine palmitoyltransferase [34]. Ethanol increased significantly ceramide content in the neonatal brain [35]; the disbalance of sphingolipid metabolism increased the astrocytes' susceptibility to tumor necrosis factor-α(alpha) (TNF-α (alpha))-induced cell death [36]. Astrocyte death induced by ethanol is associated with stimulation of sphingomyelinase (SMase) activity, as well as with ceramide accumulation and activation of stress-related kinases, c-Jun N-terminal kinase, p38 mitogen-activated protein kinase, and extracellular signal-regulated kinase pathways [37].

Based on the findings that ethanol inhibited the accumulation of PS, induced SMase-ceramide pathway, and 22:6n-3 prevented neuronal apoptosis through promoting PS accumulation and triggering sphingolipid metabolism, in this chapter, the effect of dietetic n-3 PUFA on sphingolipid turnover in the hippocampus and brain cortex and cognitive dysfunction in alcoholized animals and humans have been analyzed. The effects of in vivo exposure to ethanol and n-3 PUFA on the hippocampus have been also examined in relation to the PS status. The conjectured mechanism of hippocampus and cognitive dysfunction protection in alcoholism by fish oil n-3 fatty acids has been discussed.

Peculiarities of Sphingolipid Turnover in Brain Structures and Cognitive Dysfunction in Alcoholism

Numerous studies have shown that in both humans and animals, brain development is adversely affected by alcohol exposure. This is reflected in morphological and behavioral alterations that include mental retardation, reduction in brain size and growth rate, as well as defects in development and function of the central nervous system. Alcohol-induced degeneration occurs due to neuronal death during development and in adulthood and is related to increased oxidative stress and neurotoxic pro-inflammatory cytokines synthesis induction [38]. Oxidative stress and pro-inflammatory cytokines are important regulators of sphingolipid turnover in different cells. A strong correlation exists between changes of ceramide content and level of oxidation products in liver [39] and brain structures and cells [40, 41]. Formation of ceramides via sphingomyelin (SM) hydrolysis or from de novo pathways is observed in response to different inducers of stress. An addition of exogenous short-chain ceramides or enhancement of cellular levels of ceramides induces cell differentiation, cell cycle arrest, apoptosis, or cell senescence [42]. The ceramide, but not the other lipids, mimics the effect of cytokines [43]. The neutral SMase (nSMase) has been suggested to mediate the interleukin-1β (beta) (IL-1β (beta)) signaling in the cells [44].

Ethanol intubation of pregnant mice or ethanol addition to the neural crest-derived cell (NCCs) cultures results in ceramide elevation, SM deficiency, and increased apoptosis [45]. Apoptotic cells stain intensively for ceramide, suggesting that ceramide-induced cell death mediates ethanol damage to NCCs. Dietary substrates for SM biosynthesis from ceramide, such as betaine or CDP-choline, may prevent the ethanol-induced damage of NCCs. Single dose of ethanol administered to pregnant mice during the third trimester increases of ceramide and sphingosine contents and leads to neuronal loss in progeny brains [46].

Acute administration of ethanol to 7-day-old mice causes apoptotic neurodegeneration in the brain and accumulation of ceramide, triglycerides (TAG), cholesterol esters (ChE), and N-acylphosphatidylethanolamine (NAPE) [35]. In contrast, lipid profiles of the 19-day-old mouse brains with the features of neuroregeneration were not significantly affected by ethanol. Substantial increase of ceramide, TAG, and NAPE, as well as caspase-3 activation, has been determined in the cortex, hippocampus, and inferior colliculus at acute ethanol administration to 7-day-old mice [47]. Cerebellum of ethanol-treated animals exerts less caspase-3 activation and ceramide accumulation. Ethanol-induced caspase-3 activation and ceramide accumulation could be effectively blocked by inhibitors of key enzyme of sphingolipid synthesis de novo (serine palmitoyltransferase). These results demonstrate that de novo ceramide synthesis has an important role in ethanol-induced neurodegeneration in the developing brain.

Administration of ethanol to adult animals markedly alters the sphingolipid turnover in brain, and this effect is strictly dependent on the brain structure studied [48, 49]. Seven-day-long treatment of 3-month-old rats with ethanol had no effect on ceramide, SM, sphingosine, and glucosylceramide (GlcCer) contents in the brain cortex (see Fig. 17.1a) and increased ceramide production in the hippocampus (see Fig. 17.1b).

It is well known that ceramide accumulation in the cells can be due to the SM degradation or reduction of its conversion to sphingosine or GlcCer. However, a short-term action of ethanol on adult rats did not change the levels of newly synthesized SM, GlcCer, and sphingosine but increased the content of newly synthesized ceramide in the hippocampus (see Fig. 17.1b). Ceramide contents in the [^{14}C] serine pre-labeled hippocampus tissues of control and ethanol-treated 3-month-old rats were 761 ± 35.7 and 1,094 ± 48.2 cpm/μmol phospholipid *Pi* ($p < 0.05$), respectively. The results obtained clearly demonstrated that a short-term administration of ethanol to adult animals increased synthesis of ceramide de novo in the hippocampus, while under such experimental conditions, brain cortex sphingolipid turnover was stable.

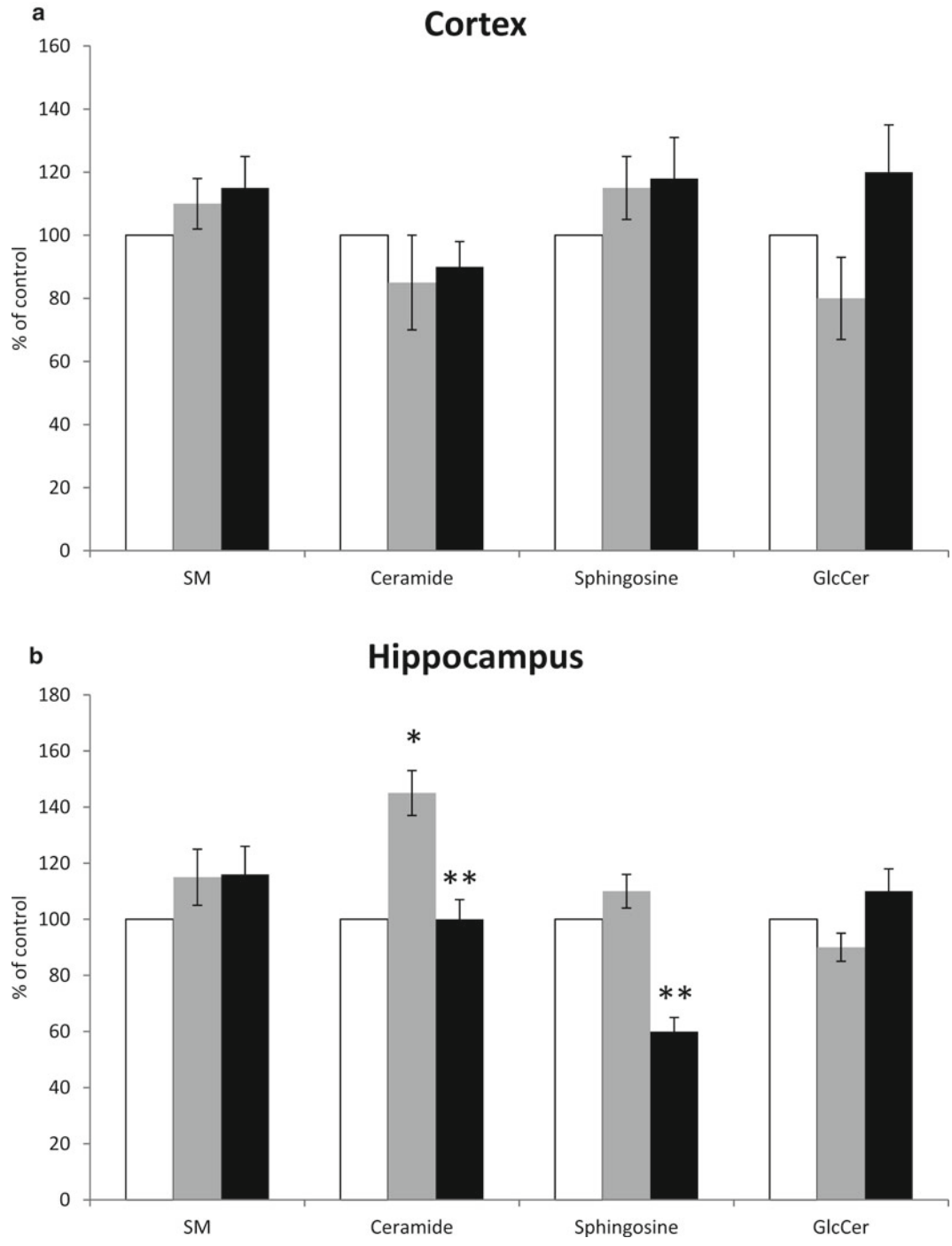

Fig. 17.1 Short-term effect of ethanol and fish-oil-enriched diet on sphingolipid turnover in the brain cortex and hippocampus. Sphingomyelin (SM), glucosylceramide (GlcCer). (**a**) Brain cortex. (**b**) Hippocampus. Open, dashed, and filled columns correspond, respectively, to control, 7-day-long alcohol-treated 4-month-old rats, and animals received fish oil in addition to ethanol. For lipid determination, the [^{14}C] palmitate-labeled hippocampus tissues have been used. Sphingolipids were analyzed as described in [27]. Results are mean ± SE of 6–8 individual experiments performed in duplicate. $^{*}p < 0.05$, ethanol-fed versus control rats, $^{**}p < 0.05$, ethanol + fish-oil-fed versus ethanol-fed

Long-term (60-day-long) feeding of rats with ethanol increased SM and ceramide synthesis and reduced the GlcCer synthesis in the brain cortex of adult 4-month-old animals (see Fig. 17.2a) [48]. However, ethanol did not increase the level of newly synthesized sphingosine and ceramide mass in the brain cortex. So, it cannot be excluded that ethanol induces ceramide synthesis de novo and newly synthesized ceramide further used for SM synthesis. These results are in line with other experiments which demonstrated that chronic exposure to ethanol stimulates the fluorescent-labeled ceramide conversion to SM in the primary astrocyte cultures [50, 51]. Taking into account that SM and SM synthesis play important role in brain development [52] and that ceramide is pro-apoptotic lipid, it is quite probable that increased SM synthesis in the brain cortex of adult animals chronically treated by alcohol is adaptive reaction to ethanol action.

Ceramide can be metabolized to GlcCer in the cells. GlcCer, in contrast to ceramide, may exert antiapoptotic effects, and treatments disturbing the balance between ceramide and GlcCer may trigger the cell death. The GlcCer synthesis and GlcCer synthase expression protect cells against ceramide-induced stress and apoptosis [53, 54]. Ethanol treatment reduced GlcCer in primary neuron cultures and SK-N-SH cells along with cell death, although it increased the GlcCer content in Neuro2A cells without apoptosis [55]. Ethanol reduced the newly synthesized GlcCer content in the brain cortex (see Fig. 17.1a) [48] and had no effect on the GlcCer level in the hippocampus (see Fig. 17.1b) [49]. Chronic exposure to ethanol increased significantly the ceramide/GlcCer ratio in the brain cortex of adult animals (see Fig. 17.2a). Ethanol-induced decrease in the GlcCer level in the brain cortex and cultured neurons may be caused by inhibition of the GlcCer synthase, as shown in other apoptotic models [56, 57]. It is not inconceivable that ethanol-induced increase of ceramide/GlcCer ratio rather than the accumulation of ceramide alone could trigger cell death in brain. However, the long-term (60-day-long) rat treatment with ethanol led to the increase of the newly synthesized ceramide content and had no effect on the GlcCer synthesis in the hippocampus of adult animals (see Fig. 17.2b) [49]. Ethanol treatment did not change the content of sphingosine, the product of ceramide degradation under ceramidase action, and decreased the level of the newly synthesized SM in the hippocampus (see Fig. 17.2b). Significant increase of the ceramide/SM ratio has been determined in the hippocampus of the ethanol-treated rats [49]. These may be related to the ethanol-induced SMase activation and SM degradation to ceramide.

Activation of acid SMase (aSMase) as well as neutral nSMase by ethanol in brain and other tissues, and cells was previously reported [34, 37, 58, 59]. The increased secreted aSMase activity in alcohol-dependent patients is implicated in alcohol-induced lipid alterations and might be relevant for the occurrence of alcohol-related disorders [59]. The mechanism by which ethanol activates SMases is not fully understood. However, the ethanol-induced increase of aSMase and nSMase mRNAs has been demonstrated. Ethanol was found to induce the secretion and expression of several cytokines including TNF-α (alpha), which is a well-known activator of nSMase [60, 61] and aSMase [62]. Ethanol could increase the nSMase activity via depletion of the content of glutathione, which is a well-known natural inhibitor of the nSMase activity. Alcohol targets ceramide, generated from aSMase activation, for gangliosides synthesis; initiates the overproduction of TNF-α (alpha) and selective mitochondrial pool of glutathione depletion; and thus increases cell sensitivity to alcohol [63]. Taken together, the generation of cytokines, the depletion of glutathione, and the production of reactive oxygen species may be responsible for the enhanced SMases activities, overproduction of ceramide, and ceramide-dependent cell death. Remarkably, a recent study reported that astrocytes treated with ethanol exhibit enhanced cell killing induced by TNF-α (alpha) or SMase [64]. Ethanol appeared to inhibit the formation of sphingosine-1-phosphate (SPP) upon TNF-α (alpha) treatment, suggesting that ethanol may shift the balance of sphingolipid metabolism in TNF-α (alpha)-treated astrocytes in favor of a pathway that increases ceramide levels over that of SPP.

These studies demonstrated that in human alcoholics or experimental animals, as well as in the isolated neurons and glial cells, ethanol consumption is accompanied by the disturbances of sphingolipid turnover, apoptotic cell death, and neurodegeneration. Neuronal loss has been observed in the

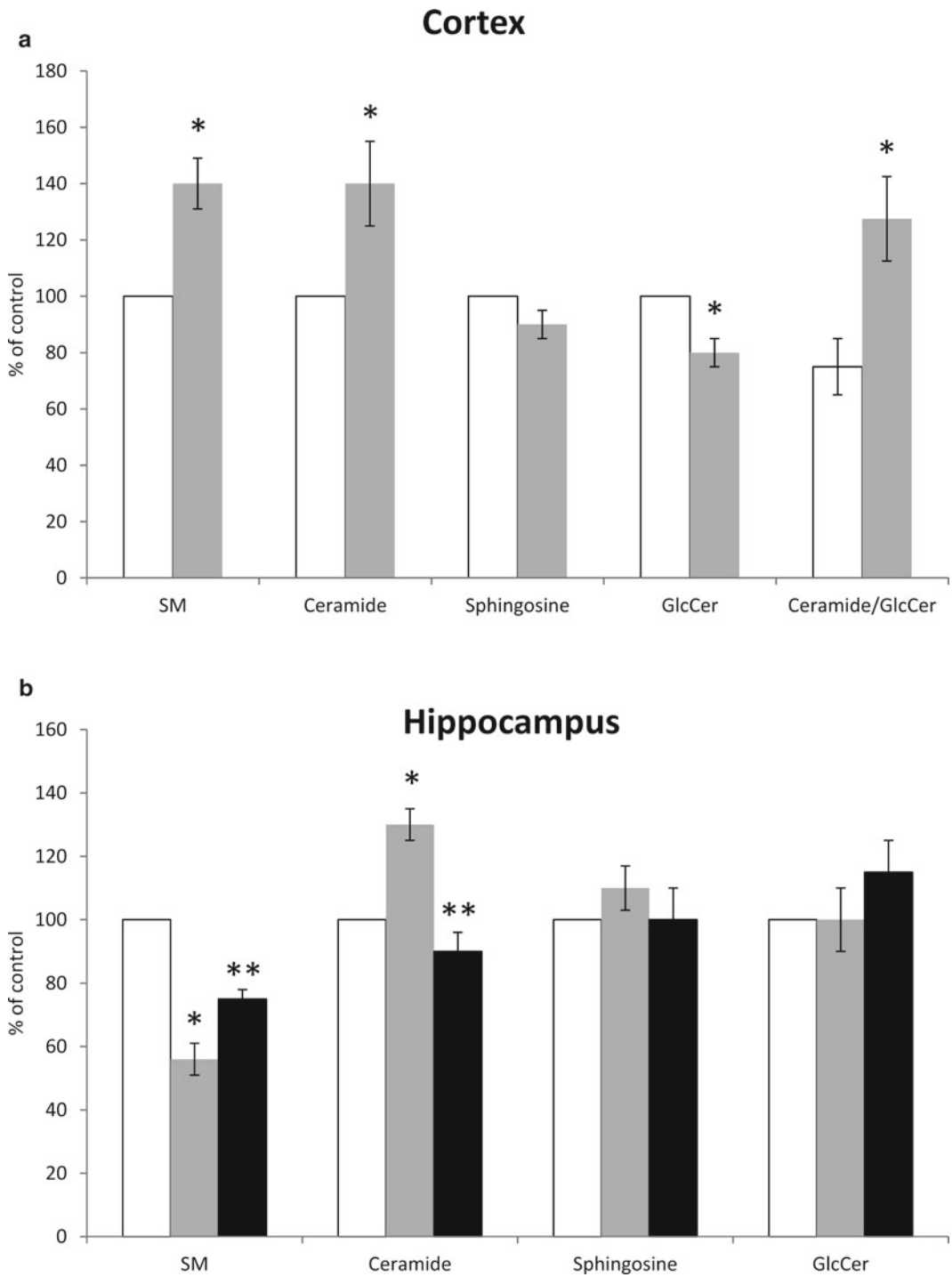

Fig. 17.2 Long-term effect of ethanol and fish-oil-enriched diet on sphingolipid turnover in the brain cortex and hippocampus. Sphingomyelin (SM), glucosylceramide (GlcCer). (**a**) Brain cortex. (**b**) Hippocampus. Open, dashed, and filled columns correspond, respectively, to control, 60-day-long alcohol-treated 4-month-old rats, and animals received fish oil in addition to ethanol. For lipid determination, the [^{14}C] palmitate-labeled hippocampus tissues have been used. Sphingolipids were analyzed as described in [27]. Results are mean ± SE of 6–8 individual experiments performed in duplicate. $^{*}p < 0.05$, ethanol-fed versus control rats, $^{**}p < 0.05$, ethanol + fish-oil-fed versus ethanol-fed

brain structures (hippocampus, cortex, and hypothalamus), which contribute to cognitive and motor deficits, and are common alcohol targets. As it was found in experiments on animals, the long-term ethanol consumption, which leads to the death of hippocampal neurons, results in a drop of lability of nerve processes, intensification of inhibitory processes, suppression of motor-conditioned reflex activity, and suppression of learning capability [65]. The cognitive functions of the animals chronically consuming alcohol were clearly depressed, as compared with those in the control animal group [48, 49]. The number of combinations of stimuli necessary for reaching the criterion of reproducibility in the group of rats consuming ethanol was considerably higher than that in the control group. The number of reactions of avoidance in the shuttle chamber in the groups of rats that received ethanol decreased on the first experimental day, as compared with the corresponding index in control animals. On the third day of training for conditioned active avoidance reflex, the latencies of avoidance reactions in alcoholized rats were significantly longer than those in the control group.

In humans, it has been determined that cognitive deficits have been seen just as in the short-term action of ethanol and in the late stages of alcoholism, too. Ethanol given acutely decreased the number of solutions with the minimum moves in the planning task [66]. Ethanol also decreased the thinking time before initiating a response, while it increased the subsequent thinking time in the same task. Under alcohol, participants recognized fewer items in the spatial recognition task. Heavy users of alcohol in contrast to moderate ones performed worse in the spatial working memory and in the pattern recognition task.

It is worth noting that alterations of sphingolipid turnover predict cognitive, affective, and behavioral symptoms of Alzheimer's disease [67]. The low serum SM level was associated with memory impairment, while high ceramide levels predicted memory impairment. Based on the study, it was suggested that these lipids could be the biomarkers of Alzheimer's disease progression [68]. Hippocampus and cognitive dysfunctions coincided with increased sphingolipid metabolism and ceramide accumulation in the brain at old age [27]. A chronic increase in intracellular ceramide can inhibit axonal elongation, receptor-mediated internalization of the nerve growth factor, and induce cell death [69]. The nSMase-mediated ceramide production in the hippocampus and brain cortex at old age activates the rate of amyloid β (beta)-peptide generation [70]. Aging is accompanied by a progressive increase in the ceramide/SM ratio and decrease of the SM level in hippocampus of the rats [27]. These data suggest that at old age, the perturbed hippocampal sphingolipid metabolism may result from a high SMase activity. The chronic inhibition of the nSMase activity by SMase inhibitor manumycin prevents ceramide accumulation in the brain cortex and hippocampus of the aged animals [70]. It has been also demonstrated that manumycin completely abolishes both the amyloid load and amyloid β (beta)-peptide accumulation, leading to a dramatic amelioration of Alzheimer's disease-like neurodegeneration. It has been hypothesized that the nSMase inhibitors may provide efficient ways to reduce the Alzheimer's disease risk associated with age. Based on these results, reasonable assumption can be made that ceramide accumulation and SM deficiency in hippocampus during chronic ethanol consumption can be the important reasons of cognitive dysfunction.

n-3 Fatty Acids Ameliorate Alcohol-Induced Disturbances of Hippocampal Sphingolipid Turnover and Cognitive Dysfunction

In both animals and humans, it has been demonstrated that long-term ethanol consumption can change the n-3 PUFA status of organism. Ethanol exerts its effect on brain, at least in part, through reducing the 22:6n-3 fatty acyl content mainly in PS [31–33]. Long-term exposure of cultured cells [71] or animals [72] to ethanol decreased significantly the PS accumulation induced by n-3 PUFA. Our results demonstrated that long-term (60-day-long) feeding of rats with ethanol decreased significantly the PS content and synthesis in the hippocampus of adult rats (see Fig. 17.3) (unpublished data). However,

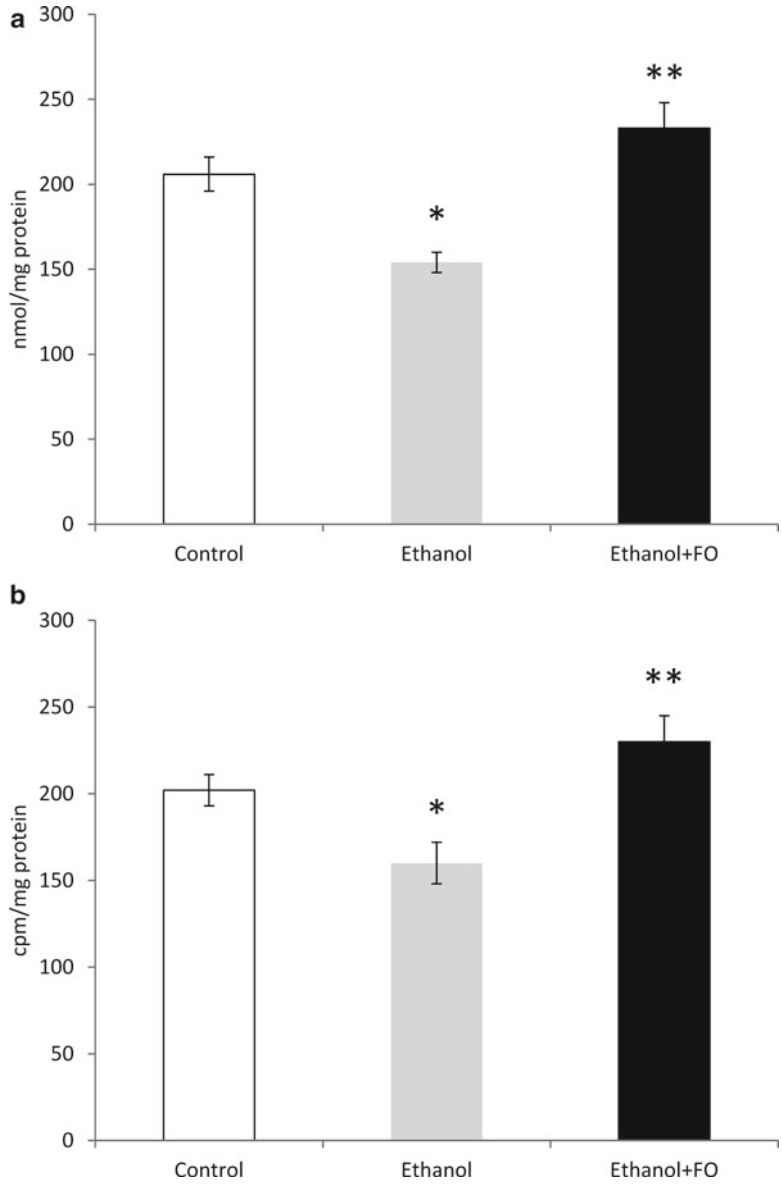

Fig. 17.3 Long-term effect of ethanol and fish-oil-enriched diet on the phosphatidylserine content and synthesis in the hippocampus. (**a**) PS content. (**b**) PS synthesis. Open, dashed, and filled columns correspond, respectively, to control 4-month-old rats, 60-day-long alcohol-treated animals, and rats received fish oil in addition to ethanol. PS content and synthesis were analyzed as described in [27]. Results are mean ± SE of 6–8 individual experiments performed in duplicate. $^{*}p < 0.05$, ethanol-fed versus control rats, $^{**}p < 0.05$, ethanol + fish-oil-fed versus ethanol-fed

feeding of ethanol-treated rats with the fish-oil-enriched diet increased the PS content and synthesis in the hippocampus up to the level observed in control animals (see Fig. 17.3) thereby improving the n-3 PUFA state of ethanol consumers. Moreover, fish oil feeding of adult rats treated with ethanol for 7 or 60 days significantly reduced elevated ceramide levels in the hippocampus (see Figs. 17.1b and 17.2b) but had no effect on ceramide production in the brain cortex of ethanol-treated animals (see

Fig. 17.1a). Fish-oil-enriched diet reduced the content of other toxic pro-apoptotic sphingolipid (sphingosine) in the hippocampus of the 7-day-long ethanol-treated rats (see Fig. 17.1b) and increased the level of the newly synthesized SM in the hippocampus of the 60-day-long ethanol-treated rats up to that observed in control animals (see Fig. 17.2b). Taking into account that fish-oil-saturated diet decreased the ceramide/SM ratio in alcoholized rats up to the level in control animals, reasonable suggestion has been made that n-3 PUFA reducing SMase activity normalizes the sphingolipid turnover and ceramide content in the hippocampus of ethanol-treated animals [49].

The ability of dietetic fish oil to nullify the ethanol-dependent disturbances of the sphingolipid turnover as well as PS content in the hippocampus made it possible to suggest that PS could play an important role in the SMase/ceramide signaling pathway. Significant decrease of PS content in the hippocampus of aged rats was in parallel with ceramide accumulation in brain and age-dependent cognitive dysfunction [27]. Dietary n-3 PUFA supplementation normalized an age-related decreased cognitive function and gave rise to a production of new polyunsaturated PS species in the brain cortex and hippocampus [26]. 22:6n-3 completely prevented N-acetylsphingosine (C2-ceramide)-induced photoreceptor and amacrine neurons death, increasing the Bcl-2 expression, precluding the mitochondrial depolarization, and simultaneously reducing the endogenous ceramide content through increased ceramide conversion into GlcCer [73]. 22:6n-3 induced downregulation of SMases expression and activities in human retinal endothelial cells [74]. A short-term feeding of mice by eicosapentaenoic acid or 22:6n-3 suppresses mitogen-induced T-lymphocyte proliferation and reduces ceramide production [75]. These results are consistent with an observation that the 8-week consumption of fatty fish increases the concentration of n-3 PUFA and reduces the ceramide content in blood serum [76].

It is important that dietetic n-3 PUFA effects on sphingolipid turnover in brain and cognitive function could be imitated by the exogenous PS administration. Administration of PS, as well as fish oil to aged rats, leads to increased PS contents in the hippocampus of the 24-month-old rats in such way that it approaches the level observed in the brain of 3-month-old animals [27]. PS administration to old rats significantly decreased the ceramide production by the nSMase in the hippocampus. Both the n-3 PUFA-enriched diet and exogenous PS addition improved cognitive decline at old age and in the ethanol-treated adult animals [26, 77]. The conditioned reflex activity of these animals became normalized to a certain extent. The number of successive combinations of stimuli necessary for the appearance of the first conditioned active avoidance reflex and for reaching the selected criterion of reproducibility of such a reaction in the group of rats receiving ethanol and fish oil was smaller than analogous indices in the group of animals consuming only ethanol. On the first and second experimental days, the number of active avoidances in the shuttle chamber in the group of animals consuming ethanol against the background of a fish-oil-saturated diet exceeded significantly the corresponding values in "pure" alcoholized rats. On the third experimental day of training of conditioned active avoidance reflex, the significantly smaller latencies of avoidance reactions were observed in animals supplied with fish oil, as compared with those in the alcoholized animal group. This is consistent with the published data demonstrating effects of the fish oil or exogenous PS on cognitive functions in animals investigated in different types of tests. Thus, chronic oral administration of bovine brain PS to aged rats improved test results for spatial recognition and passive avoidance [78]. The repeated administration of PS improved acquisition and retention of passive and active avoidance tasks in aged Wistar rats [79]. Based on these results, it has been thought that n-3 PUFA prevent ceramide accumulation in the hippocampus and normalize cognitive functions of old or ethanol-treated rats, at least in part, via the PS-dependent SMase inhibition.

It is known that upon the action of ethanol, the amounts of cytokines (TNF-α (alpha) and IL-1β (beta)) increase in the brain, liver, and blood serum; it occurs not only on the chronic ethanol consumption but on a single ethanol consumption as well [80]. The level of cytokines is normalized

relatively rapidly in the blood serum and liver, while in the brain, it remained increased for a long time after cessation of ethanol consumption. Using cells of different types, TNF-α (alpha) and IL-1β (beta) have been shown to realize their actions mostly via stimulation of the SM cycle and accumulation of ceramide [80–82]. Ethanol also decreases the level of glutathione, an inhibitor of nSMase, in the cells [83]. These data suggest that ethanol-induced modifications of sphingolipid turnover in the hippocampus are mediated by the cytokines- and redox status-dependent nSMase activation. Since the neuroprotective actions of n-3 PUFA depend to a large extent on the stimulation of the antioxidant enzymes expression and suppression of pro-inflammatory cytokines production [84], it is believed that the normalization of sphingolipid turnover in the hippocampus of alcoholized rats upon the action of PUFA-containing fish oil can be achieved at the expense of both the suppression of the SMase activity and decreased production of this enzyme inductors.

It is well documented that the PS liposomes have anti-inflammatory effects when administered to animals or added to the cell culture. Just the PS liposomes, but not the phosphatidylcholine liposomes, injected intraperitoneally to Swiss mice after the inflammatory stimulus reduced the IL-1β (beta) production [85]. A pretreatment of rats with the PS liposomes prevented the increase in the IL-1β (beta) concentration, as well as the activation of p38 and c-Jun N-terminal kinase and negative effect of the lipopolysaccharide on the long-term potentiation in the hippocampus [86]. Addition of the PS liposomes to the culture media repressed the pro-inflammatory activities in microglial cells [87]. Taking into account that the PS can decrease both the nSMase activity and the concentration of IL-1β (beta) and that the PS liposomes mimic the fish oil effects in the hippocampus, the possibility must not be ruled out that dietetic n-3 PUFA modulate the brain inflammatory state by the PS-mediated decrease of cytokine-induced ceramide production.

Conclusion

In both animals and humans, it has been demonstrated that exposure to ethanol stimulates the sphingolipid turnover in different brain structures as well as cognitive and motor dysfunction development. More pronounced changes of sphingolipid metabolism can be seen in the brain structures (hippocampus, cortex, and hypothalamus), which are well-known alcohol targets contributing to cognitive and motor deficits. However, ethanol effect is strictly dependent on the duration of exposure, ethanol dose, and age of animals or human being. Ceramide accumulation and SM deficiency are features of toxic chronic ethanol action on organism during pregnancy and at postnatal ontogenesis. The data obtained suggest that ethanol increases sphingolipid turnover in the hippocampus, mainly via oxidative stress- and cytokine-dependent activation of ceramide synthesis de novo, and SMases activities and inhibition of the PS synthesis and content (see Fig. 17.4). Taking into account that ceramide is pro-apoptotic lipid and induces the AD-like neurodegeneration [88–90], and exposure to exogenous ceramide causes deficits in cognitive and motor functions [91] imitating ethanol effects, one can conclude that ethanol-induced ceramide accumulation in the hippocampus plays an important role in its dysfunction. Ethanol-initiated disturbances of sphingolipid turnover, as well as ethanol-induced cognitive deficit, are reversible. Enrichment of the diet with n-3 PUFA of the fish oil diminishes features of oxidative stress and cytokines production and increases the PS synthesis and thereby normalizes the sphingolipid turnover in ethanol-treated hippocampus and improves the cognitive function.

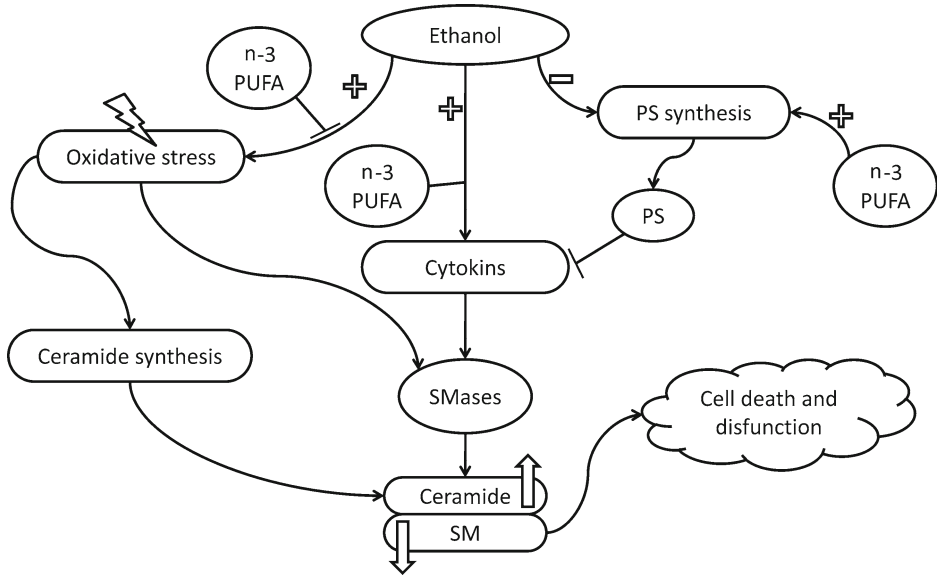

Fig. 17.4 Effects of n-3 fatty acids supplementation on the sphingolipid turnover in ethanol-treated hippocampus. Induction of oxidative stress and cytokine production following ethanol consumption results in ceramide synthesis and sphingomyelinase (SMase) activation. Ethanol-induced activation of sphingolipid turnover leads to ceramide accumulation and sphingomyelin (SM) deficiency. n-3 fatty acids supplementation improves oxidative state and cytokine and phosphatidylserine (PS) contents and thereby predict ceramide accumulation and SM deficiency in the ethanol-treated cells and cells dysfunction

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Chapter 18
Alcohol in HIV and Possible Interactions with Antiretroviral Medications

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Key Points

- This chapter explores the relationship of alcohol consumption, HIV, and antiretroviral treatment (ART) and the potential mechanisms of actions that generate and modify these relationships such as oxidative stress and consequent mitochondrial and liver damage.
- The behavioral alterations produced by alcoholism have been well studied, and there is general agreement that they affect compliance and adherence to therapeutic regimes in people living with HIV; however, the metabolic alterations produced by the synergistic interactions between alcohol and ART need further investigation.
- Findings in this field are sometimes contradictory, especially those related to mitochondrial damage and liver fibrosis. As new antiretrovirals are developed, and their effectiveness confirmed, the biological and behavioral interactions with alcohol will change, making this field even more complex.
- This chapter reviews the relevant literature, including in vitro animal and human studies, which support the associations of the variables under study. The authors recognize the need of long-term and mechanistic studies on the interactions between alcohol and ART, and their effect on oxidative stress, mitochondrial damage and liver disease, as well as the rapidly changing nature of this area of study.

Keywords Alcohol • HIV viral load • CD4+ cell count • ART

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Alcohol and HIV: Introduction

Chronic alcohol abuse is a contributing factor to HIV-1 transmission [1–3], disease progression [4, 5], and late presentation for diagnosis and early treatment [6]. The longitudinal effects of alcohol consumption in the context of antiretroviral therapy (ART), however, have not been sufficiently investigated beyond the well-documented behavioral consequences of alcoholism on ART adherence [7–9]. Nucleoside reverse transcriptase inhibitors (NRTI) have demonstrated their effectiveness as a family of antiretroviral drugs against HIV-1 for more than 15 years. NRTI in combination with non-NRTIs and protease inhibitors (PIs) are still the cornerstone of HIV treatment, despite their well-described side effects, including liver toxicity. Although the newest NRTIs are less liver-toxic with fewer side effects, the long-term multifactorial and synergistic interactions between alcohol consumption and HIV treatment have not been well documented [10, 11]. This chapter will explore the interaction between alcohol consumption and ART and the mechanistic roles of oxidative stress and mitochondrial DNA damage in explaining this interaction in people living with HIV.

Alcohol abuse was the third leading lifestyle-related cause of death in 2010 in the United States. One of the mechanisms of action that have been suggested for the deleterious effects of chronic alcohol consumption is increased hepatic oxidative stress and reduced antioxidant defense resulting in alcohol-induced liver injury [12, 13]. Globally, alcohol consumption increased throughout the 1980s and became stable afterward, with a mean adult per capita intake of 5.1 liters of pure alcohol per year [14]. In the United States, heavy alcohol use affects approximately 5% of the general US population, with up to 15% of Americans engaging in binge drinking [15]. Among people living with HIV, however, excessive alcohol consumption is more common with some studies showing a prevalence of alcohol abuse up to 50% [16–18]. Stabinski et al. [19] in a study conducted in Uganda reported that HIV infection was associated with a 50% increase in liver fibrosis (adj PRR 1.5, 95%CI 1.1–2.1; $p=0.010$) independent from hepatitis infections, a finding corroborated by a study in the United States [20]. The Ugandan study also found that liver fibrosis was associated with heavy alcohol consumption (adj PRR 2.3, 95% CI 1.3–3.9; 0.005). Use of ART, however, reduced the risk of fibrosis (adj PRR 0.6, 95% CI 0.4–1.0; $p=0.030$) in this study. The positive effect of ART on liver functioning was also confirmed in a small longitudinal study in treatment-naïve patients who were initiated on ART [21]. Moreover, the study showed a relationship between controlled HIV viral load and a significant decrease in aminotransferase levels after initiating ART, which was independent from alcohol consumption. The above studies [19–21] demonstrated an association between HIV infection, alcohol consumption, and liver disease, and the beneficial effect of ART on liver disease, independent of hepatitis infections. Since liver disease is already the leading cause of mortality among HIV-infected persons in developed countries, where it has been usually associated with hepatitis infections, additional studies are needed to elucidate the effect of long-term ART on liver disease in this context. As ART becomes available in many countries with limited resources, and other opportunistic diseases can be controlled, alcohol consumption may aggravate the underlying liver disease promoted by HIV infection and lack of adherence to ART [22–25].

The metabolism of alcohol increases production of reactive oxygen species (ROS) in mitochondria, and, as a consequence, significant oxidative stress is observed in alcoholic patients [26–31]. Increased ROS and oxidative stress is also observed in HIV-1 infection [32–38]. Moreover, the use of ART is associated with mitochondrial DNA damage, and increased oxidative stress produced by ART may be aggravated by alcoholism [32–48], as chronic alcohol consumption is also associated with increased hepatic oxidative stress and reduced antioxidant defense resulting in alcohol-induced liver injury [12, 13]. Through oxidative stress, and other potential mechanisms, alcohol directly suppresses the immune system by affecting T-cell apoptosis [49, 50], mitochondrial damage [47, 48, 51–55], T-cell responses, NK cell activity, and macrophage phagocytic activity [56]. Acting indirectly, alcohol affects immunity by causing malnutrition and promoting liver disease [57, 58]. Because of its deleterious effect on immunity, alcohol consumption, especially excessive consumption by HIV infected

individuals, who are already immune-compromised, may accelerate HIV disease progression and increase exposure to opportunistic infections.

Early observational studies, though, did not find an association between alcohol consumption and HIV disease progression [59–62]. Animal and in vitro studies, however, suggested significant effects on several aspects of the disease. Alcohol caused an altered cytokine response and reduced macrophage reactive oxygen species (ROS) production in response to HIV infection in studies with transgenic mice, which could lead to accelerated development of AIDS [63, 64]. In mice who were fed alcohol chronically, changes in the relative proportions of T-cell subsets in the thymus, increased losses of CD4+ and CD8+ cells, and susceptibility to AIDS associated pathogens were also observed [65–67]. Alcohol caused in vitro suppression of human lymphocyte proliferative response to HIV antigens and decreased the production of cytokines in a dose-related manner. In addition, in vivo exposure to alcohol caused an increase in HIV-1 replication in peripheral blood mononuclear cells (PBMCs) that was associated with a decrease in T-helper and suppressor cell function [68, 69]. The effect of alcohol on in vitro cultures of isolated human brain microvascular endothelial cells (MVECs), a major cellular component of the blood–brain barrier, was tested in combination with the proapoptotic potential of various HIV-1 proteins. This study demonstrates the potential of alcohol for inducing apoptosis of human MVECs when combined with HIV-1-specific proteins, suggesting a synergistic effect in increasing HIV-1 capacity for neural invasion and neuropathogenesis [70]. Simian studies have also provided evidence that chronic alcohol intake before and during HIV infection results in higher viral set point, more rapid progression to end-stage disease, and exacerbation of the AIDS wasting syndrome through increased expression of TNF-α and atrogin-1 [71–73].

Several studies on alcohol and HIV disease progression after the introduction of ART have established that alcohol results in reduced viral load response to treatment, decreased CD4+ cell reconstitution, and poorer adherence to ART [74–76]. A cross-sectional analysis of HIV-1 infected drug users found that heavy alcohol users (defined as alcohol intake ≥ (equal or less) 3–4 times per week), who were receiving ART, were four times less likely to achieve undetectable viral load and two times more likely to have CD4+ cell counts below 500 cells/μL (microliter) than moderate drinkers or abstainers [76]. A small prospective study of HIV + patients receiving ART found no difference in the proportion of those who attained undetectable viral loads or the mean CD4+ cell count between abstainers, moderate alcohol drinkers (<60 g/day), and heavy alcohol drinkers (>60 g/day) [77]. However, a larger longitudinal study of HIV + persons with a history of alcohol problems found that alcohol use was a significant predictor of poorer adherence to ART [75] and was negatively associated with HIV viral load suppression [78]. A prospective study of 161 HIV + women on ART also found that poorer adherence to ART was significantly associated with virologic failure and that alcohol use was a significant predictor of lower adherence [74].

These findings suggest that alcohol may accelerate disease progression through poorer adherence to ART treatment; however, the report by Samet et al. [4] of a 7-year prospective study on the association between heavy alcohol intake and lower CD4+ cell counts in an HIV-positive cohort who were not receiving ART indicates that alcohol may directly influence disease progression through an effect on CD4+ cell count. Evidence of the promotion of T-cell apoptosis by alcohol offers a plausible mechanism by which this may occur [49, 50]. In addition, alcohol consumption has been found to decrease mitochondrial DNA (mtDNA) and promote T-cell apoptosis through increased systemic oxidative stress [49].

Baum et al. [79] examined the associations of alcohol use with HIV disease progression in a prospective, 30-month, longitudinal study of 231 HIV-positive persons. The study found that those who were frequent alcohol users (≥ (more or less) 2 drinks daily) were 2.91 times (95% CI:1.23–6.85, $p = 0.015$) more likely to present a decline of CD4+ cell count to ≤ (equal or less) 200 cells/μL (microliter), independent of baseline CD4+ cell count and HIV viral load, antiretroviral use over time, time since HIV diagnosis, age, and gender. Frequent alcohol users who were not on ART had increased risk for CD4+ cell count decline to ≤ (equal or less) 200 cells/mm^3 (HR = 7.76:95% CI:1.2–49.2, $p = 0.03$)

and higher HIV viral load over time (β (beta) =0.259, $p=0.038$). The significant effect on HIV viral load was maintained in those receiving ART (β (beta) =0.384, $p=0.0457$) but not in those without ART. These results suggest that frequent alcohol intake accelerated HIV disease progression through a direct effect of alcohol consumption on CD4+ cell count decline independent of ART. In contrast, the effect of alcohol abuse on HIV viral load appeared to be through reduced adherence to ART [79].

Mechanisms of Interaction Between Alcohol, HIV, and ART

Cellular mitochondria are protected from radical-mediated oxidative damage by endogenous and exogenous antioxidants [80–84]. Deficiencies in the antioxidant enzymatic system and micronutrients required for antioxidant defense in HIV-infected persons result in increased oxidative stress which contributes to impaired mitochondrial toxicity [34–41]. Moreover, mitochondrial DNA damage and increased oxidative stress produced by ART may become more severe with alcoholism [32–48, 85, 86]. The burden of liver disease in HIV-infected patients is expected to increase as the number of patients living with the disease continues to rise [87]. A better appreciation for alcohol's effects on HIV and liver disease may increase utilization of alcohol cessation interventions, thus improving treatment outcomes. This section will explore the literature that investigates the combined effects of chronic alcohol consumption and antiretroviral therapy on oxidative stress, antioxidant status, mitochondrial toxicity, and liver hepatocellular injury and function to provide the basis for potential preventive therapeutic approaches to protect the liver from alcohol and antiretroviral drug-induced injury.

HIV infection is characterized by increased oxidative stress [32–35]. Increased generation of both oxygen radicals and pro-inflammatory products, including cytokines, occurs early after infection with HIV-1 [88–91]. HIV-1 infection and proliferation causes chronic immune activation which contributes to an increase in the production of ROS. As a consequence of the increased antioxidant demand, the major antioxidant defense enzymes are altered, including superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPX) [32–35], as are the major nonenzymatic antioxidants, most notably glutathione, vitamins E and C, carotenoids, selenium, and zinc [37–41]. As the antioxidant capacity is stretched, markers of oxidative damage including products of lipid peroxidation (malondialdyde or MDA), protein carbonyls, and lymphocyte nuclear and mitochondrial DNA damage accumulate [42, 43].

Antiretrovirals and alcohol are each associated with increased oxidative stress, decreased antioxidant status, and mitochondrial damage, leading to hepatocellular injury. With the advent of ART, HIV disease has become a manageable, chronic disease. The additive or synergistic effect of antiretrovirals and alcohol on liver injury, in the context of HIV-1 infection, in itself a condition of high oxidative stress, needs further investigation.

Alcoholism, Oxidative Stress, and Mitochondrial Damage

Although the consumption of alcohol in the general population has declined in the last 20 years [92], approximately one in seven Americans (14.2%) met the criteria for alcohol dependence during their lives, with as many as half of those meeting the criteria for current alcoholism [93]. Similarly, alcoholism is prevalent in the HIV-positive population. While moderate consumption of alcohol has been associated with health benefits [26–28, 94–96], alcohol abuse has been shown to lead to liver disease, even after long-term abstinence [97].

Increased oxidative stress is one of the mechanisms of liver damage in chronic alcoholism. Metabolism of alcohol increases ROS production in mitochondria through complex I (NADH coenzyme Q reductase) of the electron transport chain. Alcoholics have difficulty compensating for excessive alcohol-induced

free radical production, generating significantly higher products of oxidative stress, including serum MDA, 8-iso-prostaglandin F2 alpha, protein carbonyl content, nitrite/nitrate, diene conjugates, and homocysteine, despite higher activity of the enzymatic antioxidant defense, superoxide dismutase (SOD), and glutathione peroxidase (GPX) [98]. In addition, increased endogenous and peroxide-induced DNA damage in lymphocytes are also observed in alcoholics when compared to controls. These endogenous lymphocyte markers were significantly correlated with serum MDA and protein carbonyl content in chronic alcoholics [99]. Alcohol abstinence was associated with lowering of serum markers of oxidative stress, significantly decreasing these markers as the length of abstinence increased.

The increased generation of oxygen radicals is the mechanism by which alcoholism causes mitochondrial toxicity [100] and accelerates mitochondrial DNA damage. Alcohol induces mtDNA depletion and increases oxidative modification of mtDNA. Additionally, alcoholics with microvesicular steatosis have an increased presence of a common 4977-base pair deletion in hepatic mitochondrial DNA associated with oxidative damage [52–55]. There is some evidence that this may lead to impairment of mitochondrial function and microvesicular steatosis [52]. Mitochondrial oxidative damage caused by alcohol consumption can lead to decreased reoxidation of the reduced NADH molecules produced during ethanol metabolism to acetate. This leads to a decrease in the NAD+/NADH ratio, inhibition of mitochondrial β (beta)-oxidation, and microvesicular steatosis. This process accelerates normal oxidative aging of mtDNA [53, 101] and decreases levels of major proteins of the oxidative phosphorylation pathway including reduced nicotinamide adenine dinucleotide dehydrogenase, cytochrome oxidase, and mitochondrial complex I and IV [102].

Comparisons of oxidative stress and antioxidant status in alcoholics and controls have found that, in alcoholics, when markers of oxidative stress increase, antioxidant status deteriorates [84, 103, 104]. In a cohort of 102 alcoholic patients without severe liver disease, who were followed before and after 21 days of withdrawal treatment, plasma concentrations of alpha-tocopherol, ascorbic acid, and selenium were lower in alcoholics than in 417 healthy men who consumed only low or moderate amounts of alcohol ($p \leq$(equal or less) 0.001). Serum MDA was also higher in alcoholics ($p \leq$(equal or less) 0.001). Plasma concentrations of alpha-tocopherol and selenium remained unchanged after the withdrawal period, whereas MDA decreased ($p \leq$(equal or less) 0.001). Ascorbic acid concentrations also decreased ($p \leq$(equal or less) 0.01) which suggested a specific effect of alcohol on antioxidant vitamins, independent of nutritional status, and after adjusting for lipid profile and nutritional intake [103].

While many studies confirm the presence of elevated oxidative stress in HIV [32–38], limited data are available on the synergism of ART and alcoholism on oxidative stress in the context of HIV disease. In an animal study, when the group of mice treated with alcohol and the HIV Tat protein was compared to the control group or mice treated with alcohol or Tat alone, those treated with alcohol and Tat synergistically increased expression of inflammatory cytokines, MCP-1, ICAM-1 mRNA levels, and selectively activated redox-regulated transcription factors. This study showed that HIV-1 Tat and alcohol can amplify cellular effects, leading to alterations of redox-regulated inflammation [105]. Using another mouse model of HIV infection to study mechanisms of oxidative injury, Potula et al. [106] demonstrated that alcohol administration enhanced HIV viremia and suppressed immune response. In summary, although alcoholism alone is associated with increased oxidative stress and increased mitochondrial and liver damage, there is limited data on the synergistic effects of alcoholism on these factors in the context of HIV disease.

Antiretrovirals, Oxidative Stress, and Mitochondrial Damage

Antiretrovirals have generally been described as increasing oxidative stress and damage [107–110], although some studies have found increased antioxidant capacity and DNA damage repair [111–113]. Although the effect of different types of antiretrovirals on oxidative stress may vary, PIs have generally

been found to increase the production of ROS including superoxide and peroxide and are associated with endothelial dysfunction and dyslipidemias leading to increased cardiovascular risk [108, 114]. NRTIs have a well-established effect on mitochondria that result in increased measures of oxidative damage including lipid peroxidation products, protein carbonyls, and mitochondrial damage [109, 110]. Studies of ART use that combine several types of antiretrovirals have shown increased oxidative stress as well. A study of oxidative stress in 85 HIV-positive patients who were either ART naïve or on three different ART regimens showed increased lipid peroxidation, as measured by MDA, in the HIV-infected patients vs. healthy controls and in the ART-treated groups compared to the ART-naïve group [115]. Exposure to ART has also been found to increase the generation of ROS in human aortic endothelial cells [116].

The advent of ART has transformed HIV infection from a fatal condition to a chronic viral disease [87]; however, antiretroviral effectiveness is limited by the hepatotoxicity of some NRTIs and PIs [115–119]. Although the literature is inconsistent on the effect of ART on liver damage, the effect is thought to depend on the stage of HIV infection, the type and length of use of antiretrovirals, and their success in controlling the HIV virus. While some studies have reported that PIs were protective of liver fibrosis [120], others showed that PIs were associated with development of impaired glucose tolerance, hyperinsulinemia, and dyslipidemia [121, 122] which are implicated in hepatic steatosis. Studies in HIV-infected cohorts using PIs have also reported liver failure [123, 124]. A recent French report of two case studies described a rapid evolution of liver steatosis to cirrhosis in HIV-positive patients without viral hepatitis, despite adequate HIV control, and for whom the only risk factor for liver injury was the chronic use of an ART regimen that included PIs, NRTIs, and NNRTIs [125].

It is generally recognized that NRTIs are associated with liver disease, and one of the mechanisms proposed for increased liver steatosis, among other adverse effects of treatment, is mitochondrial toxicity [10]. There are a number of mechanisms by which NRTIs cause mitochondrial toxicity, including direct inhibition of mtDNA polymerase γ, termination of elongation of mtDNA during transcription by incorporation of NRTI triphosphate into the growing chain, and persistence of the NRTI analogs in mtDNA due to inefficient excision. NRTIs have a high affinity for DNA polymerase γ (gamma), the regulatory enzyme of mtDNA replication, but not for nuclear DNA polymerase γ (gamma). Inhibition of this enzyme downregulates mtDNA production and reduces the ability to repair mutations produced by respiratory and immunological oxidative stress [10, 126]. NRTIs, especially d4T, have a rare but serious adverse reaction, the development of lactic acidosis and hepatic steatosis [117, 127]. Mitochondrial toxicity may play a role in these adverse side effects. NRTI incorporation into mtDNA results in mtDNA depletion decreased mitochondrial protein, and ATP synthesis, as well as an increased flux of ROS into the mitochondria. Increased mitochondrial ROS influx and markers of mitochondrial oxidative stress were demonstrated in HepG2-cultured human hepatoblasts treated with d4T [118]. Increased oxidative stress due to mitochondrial toxicity may affect the pathophysiology of HIV disease and the cellular damage seen in AIDS [119]. Many types of cells and organ systems are affected by mitochondrial disease, but liver cells are especially vulnerable because of their dependence on oxidative metabolism to render their functions.

Newer nucleoside and nucleotide agents used to treat HIV include lamivudine, emtricitabine, abacavir, and tenofovir. They are weaker inhibitors of mtDNA polymerase γ (gamma). These NRTIs have a lower risk of events related to mitochondrial toxicity and are becoming the NRTIs of choice [123, 128–130]. The standard of care has moved away from using hepatotoxic thymidine analog-based ART regimens due to lipodystrophy; hence, ddI, ddC, and d4T are no longer prescribed as the preferred first line of treatment in the United States [131]. As new and less toxic antiretrovirals are developed, more information is needed on their interaction with alcohol consumption in HIV-infected persons.

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Chapter 19
Popular Energy Drinks and Alcohol

Erin C. Duchan

Key Points

- Since their introduction to the general public 25 years ago, energy drinks have continued to grow in popularity. This is especially true among 18–35-year-olds who frequently use energy drinks as a mixer with alcohol.
- The most common active ingredients in energy drinks are caffeine, taurine, guarana, and ginseng although they may contain a variety of other substances.
- The combination of alcohol and energy drinks is considered "risky drinking" due to increased alcohol absorption, a propensity to consume larger volumes of alcohol, decreased awareness of alcohol-induced impairment, and a higher rate of alcohol-related consequences.
- The sale of alcohol mixed with caffeine was banned in the United States in 2010 following a review of their safety.

Keywords Energy drink • Caffeine • Taurine • Guarana • Alcoholic energy drink

Introduction

Since their introduction to the European beverage industry 25 years ago and to the United States more than 10 years ago, energy drinks have been gaining popularity, especially among adolescent and young adult consumers. Currently, there are over 300 different energy drinks on the market [1], with new beverages being added regularly. Energy drinks are widely touted for their ability to increase energy levels and enhance cognitive and athletic performance. By promoting this aspect, the consumer marketing of energy drinks frequently targets the 18–35-year-old demographic. With popular lore conveying the expectation that caffeine will counteract the sedating effects of alcohol, energy drinks have become increasingly popular to mix with alcohol, especially in young adults. This is demonstrated by a survey revealing that, of college students who had consumed alcohol in the last 30

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days, almost one-quarter reported doing so in combination with an energy drink [2]. Despite the frequency of use, there are several concerns regarding the consumption of energy drinks mixed with alcohol including increased absorption of alcohol, drinking higher volumes of alcohol per drinking session and drinking alcohol more frequently, decreased awareness of alcohol-related impairment, and increased rates of alcohol-related consequences.

What Is an Energy Drink?

The concept of energy drinks is loosely based on Asian "tonic drinks" containing taurine, vitamins, and minerals that were popular in the Far East in the early 1980s. In 1987, the first mass-marketed energy drink, Red Bull, was introduced in Austria. This new energy drink contained sugar, caffeine, and taurine. Initially, the energy drink was widely promoted to increase alertness and improve cognitive performance; however, it rapidly found success among partygoers as a mixer with alcohol. Following a rise to popularity in the European market, Red Bull was introduced to the United States 10 years later, where it was similarly embraced, leading to the development of competing energy drinks [3].

Ingredients in Energy Drinks

The main active ingredient in most energy drinks is caffeine, although many energy drinks also contain varying amounts of taurine, guarana, and ginseng. Other ingredients less commonly found in energy drinks include carnitine, ginkgo biloba, green tea, branched chain amino acids, and inositol [4]. Energy drinks are not regulated by the US Food and Drug Administration (FDA); thus, the ingredient amounts may not be readily known to the consumer.

Caffeine

Caffeine (1,3,7-trimethylxanthine) is a naturally occurring central nervous system stimulant found in the seeds, fruits, and leaves of more than 63 plant species. The three most commonly extracted sources are the *Coffea arabica* (coffee bean), *Cola acuminate* (kola nut), and *Camellia sinensis* (tea leaves) plants [5]. Coffee beans, the most common source of caffeine, contain 1–2% caffeine [1, 5, 6]. Caffeine is a lipid-soluble purine that is well absorbed following oral ingestion. The onset of action of caffeine is 15–45 min following ingestion, and the peak plasma concentration is obtained within 1 h, regardless of the dose. Once ingested, it is 36% protein bound and is widely distributed throughout the body, with a volume of distribution of 0.6 L/kg of body mass. The half-life of caffeine is 4–5 h. Caffeine is metabolized in the liver by the isoenzyme CYP1A2, primarily by demethylation of 1,3,7-trimethylxanthine to 1,7-dimethylxanthine (theophylline), but other active metabolites include theobromine and paraxanthine [7].

Energy drinks are generally available in 8-, 16-, or 24-mL cans that contain anywhere from 80 to more than 500 mg of caffeine. In addition to these beverages, there are high-concentration energy shots: a 1–2.5 oz bottle that contains between 90 and 170 mg/oz of caffeine. Finally, the newest additions to the energy drink market are single-swallow ultra shots that are less than 1 oz and provide between 90 and 240 mg/oz of caffeine [1].

Some studies report that moderate amounts of caffeine have potential health benefits, including reducing the risk of type 2 diabetes, Parkinson's disease, liver disease, colorectal cancer, and improving immune function; however, the evidence is equivocal [5, 7]. Caffeine is also reported to have ergogenic effects which can enhance athletic performance; however, this claim has been contested [8, 9].

Individual responses to caffeine vary widely. Some individuals are nonresponders while others experience significant side effects at similar doses. Adverse effects are numerous, including arrhythmias, psychomotor agitation, headache, and irritability, but are generally not experienced at less than 3 mg/kg body weight [5, 7]. Unfortunately, consuming more than 3 mg/kg body weight may be easily achieved, especially in adolescents, thin individuals, or those consuming multiple energy drinks in one sitting. For example, a 100-kg (220 lb) person who drinks 150 mg of caffeine obtains only 1.5 mg/kg whereas a 50-kg (110 lb) person drinking the same amount of caffeine would consume 3 mg/kg. Serious adverse effects including hypertension, heart attack, and seizures are more likely in individuals who consume other foods or drinks containing caffeine as well as individuals with cardiovascular disease or those who smoke [5, 7, 10–17]. Caffeine toxicity is dose dependent, and fatalities have been reported at very high dosages of greater than 150–200 mg/kg.

Taurine

Taurine (2-aminoethane sulfonic acid) is a conditionally essential amino acid that is synthesized from cysteine and methionine in the liver and brain and is also found in a variety of dietary sources, including meat, fish, and dairy products. A non-vegan diet typically supplies between 20 and 200 mg/day. Similar to caffeine, peak plasma concentration is reached in 1 h. Within the body, taurine is stored primarily in skeletal muscle and myocardium but is also found in the retinas and blood [11, 18]. At physiologic levels, taurine is reported to function in bile acid conjugation, calcium regulation, carbohydrate metabolism, osmoregulation, platelet aggregation, and retinal photoreceptor activity. Additionally, taurine is reported to have antioxidant effects [18].

The content of taurine in energy drinks is not always declared. However, among energy drinks reporting this information, the content of taurine ranges from 9 to 120 mg/oz, with the majority of drinks reporting taurine content on the higher end [19].

Although the health benefits of taurine have not been extensively studied, human clinical trials suggest taurine may be effective for managing alcohol withdrawal, congestive heart failure, and cystic fibrosis. Taurine supplementation of 300 mg to 2 g/day has also been used in the management of diabetes, epilepsy, hypertension, cardiac arrhythmias, hepatitis, and anxiety despite the lack of supporting scientific evidence. While there is no established safe upper limit, the adverse effects of taurine are rare and include mild diarrhea and constipation [18, 19].

Guarana

Guarana, also known as Brazilian cocoa or zoom, is derived from the fruit seeds of the *Paullina cupana* and *Paullinia sorbilis* plants native to Brazil and other regions of the Amazon. The seeds are crushed and dissolved in water or juice to make a paste that can be added to beverages. The caffeine content of guarana ranges from 3.6% to 5.8%, more than twice the amount of caffeine found in coffee beans. The seeds of guarana also contain small amounts of theophylline, theobromine, tannins (primarily catechutannic acid and cetechol), and timbonine [20, 21]. However, because of the high caffeine content, the effects of guarana are primarily attributed to caffeine.

The guarana content of energy drinks varies widely, ranging from 1.4 to 400 mg of guarana per 240-mL can [11, 22]. The FDA recognizes guarana as a generally safe food additive at the typical stimulant dose of 1 g per day. However, guarana use can cause excessive nervousness and insomnia in individuals sensitive to caffeine or consuming caffeine from other sources, and if consumed in doses greater than 3 g/day, caffeine toxicity can result [20, 21, 23–25]. For these reasons, guarana should not be used by individuals who are pregnant or lactating or who have anxiety disorders, hyperthyroidism, glaucoma, cardiovascular diseases, or bleeding disorders [20, 21].

Asian Ginseng

Ginseng refers to several species of plants of the genus *Panax*, including the two primary species: American ginseng (*Panax quinquefolius*) and Asian ginseng (*Panax ginseng*) [26, 27]. Dried ginseng roots are used to make ginseng supplements because the ginseng roots contain pharmacologically active saponins (ginsenosides or panoxosides). The amount of ginsenosides in the root of the ginseng plant varies based on the species of ginseng plant, the age of the root (ginsenosides are more concentrated in older plants), the season of harvest (fall yields the most ginsenosides), and the method of preservation or curing. In addition to ginsenosides, ginseng root also contains variable amounts of methylxanthines, volatile oils, sterols, acetylenes, polysaccharides, starch, flavonoids, peptides, thiamine, riboflavin, vitamin B12, pantothenic acid, biotin, trace minerals, enzymes, and choline [24, 28]. Furthermore, following ingestion, ginsenosides are metabolized by gastrointestinal microflora, resulting in pharmacologically active metabolites. These factors complicate the interpretation of research data and may explain the variability of the reported health benefits of ginseng.

The amount of ginseng in energy drinks is typically 25–100 mg per 8 oz, which is below the typical recommended dietary supplement dosage. Although studies have not consistently shown definitive health benefits of ginseng [24, 26–28], there are claims that 100–200 mg/day of ginseng can improve menopausal symptoms, cognitive abilities, mood, sexual function, and immune function and reduce the risk of certain cancers. Ginseng is usually considered safe when used for short periods of time; however, side effects are more likely to occur if ginseng is consumed for more than 3 months. Adverse effects include palpitations, menstrual changes, insomnia, headache, dizziness, mania, and edema [20]. Ginseng also may interact with medications, especially those metabolized in the liver by the cytochrome P450 system, including certain blood pressure medications, anticoagulant medications, antipsychotic medications, antidiabetes medications, and antidepressant medications [27].

Mixing Energy Drinks with Alcohol

Sociodemographic Factors

Energy drinks have found their niche with the college-aged crowd, although use is rising in teenagers as well. More than half of college students in the United States report regularly consuming energy drinks [29], and among 12–17-year-olds, 31% report regularly consuming energy drinks [30]. One explanation for this may be advertising featuring celebrities, scantily clad women, and adrenaline-fueled athletics that appeal to a younger demographic. Energy drink manufacturers also sponsor a variety of athletes, athletic events, and competitions that appeal to the teenage and young adult market, such as skateboarding, wakeboarding, snowboarding, and BMX biking. With slogans such as the

energy drink will "give you wings," "party like a rockstar," and "unleash the beast," these beverages have become prominent in the daily routines of adolescents and young adults.

With the increasing popularity of energy drinks, there has also been a rise in the popularity of energy drinks mixed with alcohol. Energy drinks mixed with alcohol originated as a trendy fad at dance clubs; however, over the years, they have become ubiquitous at clubs, bars, and college campuses. Energy drinks mixed with alcohol have even given rise to bottled, premixed alcoholic energy drinks that were sold at grocery stores and convenience marts. These premixed alcoholic energy drinks include beer with added caffeine and malt or distilled spirit-based beverages mixed with caffeine, guarana, ginseng, and/or taurine. The malt and spirit-based beverages were available in a wide variety of fruit flavors, including watermelon, fruit punch, and blue raspberry, that characteristically tend to appeal to younger drinkers and females.

Energy drinks mixed with alcohol have gained the most popularity with college-aged drinkers and have become enmeshed in the subculture of partying on college campuses across the world. Fifty-four percent of university students surveyed in the United States reported mixing energy drinks with alcohol and 49% commonly consumed 3 or more energy drinks with alcohol while partying [29]. In Canada, 72% of university students surveyed reported deliberately mixing alcohol with an energy drink and 19% reported doing so during the week prior to the survey [31]. In France, 25–40% of young people report consuming a mixture of energy drinks with alcohol while partying [32]. A survey of Italian college students found that 85% of energy drink consumers had mixed these substances with alcohol in the past month [33]. In Argentina, alcoholic energy drinks have become synonymous with partying, to such detriment that the senate has proposed banning energy drinks in nightclubs [30]. And in Sweden, energy drinks have labels that warn against mixing energy drinks with alcohol and cannot be sold to children less than 15 years of age [30].

Individuals who consume alcohol mixed with an energy drink are more likely to be Caucasian and male [34, 35]. Those who participate in intramural athletics or are involved in fraternities and sororities, collegiate social organizations, are also more likely to consume alcohol mixed with an energy drink [36]. When compared to consumers of energy drinks without alcohol, persons drinking energy drinks mixed with alcohol are more likely to be young adults [37].

Physiology of Alcohol Absorption when Mixed with Caffeine

There is a modern twist to the old adage that a cup of coffee can help a person to sober up. The new widespread, but misplaced, notion is that consumption of alcohol mixed with an energy drink will offset the central nervous system depressant effects of alcohol. Thus, drinkers mixing alcohol with an energy drink may mistakenly believe that they can consume a larger volume of alcohol before experiencing impairment or may perceive that they are less intoxicated than they actually are. In reality, caffeine has no effect on the metabolism of alcohol by the liver and thus does not reduce breath alcohol concentrations or reduce the risk of alcohol-attributable harms [38]. In fact, the combination of alcohol with an energy drink increases the effects of alcohol by increasing the absorption. The carbonation present in energy drinks also increases the rate of alcohol absorption in the gastrointestinal tract [39]. Additionally, diluted concentrations of alcohol are emptied into the small intestines more rapidly than higher concentrations of alcohol and, once in the small intestines, are absorbed at a faster rate [39]. All of these factors result in increased absorption of alcohol when consumed with an energy drink. Once the alcohol and caffeine are absorbed, the physiologic response to both substances is individual, depending upon body weight, sex, general health, hepatic function, nutrition, prior exposure, and medication use (both over-the-counter and prescription). Therefore, it is impossible to predict a safe level to consume or to predict the level of impairment that may arise from consumption of an energy drink mixed with alcohol.

Alcohol Mixed with Energy Drinks Constitutes Risky Drinking

The factors that contribute to increased absorption of alcohol when mixed with energy drinks are only one of the reasons that the consumption of energy drinks mixed with alcohol constitutes risky drinking.

Another reason that mixing alcohol with energy drinks is considered risky drinking is that those who consume alcohol mixed with an energy drink drink more frequently and in larger quantities. Those who drink alcohol mixed with an energy drink report twice as many episodes of weekly drunkenness [36]. Individuals who consume alcohol mixed with an energy drink are more likely to have heavier alcohol consumption patterns [40]. In a survey of university students, participants reported drinking significantly more alcohol with it was mixed with an energy drink than when it was served alone [41]. One explanation for this may be that caffeine can diminish the sedative effects of alcohol, allowing the consumer to remain awake, and to continue ingesting alcohol, for a longer period of time. It has been well established that during binge drinking episodes, the drinker is at risk of serious injury, sexual assault, drunk driving, and death. However, when the alcoholic beverage is mixed with an energy drink, a new concern arises of caffeine toxicity.

A third reason that combining alcohol with energy drinks is considered risky drinking is that it is associated with decreased awareness of the physical and mental impairment caused by the alcohol [41]. A field study conducted in a United States college bar district found that patrons who had consumed alcohol mixed with energy drinks were three times more likely to leave the bar highly intoxicated and four times more likely to drive upon leaving when compared to those who had only consumed alcohol [42]. There are reports that the subjective perceptions of alcohol intoxication are less intense after the combined ingestion of alcohol with an energy drink when compared to alcohol alone because the ingredients in energy drinks give the drinker a false sense of physical and mental competence [41]. However, objective measures of motor coordination and visual reaction time fail to support this opinion [32]. In a study comparing maximal effort and physiological indicators, such as blood pressure, heart rate, and oxygen uptake, after consumption of either alcohol or alcohol mixed with an energy drink, there was no significant difference between groups and the energy drink did not reduce the effects induced by alcohol [43]. In fact, when compared to consumption of a placebo, drinking alcohol mixed with an energy drink resulted in a lower performance in visuospatial constructs and language performance [44]. Furthermore, studies have shown that the addition of caffeine to alcohol does not enhance reaction time [36].

A final reason that combining alcohol and energy drinks is considered risky drinking is that, even after adjusting for alcohol consumption, drinkers who consumed alcohol mixed with an energy drink had dramatically higher rates of alcohol-related consequences [32]. This includes taking advantage of others or being taken advantage of sexually, riding with an intoxicated driver, being physically injured, and requiring medical treatment [2]. Even without the added alcohol, consumers of energy drinks are more likely to consume alcohol more frequently and in greater volumes to experience alcohol dependence and alcohol-related problems and to have used nonmedical prescription medications [37, 40]. Caffeine has been associated with impulsivity among college students, including sexual activity, marijuana use, not wearing seatbelts, smoking, and illicit prescription drug use [33]. These behaviors may be further enhanced by consuming alcohol with energy drinks [2]. Additionally, young individuals tend to have a sense of immortality and a less mature judgment in regard to sexual activity and risk-taking behavior, which could be further exaggerated by the consumption of alcohol, especially when combined with caffeine.

These factors all contribute to consumers of caffeinated alcoholic beverages being more intoxicated than those who consume the same volume of alcohol without the mixer.

Premixed Alcoholic Energy Drinks

Sharing the shelf with energy drinks at grocery stores and convenience stores were premixed drinks that contained an energy drink mixed with alcohol, usually vodka, with an alcohol content ranging from 6% to 12%. In late September, 2009, 18 state attorneys general requested that the US FDA review the safety of premixed caffeinated energy beverages following an increasing number of reports of young people becoming seriously ill after drinking caffeinated energy beverages. These reports included injury and death believed to be the result of consumption of alcoholic energy drinks. Another concern raised during the debate was that the packaging of the alcoholic energy drinks was nearly identical to that of plain energy drinks, making it easier for youth to obtain and hide the alcohol or for consumers to mistakenly purchase the alcoholic energy drink. In November 2009, the FDA launched an investigation, notifying nearly 30 manufacturers of caffeinated alcoholic beverages that the agency would be looking "into the safety and legality of their products" [45]. One year later, the FDA ruled that the addition of caffeine to alcoholic beverages is unsafe, and under the Federal Food, Drug, and Cosmetic Act, the addition of caffeine is unlawful. Following this ruling, the FDA sent warning letters to four beverage companies that manufacture alcoholic drinks with a high caffeine content. These letters informed the companies that they were required to remove the caffeine from their product or remove their beverages from store shelves. Following this ruling, a United States Department of Health and Human Services survey reported that the consumption of flavored alcoholic beverages in 2010, including those with added caffeine, decreased from 53.4% to 47.9% [46]. Despite this ban on premixed caffeinated alcoholic beverages, there are no rules against bars or individuals mixing energy drinks with alcohol. The FDA regulates premixed caffeinated energy drinks and is responsible for ensuring the mixture is generally recognized as safe; however, the FDA has no such oversight on drinks mixed by individuals.

Conclusions

Introduced to the United States in 1997 following European success, energy drinks are functional beverages marketed to increase energy levels and performance. Energy drinks contain modest to relatively high levels and concentrations of caffeine along with varying amounts of other ingredients, most commonly guarana, taurine, and ginseng. Common adverse effects of caffeine include arrhythmias, psychomotor agitation, headache, and irritability although more serious adverse effects, including death, have been reported. While guarana, taurine, and ginseng are generally recognized as safe dietary supplements, there is no regulatory oversight on the quantity contained in energy drinks, and there are limited studies evaluating the safety of these additives when consumed in large quantities.

The consumption of alcohol mixed with energy drinks has increased in popularity following the marketing success of energy drinks. The use of energy drinks mixed with alcohol can be considered high-risk for many reasons, including increased absorption of alcohol, association with heavier alcohol consumption patterns, decreased awareness of impairment from alcohol, and higher rates of alcohol-related consequences. Although no longer available as premixed beverages in grocery stores, convenience stores, or liquor stores, energy drinks mixed with alcohol remain pervasive and popular in clubs, bars, and restaurants. Health-care professionals and nutrition experts need to be knowledgeable of the dangers of energy drinks mixed with alcohol as their consistent popularity proves this may not be a passing trend.

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Chapter 20
The Psychological Synergistic Effects of Alcohol and Caffeine

Ambereen Ameer and Ronald Ross Watson

Key Points

- Caffeine masks depressive symptoms of alcohol.
- Caffeinated alcoholic beverages do not reduce drunkenness or after effects.
- Caffeine and alcohol consumed in conjunction can result in higher rates of drunk driving and or violence.

Keywords Caffeine • Alcohol • Caffeinated alcoholic beverages • Psychological effects

Introduction

Any substance that humans consume has some sort of psychological effect upon them. Generally, most people seek out the ones that can elicit feelings of comfort, pleasure, or the sense of improving one's health. Yet, some individuals seek out various substances to dull one's senses or to rouse them. Alcohol and caffeine can serve those purposes perfectly well. Together, the effects of caffeine and alcohol may have some impact upon the mind and behavior, which may be positive or negative. While the consumption of either type of drink in subsequent usage is not a new concept, the mixing of both beverages is a newer phenomenon, and the noticeable effects are causing physicians, health officials, and law and policymakers take notice and take action. Due to the newness of the concept, research upon the subject is only in its burgeoning stages, yet it is sure to develop subsequent studies in due time based upon its generation of consumers. An entire industry of caffeinated alcoholic beverages (CABs) has arisen in recent years, primarily targeting college-aged individuals and has seen widespread success among this population. This chapter will examine the psychological effects of alcohol and caffeine, who are the most likely users, its safety and health implications, current legislation in place and developing policy, and what the future of this type of consumption can have in coming years.

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Background

One of the first questions we must investigate in this study is how did this concept originate. Based upon an established opinion, the consumption of caffeine after drinking alcohol as a sobering agent is a means to cure the after effects (or a hangover) of a previous night's drinking. When the blood alcohol content reaches about .10%, parts of the central nervous system have begun to "shut down." Normal reaction times have decreased, judgment and reasoning have become impaired, and inhibitory responses have diminished [1]. As consumption increases, more of the CNS loses function and excessive drinking can result in death. It is advisable for individuals who plan on drinking to eat before and during consumption because the alcohol absorption slows. However, caffeine is a known stimulant and users may think that the stimulant effects will cancel out alcohol's depressant effects or at least diminish it. With this thought process in mind, alcohol manufacturers and companies introduced the concept of caffeinated alcoholic beverages, which can reduce the feelings of intoxication based on the stimulant effects of caffeine. In another scenario, a person who ingested copious amounts of alcohol may feel the hangover effects of alcohol the following morning. Caffeine has an analgesic component to it which may alleviate headache and other feelings of malaise [2]. Again, the manufacturing companies must have assumed that the caffeine component would reduce after effects of alcohol, and thus, caffeinated alcoholic beverages were born.

Basic Effects of Caffeine and Alcohol

Although the idea of an alcoholic beverage that did not contain the negative components or risks sounds highly marketable, it does not necessarily mean that the science is correct. In a study conducted in 1998, researchers investigated the effects of ethanol and caffeine on operating behaviors in rats. The study found that the caffeine, on the whole, served to augment the effects of ethanol in terms of attention span, accuracy, and latency. Yet, the caffeine did stabilize components such as the lengths of pauses and response rate [3]. Another study indicated that caffeine and alcohol "significantly reduced subjects' perception of headache, weakness, dry mouth, and impairment of motor coordination but did not significantly reduce the deficits caused by alcohol on objective motor coordination and visual reaction time" [4]. Further research is currently being conducted; however, most data indicates that CABs do not reduce drunkenness. Rather, it only covers the effects but CNS depression occurs. In terms of curing hangovers, caffeine seems to be a continually used method to cure the headache aspect of a hangover. Since the caffeine constricts blood vessels in the brain, it aids in reducing headaches [1]. However, caffeine alone does not cure the hangover. Instead, the caffeine only exacerbates it due to its diuretic properties and results in further dehydration. CABs are no exception to this rule.

Based on anecdotal data, these particular beverages worsen the hangover effects, perhaps due to alcohol and caffeine serving as a doubled dose of diuretic. Interestingly enough, people who consumed CABs the preceding morning from alcohol consumption were "able to drive better than the people who were randomized to straight alcohol" [5]. The same researchers noted that the presence of caffeine and absence of alcohol resulted in better driving. The long-term effects of the drinks are still being researched. Studies that tested the effects of alcohol being coadministered with caffeine on the plus maze discriminative avoidance test (PMDAT). Caffeine did not inhibit any decrease in learning caused by ethanol but ethanol did prevent the anxiety that can be caused by caffeine [6]. The immediate onset of CABs is mainly the reduction of perception of intoxication, as noted throughout this chapter. CABs will allow the consumer to feel more sober than they really are, but in actuality, they are experiencing the "wide awake drunk" phenomenon that indicates the user is alert but still experiencing the depressant effects of alcohol. Due to the caffeine content in CABs, there has been concern that these beverages will serve as a gateway drug to actual alcohol in the future. Based on the perceptions and myths behind CABs, it may

appear as a "starter" drink that a new alcohol consumer can drink based on its so-called lowered effects of drunkenness. Since the perceptions of elongated sobriety are experienced, new consumers will practice learning how to drink "responsibly" with CABs before trying hard liquor.

Healthcare and Legal Perspectives

Researchers at the National Institute on Alcohol Abuse and Alcoholism (NIAAA) have stated that CABs is akin to "drinking a bottle of wine in a can" [7]. Physicians and public health officials are particularly concerned about the impact CABs have upon issues such as drunk driving, injury or death caused by violence, and alcohol poisoning because the effects of intoxication are not felt and the consumer will assume they are well enough to continue drinking, despite the fact they are just as inebriated (if not more so) as they would be by drinking straight liquor. Even though the FDA has taken initiatives to block the marketing of CABs, their efforts may not truly come to fruition. Customers at bars or alcohol consumers will simply mix a caffeinated beverage into their drink or will consume a caffeinated beverage, such as coffee after a few glasses of wine. In order to reduce this type of drinking behavior, there must be an increased awareness about the effects caffeine and alcohol can have together upon the brain and what the health implications can result from CABs and mixed alcoholic beverages.

Based on the information collected, alcohol and caffeine consumed together does not reduce the effects of inebriation nor does it act as a sobering agent. Despite the contrary research claims, CABs such as Four Loko, Joose, and Red Bull and vodka have seen tremendous success in recent years. The average users are college aged (ages 18–24) at approximately 34%. However, usage via self-report stated about 39–57% in the past month. Fifty-four percent of college students have professed to mixing alcohol with their energy drinks at parties [8, 9]. However, the success of CABs has been short-lived. In November of 2010, the FDA moved to completely ban the sale of Four Loko because of its increased alcohol content and high-risk accidents [10]. The FDA has intervened with the sale of CABs due to surfacing reports that consumers that mixed caffeine with their alcoholic drinks were three times more likely to binge drink and were four times more likely to drive under the influence [11]. Crimes of sexual assault were twice as likely to occur when CABs were consumed by victims and or perpetrators [12]. The question arises if it was right for FDA to limit or ban the sale of CABs. Critics argue that if it is legal to ban CABs, then alcohol should also be banned or at least limited. Other opponents have stated that the government should not interfere with what Americans choose to consume and since both substances are legal, it is an infringement upon personal freedoms. While the debate continues on, most health-care providers and public health officials maintain that since CABs do increase risky drinking behaviors and do not have a positive effect upon health in general that prevention of sales and manufacturing is the safest choice.

Psychological Effects

Since caffeine and alcohol are classified as differing substances, examining their similar and differing effects upon the brain may provide insight as to why taking both substances in combination may not be prudent. Alcohol is mainly linked to its interference with gamma-aminobutyric acid (GABA) and at its receptor. Since alcohol is a depressant, it actually augments the inhibitory effects of GABA, which (normally) reduces action potentials and neuron activity. There have also been noted effects of alcohol upon increasing dopamine levels, which result in pleasurable feelings. The release of dopamine in the early stages of alcohol consumption may contribute to the "buzzed" feelings that most drinkers seek. Because of the initial "buzz" that most drinkers get akin to stimulation and then the

subsequent depressive symptoms, scientists have classified alcohol to have a bimodal phase. This means that alcohol can act as a stimulant and then as a depressant. Since the stimulant effects are relatively brief, it is more appropriate to categorize alcohol as a depressant. Caffeine, meanwhile, is largely associated with the neurotransmitter adenosine. In order to enhance alertness, the caffeine inhibits the adenosine receptors, which induces sleep [13]. Returning to the subject of GABA, adenosine is an inhibitory neurotransmitter, namely, inhibiting glutamate [14]. Interestingly enough, alcohol also inhibits glutamate, which is an excitatory neurotransmitter. Glutamate is primarily linked to learning and memory and alcohol use, as many people are aware, impedes both mental processes [15]. Another similarity between caffeine and alcohol is that caffeine also increases dopamine levels and has been seen to elevate mood. Since caffeine stimulates dopamine level, another theory about CABs may be that the caffeine in the beverages may be thought to extend the "buzz" phase of alcohol and delays the depressant phase. However, since caffeine inhibits glutamate as well, the combination of both substances may increase the inhibition rate of glutamate. Thus, it impairs memory and learning faculties. If both substances are depressing two core aspects of brain function, why do people choose to drink both substances? The placebo effect may be the very reason since the belief that caffeine will alert one's senses after they have been dulled by alcohol can perhaps make an individual feel more capable of performing regular tasks.

Of course, not all studies are consistent with one another, and differing reports have stated that caffeine may actually reduce the hypnotic effects of alcohol with respect to the adenosine receptors. The A_{2A} receptor (when activated) proves to be particularly useful in cutting the effects of an ethanol-caused coma, especially with a nonselective adenosine receptor antagonist-type caffeine. The researchers deemed that drinking caffeine is helpful in acting against alcohol [16].

Surveillance Results and Statistics

The Behavioral Risk Factor Surveillance System survey recently collected information that over one half of the adult population drank an alcoholic beverage in the past 30 days in the United States [17]. While the figure may seem to be excessive, one must account that there may be underreporting of data or participants may overestimate or underestimate the amount they have drunk in this time. The same report, however, also indicated that about 15% of this adult population binge drinks and another 5% stated they "drink heavily." In respect to caffeine consumption, North American adults consume about 75% of caffeine via coffee. The last 25% is through beverages such as sodas, teas, energy drinks, and cocoa products [18]. There is no current scientific data that can clearly state whether caffeine is more popular than alcoholic beverages, but based on the variety of caffeinated beverages that range from tea to energy drinks and the attempts to lower under aged drinking and excessive alcohol usage, it may be that caffeinated drinks are more widely consumed than alcohol is. Since there seems to be a greater population drinking caffeine as opposed to alcohol, it poses the question as to which substance results in more dependence. To examine the dependence of caffeine, a scientific study observed the variables of withdrawal, tolerance, and reinforcement. In terms of withdrawal, the researchers concluded that the withdrawal symptoms for caffeine intake did not comply with amount ingested. For tolerance, the results indicated that humans mostly became partially tolerant to sleep but only for a small portion of participants. Lastly, caffeine showed to be a low or moderate reinforce of stimuli. The conclusion was reached that while some criteria of dependence were met. Caffeine is the least "addictive" substance, compared to drugs such as benzodiazepines, barbiturates, cocaine, and others [19]. Alcohol, on the other, is a widely known drug that causes many problems in terms of health, society, and economy. Aspects of positive and negative reinforcement play a large part in alcoholism and relapse in sobriety. Also, the alcohol can alter brain chemistry and changes the "motivational processes, including arousal, reward, and stress" [20]. Based on this knowledge, it is blatantly obvious that alcohol results in more

dependence rather than caffeine, although there are a higher percentage of people that consume caffeine. However, caffeine and alcohol both have a role on interfering with neurotransmitters such as dopamine, as previously mentioned. Since both substances increase dopamine levels, it may be that alcoholics (when not drinking) choose caffeine as an alternate drink of choice based on its ability to induce its pleasurable emotions. Coffee in particular has chlorogenic acid quinides that raise adenosine levels. This induces an antidepressant and anxiolytic sensations that can lead to a reduced alcohol intake [21, 22].

In regard to alcohol and caffeine consumption, a 1977 study revealed that both men and women who drank heavily also drank excessive amounts of coffee two times more than those that did not drink heavily [23]. For recovering alcoholics, caffeine also seems like a reasonable alternative to alcohol again due to its increase of dopamine levels. Only with caffeine, the depressant effects of alcohol are not present [22].

Conclusion

In conclusion, the data and information that surrounds the psychological effects of caffeine and alcohol mainly indicates that caffeine does not play a role in acting as a sobering agent nor does it cancel out any depressant effects of alcohol. Rather, it can result in further intoxication and can amplify the depressant effects. Evidence from ongoing or current research demonstrates that CABs have a tendency of increasing risky behaviors such as intoxicated driving, forced or unsafe sexual behavior, or excessive consumption that can lead to alcohol poisoning or an alcohol-induced coma. Caffeine and alcohol can result in impaired levels of learning and memory because of the effects upon neurotransmitters such as glutamate. In essence, alcohol and caffeine taken in combination negatively impacts mental faculties and can have a direct impact upon public and personal health.

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Chapter 21
Alcohol and Smoking: A Correlation of Use in Youth?

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Key Points

- A synergistic effect has been rising among adolescents with using alcohol and tobacco.
- There have been a multitude of reasons such as the availability, accessibility, and expectancies attached to the drugs.
- In addition to these factors, there are peer, home, genetic, and media influences that have been proven to increase the co-usage of the drugs.

Keywords Alcohol • Tobacco • Adolescent • Youth • Smoking • Drinking

Introduction

Adolescents abuse tobacco and alcohol more than any other drug [1]. Young people are more likely to experiment with these drugs prior to doing so with other drugs due to their availability and the peer expectancies surrounding them. The high risk of co-using these drugs is exemplified by the US National Household Survey, which indicates that people within the age cohort of 18–24 years show a high usage of both drugs. This epidemiology study reveals that within that age group, 19.4% of men and 12.5% of women co-use alcohol and tobacco [2]. Each drug is easily accessible for adolescents, and through this availability, the joint usage of the drugs becomes apparent. In 2010, 61.1% of eighth grade students admit that alcohol would be "easy" or "fairly easy" to obtain and 55.5% state the easy ability to obtain cigarettes. In regard to 10th graders, there is an increase in usage of both substances: 80% for alcohol and 69.4% for cigarettes. These high usage rates are the most prominent of all drugs, including PCP, MDMA, amphetamines, tranquilizers, and heroin with abuse percentages as low as 12.6% [3]. Thus the difficulty of access among these drugs in comparison to alcohol and cigarettes would leave adolescents prone to use the more available substances. On the contrary, since these two drugs are so easily accessible, adolescents can and do use them together, therefore causing a synergism which will be the focus of this review.

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Expectancies

Expectancies are a main reason why adolescents experiment with drugs. Peer pressure is a major cause of why kids try a drug for the first time. For example, if an individual foresees a particular outcome from the drug, then he or she is more likely to try it. A recent study examines the different expectancies of alcohol among adolescents who are non-substance users, tobacco-only users, alcohol-only users, or co-users of tobacco and alcohol. The results of this study reveal that co-users have the greatest expectancies of alcohol's effects, and the co-usage is about double in comparison to the non-substance users. This study also examines adolescents' expectancies surrounding tobacco and the results show similarities. The co-users have the highest prevalence and show a higher rate than even the tobacco only [4]. These results provide more evidence that adolescents who smoke and drink have higher expectations of the substances' benefits. This suggests that the adolescents use both drugs to reach the ultimate experience from each drug because of this high level of expectancy. Alcohol is associated with providing a pleasurable experience, whereas smoking cigarettes is thought to instill more of a relaxing feeling in the person. Furthermore, an individual who smokes believes that he or she can enhance the experience by drinking [5]. Additionally, another study investigates male smokers, ages 18 through 30, and their alcohol desires after having either a denicotinized or regular cigarette. The double-blind study disabled the participants and investigators from knowing if the participants were receiving a regular or denicotinized cigarette [6]. Overall, the results proved that more nicotine consumption causes a greater desire for alcohol. These results are also consistent with a recent study among 7th- to 12th-grade students evaluating alcohol use by current smokers. The data show that around 95% of the smokers use alcohol while one-third of the drinkers smoke [7]. However, it should be recognized that these data are different than that for the general population of adolescents. For example, these results indicate that smokers use and crave alcohol because of the feelings they receive from concurrent use. These experienced feelings can encourage an adolescent to use the drugs together in order to receive both a pleasurable and relaxing experience. An animal model study gives further evidence that many smokers seek alcohol after ingesting the nicotine from the cigarette [8]. This model uses animals that are able to self-administer alcohol and are evaluated after being treated with low, medium, and high doses of nicotine. The animals that had the highest exposure to nicotine demonstrated more than double the alcohol intake in comparison to the animals in the control group who were maintained through injection of simple saline solutions [8]. This study indicates that nicotine can encourage an individual to use alcohol in order to partake in a more rewarding experience. It can be inferred that adolescent animals would behave the same way but possibly choose to self-administer more alcohol than the adults because they have been found to be less affected by the drugs withdrawal symptoms [9, 10]. Another animal model was performed to analyze the different effects of nicotine and alcohol on adolescents in comparison to adults. The results do show that adolescent animals self-administer more nicotine than adults [1].

Psychosocial

Certain adolescents are more at risk for abusing alcohol and tobacco than their peers. This phenomenon may be analyzed through the psychosocial profile of an individual. For example, it has become apparent that personality, peer pressure, and family modeling promote the co-usage of these substances. First, the personality type of an adolescent can depict the type of behavior usually exhibited in relation to these drugs. Adolescents may become more vulnerable to initiating usage of the drugs if they exhibit behaviors classified as impulsive or neurotic. Furthermore, young people who admit to using both drugs have a higher prevalence of risk-taking and violent behavior patterns than those who do not use the substances. However, they are also more likely to have drug use problems later in their

lives if they consistently drink and smoke throughout their adolescence [11]. For example, these particular adolescents may participate in selling drugs, stealing, or using predatory violence. Among 23-year-olds classified as "early highs" (those who admitted to more than average use of tobacco and alcohol at age 13 and have increased usage of both substances), 18.3% were selling drugs. This same study also looked at nonusers in which 0.0% sold drugs and "normative users" (smoke a few times a year and drink one to times a month) in which only 3.7% sold. In regard to stealing, 15.8% of the "early highs" admit to stealing, 2.7% of nonusers, and 8.1% of "normative users." Lastly, almost a quarter of the "early high" group has shown predatory violence by age 23 when compared with 7.0% of nonusers and 7.4% of normative users [11]. Clearly, it can be deduced from this study that those who co-use alcohol and tobacco at a young age and into adulthood have a higher probability of exhibiting these sensation-seeking behaviors. Another recent study shows that among a group of early and mid-adolescents, ages 11 through 14, sensation seeking increases with nicotine and alcohol use [12]. "Sensation seeking" implies seeking a variation of situations and experiences in areas such as financial, legal, or social to receive a particular intense sensation [13]. A similar study performed in 2007 shows that among a group of adolescents characterized as non-substance users, tobacco-only users, alcohol-only users, or co-users, the co-users have the highest level of novelty seeking. Almost a quarter of the co-users engage in behaviors that are considered high risk [14]. Additionally, a recent study reveals that among adolescents who have initiated smoking in comparison to those who have not, there is a significantly higher rate of impulsivity and novelty seeking [14].

The personality profile of an adolescent can help predict the behaviors which can be characterized as risky or sensation seeking and depressive. Among a group of college undergraduates, it was found that those who used tobacco within the last 30 days, 44% were moderately depressed. Similarly, among those who binge drank, 60% were moderately depressed [15]. These data indicate that some adolescents are choosing to use alcohol and tobacco as an escape from their current mental state. It can therefore be inferred that those who are depressed have an increased risk of co-using tobacco and alcohol.

Peer Influences

Peer pressure has been known to cause individuals, especially adolescents, to try a drug for the first time and later cause potential dependency. Young people are highly influenced by what they perceive to be popular or socially acceptable. Thus individuals become more at risk when their peers are smoking and drinking around them [14]. There was a cohort study measuring the various determinants of the initiation of smoking only through adolescent years. The results revealed that smoking by parents, siblings, friends, and teachers or school staff all impacted the prevalence of smoking initiation. One study compares adolescents who have and have not tried smoking. It reveals that among the children who have smoked, 29.6% of their peers smoke, whereas adolescents who have never smoked only had 14.9% of their friends smoke. The study also demonstrates double the prevalence of alcohol use in children who have smoked versus those who have never smoked [14].

A recent study by Mrug and colleagues [16] examines the effects of other-sex relationships on smoking, drinking, and sexual behavior. Boys with all male friends had a higher alcohol use than if their clique consisted of half girls or all girls. In contrast, among the girls, there was a higher prevalence for drinking if their clique mainly consisted of boys. However, the results for probability of smoking behaviors varied for the boys from their drinking tendencies. Boys show a much more equal distribution of smoking probability among the different gender-based cliques. The largest prevalence dictator of boys' smoking probability is being part of an all girl clique. The girls also show the same results from their drinking behavior and have the highest prevalence when they are in an all boy clique [16].

Romantic relationships have been found to have an impact on substance use of alcohol and cigarettes [17]. Marriage among young adults is negatively associated with drinking and smoking. It has

also been found that among adolescents living together, they showed a lower prevalence of drinking. Cigarette use was only shown to be lower when the young adults were married [17]. Although the average age for marriage is 27.1 years for men and 25.3 years for women, [18] adolescents who choose to marry younger may experience the possible benefit of decreased drug usage.

Home Influences

Adolescents are also highly influenced by their home environment. A study's results show that parental use of both drugs among adolescents classified as co-users is 54.9%, family tobacco use is 78.5%, and there is increased risk if a parent has had an alcohol problem [11]. Alcoholics have a three times higher prevalence than nonalcoholics to smoke [11]. In addition, tobacco users are four times more likely to use alcohol than non-tobacco users [19]. Therefore, it may be inferred that adolescents of alcoholics are at a greater risk of developing a co-use of the drugs than those without alcoholic parents. In comparing nonusers of alcohol and tobacco with "early highs" (those who used excessive alcohol and tobacco at age 13 and continued), family background appears to be the most intact and positive among the nonusers. Almost three-quarters of the nonusers report having good family relationships, whereas among the "early highs," only 39.3% even have nuclear families [11]. Dalton and his colleagues [20] performed an observational study giving preschool children the opportunity to buy any items in a grocery store. The items were all props and they included alcohol, cigarettes, and other products. Over a quarter of the children bought cigarettes and 61.7% bought alcohol. These data is directly related to whether or not the parents drink and smoke. The children were more likely to buy cigarettes and alcohol if their parents smoked, drank, or watched PG-13 or R-rated movies [20]. This study provides reason that these same children would have a higher probability of co-using the substances through adolescence and even adulthood. It also may confirm that the type of information a child receives in their family environment may register to be normal behavior.

Media

An additional study investigates the effects of media on adolescents drug use. Children with restrictions on watching R-rated movies had a lower prevalence of trying alcohol and smoking. The adolescents who had no restrictions showed a prevalence of 35% in regard to smoking and 46% with alcohol. Among the children who had complete restrictions, only 2% had tried cigarettes and 4% had tried alcohol [21]. Thus adolescents mirror the behavior they see in media and that in turn can influence their choices in using drugs. Additionally, adolescents also browse through magazines and pick up ideas on what is popular and perceive them to be normative behavior. A study analyzes the top magazines read by youth found that Sports Illustrated with 5.3 million youth readers had 401 estimated alcohol and tobacco advertisements a year [22]. Thus through many drug advertisements in a sports magazine, popular magazines chosen by youth, alcohol and tobacco companies are able to target them much more readily. The media may be able to manipulate adolescents into co-using alcohol and tobacco when they display popular movie stars or sports players using them.

Socioeconomic Status

Socioeconomic status has proven to be an indicator of drug usage among adolescents. In a recent study of 13-year-old children, alcohol use increased with higher socioeconomic status [23]. A few possible explanations for this were given in the study. First, the more money available to an

adolescent, the more alcohol he or she can purchase (from an older sibling, peer, etc.). Second, some wealthy children are left feeling isolated and possibly underachieved in academics [24]. Third, many affluent families have less supervision of children after school. A possible explanation of this phenomenon could be the feeling of safety within their neighborhoods. Furthermore, decreased supervision can lead an adolescent to experiment with alcohol lying around at home [25]. Adolescents with mothers of higher levels of education were less likely to drink alcohol [23]. An explanation could be that more educated mothers provide their children with information on alcohol and its consequences. Mothers have the tendency to be the voice of reason with health-related issues for the family. However, it was found that in regard to adolescents' smoking habits, the lower socioeconomic status, the more likely the child was to smoke. This is consistent with multiple other findings, so the greater the education of the mother, the lower cigarette consumption [23]. This article's conclusions are that the mother's education and knowledge have an impact on both the drinking and smoking habits of the offspring. However, more research should be done on what different factors play into the wealthier families having a higher rate of alcohol consumption among their adolescents. A feasible reasoning behind the difference with socioeconomic status and smoking in comparison to drinking is illustrated in this study. Melotti and his colleagues [23] believe that the health community demands a zero smoking tolerance among adolescents, whereas alcohol is not as heavily advertised in regard to health consequences.

Another study provides more data on substance use and socioeconomic status among young adults. Casswell and his colleagues [26] performed a cohort study in New Zealand and used three different methodologies in measuring socioeconomic status: education, occupation, and income. There were different ages that were ultimately examined: 18, 21, and 26 years old. The results show that less-educated participants of every age group drank more in one sitting than any other education level. The researchers reason that the higher consumption within one occasion can explain the difference in life expectancy between socioeconomic status [26]. Those living in a lower socioeconomic class have a reduced life expectancy which is consistent with smoking behaviors among young adults as well. A study in Finland was performed on recognizing the relationship of socioeconomic status and smoking among adolescents over time. Doku and his colleagues [27] found that among 12–14- and 16–18-year-old girls who are categorized as having poor school performance or not in school (16–18-year-old group), smoking has a 60% prevalence. The 12–14-year-old boys with poor school performance show approximately 46% prevalence of smoking [27]. Both of these studies mentioned above recognize the prevalence of drug use among adolescents with lower socioeconomic status. The prevalence of drug use, and in particular alcohol and cigarettes, is directly related to socioeconomic status. Possible co-use of alcohol and tobacco was apparent in these studies but not explicitly mentioned [27].

A later study measured the socioeconomic status of a cohort of adolescents followed through their young adulthood. Their socioeconomic status was measured in regard to the parent's education level, the child's academic achievements in seventh grade, and college graduate status. Orlando and his colleagues [11] began the research when the children were 13 and continued until age 23. They found that as a group, the kids who were smoking a few times a month and drinking at least once a month during 7th grade increased their behavior and smoked weekly and drank more at age 23. It was also found that more than half of this cohort had poor grades in seventh grade. This prevalence is greater than among any other respective group in the study. Additionally, the same cohort referenced as "early highs" only had 2.7% of them graduated from college. The "normative users," who were classified as only smoking a few times a year and drinking a couple times a month at age 23, had almost 25% of their group graduate college. Only 32.8% of the "early highs" indicated having educated parents, whereas 48.0% of the nonusers declared their parents as being educated [11].

Early Co-usage

There is evidence to support that adolescents begin co-using the substances relatively early, developing risk factors for later substance use and dependence. A recent study examining a group of adolescents, the first puff of tobacco among non-substance users is 12.86 years, tobacco-only users is 11.15 years, alcohol-only users is 12.66 years, and concurrent users 11.64 years. Additionally, the same study asked the adolescents when was their first sip of alcohol. The results show similar results that beside the tobacco-only users, the concurrent users have the youngest age of usage at 13 [4]. The relatively close age difference between the onset of smoking and drinking suggests that adolescents try them within the same time frame and therefore may have a higher probability of co-using the substances. Also, these results indicate that concurrent users begin their alcohol and tobacco use at a very young age. If adolescents begin co-using drugs at a young age, they are likely to develop a tolerance to each substance. Co-usage of alcohol and tobacco may cause a cross-tolerance within the individual. Cross-tolerance is defined as maintaining the addictions to both drugs while increasing the dosages of each. An animal study was performed on female adolescent mice testing cross-tolerance between nicotine and alcohol. The results reveal that the female mice that received the alcohol for 4 days developed a cross-tolerance to nicotine. This cross-tolerance is seen through body temperature and activity [28]. Furthermore, because there is cross-tolerance of alcohol and tobacco, there has been research performed evaluating the success rates of cessation of smoking and abstinence from alcohol. A recent study evaluated alcoholics receiving alcohol abuse treatment and the effects of cigarette cessation on the patients [29]. The results reveal that the patients who decreased their smoking habits also decreased their likelihood of alcohol relapse [29].

Genetics

Evidence links alcohol and nicotine together in regard to the mechanisms of each substance. Through research on genetics, neurobiology, and the psychosocial of individuals, much has been learned about why adolescents are co-using the drugs.

Nicotine, the addictive ingredient in cigarettes, and alcohol both act on the mesolimbic-dopamine system of the brain. This section of the brain deals with the rewarding feeling that an individual can experience. A pleasant feeling comes from the neurotransmitter called dopamine. Dopamine releases itself from one cell and then moves to various receptors on surrounding cells. A recent research study analyzes the relationship between alcohol and tobacco consumption with the D2 dopamine receptor and dopamine transporter gene halotypes [30]. The study examines a group of males with the diagnosis of alcohol dependence. The results reveal that D2 receptor gene single nucleotide polymorphisms had smoking and drinking behaviors linked to them. A single nucleotide polymorphism is when there is a variation in a DNA sequence within a nucleotide [30]. Additionally, there has been a research study on adolescents with previous smoking experience. The results reveal that possessing additional DRD2 A1 alleles influence the progression of smoking among adolescents who had previously smoked. The DRD2 A1 allele is a dopamine receptor cell that also influences the regulation of dopamine throughout the adolescent's brain [31]. Lastly, it has been proven that among adolescent children of alcoholics, the same dopamine receptor gene influences possible substance abuse by the child. The study revealed that boys with the DRD2 A1 allele get drunk more, try more substances, and become addicted to tobacco more than adolescent boys with a different allele. The results indicate that possessing the DRD2 A1 allele places children at risk in co-abusing alcohol and tobacco [32]. Overall, genetics play a role in determining the possibility of an adolescent in becoming a co-user of alcohol and tobacco.

Conclusion

Adolescents who use alcohol and cigarettes are at a high risk in developing abusive behaviors in adulthood [11]. There are multiple trajectories that lead young people into trying cigarettes and alcohol for the first time. The home environment of an individual can promote risky behavior and therefore increase the probability of doing drugs. The home is usually thought of as a place of health advisement, especially by the mother, [25] and when adolescents look to their parents for modeling behavior and do not receive it, they become likely to experiment with drugs. Additionally, many children surround themselves with people of similar interests and therefore can readily engage in drug use if their peers are. Peer influence is a major factor in co-using alcohol and cigarettes. It can be further seen from a recent study on college freshmen students. Many participants admit to smoking and drinking in order to "fit in" in relation to their peers. This study dives further into a new concept that college students are "play" smoking. This concept means that they only smoke at parties in order to initiate conversations with other people. Some students reveal that smoking becomes normalized in a party setting and can be seen as being a "package deal" [33]. Among some adolescents, their environment becomes the largest indicator of the behaviors they will do, like using alcohol and tobacco.

Most of smoking adolescents also use alcohol and it is said to cause a more relaxing and delightful experience, yet there are multiple health consequences on their brains, lungs, liver, and heart [34–37]. An individual's brain undergoes massive growth throughout the adolescent years. The major sections of the brain are the frontal lobes, the hippocampus, and the cerebellum. The frontal lobes are helpful to make decisions, control impulses, and comprehend; the hippocampus for storing memories; and the cerebellum for maintaining balance. All of these are impaired when an adolescent drinks [37]. In regard to smoking, it was found that the adolescents who smoked for any amount of time have a poorer memory than those who do not smoke [36]. A recent study looked at the health effects of alcohol use disorders and found an association with liver injury and heart/lung symptoms in association with cigarette smoking among adolescents [35]. Another study analyzed adolescents with congenital heart disease and found that half of them have smoked and drank [34]. Clearly, there are a multitude of adverse health effects of co-using alcohol and tobacco, and there are many factors that place an adolescent at experiencing the synergistic effect of tobacco and alcohol.

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Chapter 22
Are There Physiological Correlations Between Alcohol and Tobacco Use in Adults?

Cynthia Lee and Ronald Ross Watson

Key Points

- The correlation between alcohol and tobacco usage has been observed throughout the years.
- The reasons for the correlations are still unknown, but there is increasing data being collected on the co-occurrence of these events.
- Possibilities that have surfaced are that this co-use may be caused from physiological reasons, especially dealing with the mesolimbic dopamine system.

Keywords Alcohol • Tobacco • Alcohol dependence • Co-occurrence • Co-use

Introduction

Often times, an alcohol user is also a tobacco user and likewise. These substances have significant effects on life. Understanding the history of these substances helps illustrate the relationship, or lack thereof, of these two drugs.

The usage of alcohol has existed for thousands of years. Mead, possibly the oldest alcoholic beverage, seems to have appeared during the Paleolithic Age, 8000 BC [1]. Berry wine and beer were used as far back as 6400 BC, and grape wine made its appearance sometime between 300–400 BC [1]. There are many ways to obtain alcohol; fermentation and distillation are most commonly exercised. There are also various forms of alcohol: beer, wines, and distilled spirits. Alcohol use is worldwide, and social factors play a large role.

The trends of alcohol use have generally decreased through the years. Before the American Revolution, people drank more alcohol than water. However, drunkenness was still frowned upon.

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After the American Revolution, people started to view alcohol as evil, thus making alcohol the first psychoactive drug demonized by Americans [1]. The temperance movement began soon after. Benjamin Rush discussed how heavy drinking led to health problems. He also stated that alcohol damaged morality and addiction to it was a disease [1]. Temperance societies heavily promoted abstinence from distilled spirits but allowed moderate drinking of beers and wines. However, a few years later, total abstinence was pushed. Prohibition laws were passed in individual states in 1851. In 1917, 64% of Americans lived in areas that were considered "dry"; however, people drank illegally in speakeasies, private clubs, and through patented medicines [1]. The 18th Amendment of the Constitution was ratified on January 1919. This amendment banned the sale of alcohol. A year later, national prohibition went into effect. People still continued to drink and sell alcohol illegally, and law enforcement was not effective. The prohibition did lead to a decrease in alcohol dependence and alcohol-related deaths [1]. The 18th Amendment was repealed by the 21st Amendment in 1933 due to the lack of law enforcement and decrease in revenues from alcohol taxation. Alcohol per capita sales and consumption increased until after World War II [1]. Alcohol was regulated after 1933. Most states started allowing beer sales after the prohibition ended. Mississippi was the last dry state until becoming wet in 1966. Drinking ages were lowered in some states but raised back to the age of 21 in consequence of increased drinking rates and alcohol-related traffic accidents [1]. Alcohol is also taxed. There are federal and state taxes as well as licensing fees that make up about half the amount of an alcoholic beverage. The higher the taxes, the less consumed. U.S. alcohol consumption peaked in 1981 and then started to decline. A third of Americans abstain from alcohol consumption. The average consumption per drinker is three drinks per day [1].

The American Indians had many names for tobacco, but the Spanish adopted *tabaco*, which possibly could have come from an Arawak term they come across in the Carribean. The Spanish word could have also been derived from the Arabic word *tabbaq* [1]. Europeans did not know of tobacco until 1492 when Columbus first came to the "New World." The natives handed the dried leaves of the tobacco plant to his people, but they threw them away because they did not know what the leaves were. Europeans who first encountered tobacco thought it was disgusting, but even with this perception of the plant, its usage spread [1]. Again, social factors played a large role in the assimilation of tobacco into popular culture. What initially started with trade with American natives made way for prosperity. Tobacco helped people become wealthy and powerful.

Tobacco use has also been declining throughout the years. Most people used it because it was promoted as a treatment for any ailment. However, when it was discovered that tobacco had many negative effects on the body, usage declined [1].

Drinking and smoking are often initiated by social factors. There are often correlations seen between tobacco and alcohol use. These correlations are typically of a physiological nature. Studies have shown there is a correlation between dependence and the amount of alcohol consumed during a certain period of time. There are studies that show a psychological correlation between the two as well, mostly pertaining to psychosocial factors.

Many research studies have shown that sociocultural factors influence the initiation and continued use of alcohol and tobacco among adolescents and adults. The 1997 National Household Survey on Drug Abuse found that adults who reported binge drinking within the past 30 days were twice more likely to be current smokers than those who did not binge drink [2]. Individuals who never or rarely drink (less than 12 drinks per year) are not likely to smoke. Table 22.1 displays that only 13.4% of current smokers were never drinkers. The percentages increase as drinking increases. Moderate drinkers may have more opportunities to smoke than nondrinkers if their family, peers, and social acquaintances use both substances [2].

There is research available that suggests that drinking prompts smoking. In one study, participants were to use small, portable computers to record when they smoked and what other activity they were doing alongside. The computers also "beeped" to have the participants record what they were doing during the period in which they were not smoking. Individuals were twice as likely to report recent drinking while smoking [2]. Drinking could possibly influence smoking by releasing inhibitions that

Table 22.1 Tobacco use among four categories of adolescent and adult alcohol users in the general population

Alcohol use history	Current smoker (%)	Former smoker (%)	Never smoker (%)
Ages 12–17			
Current drinker	58.1	23.4	18.5
Former drinker	23.8	37.9	38.3
Never drinker	05.6	11.2	83.2
Binge drinking[a] In past 30 days			
Yes	76.8	17.9	05.3
No	14.1	18.8	67.2[b]
Ages 18 and older			
Current drinker	36.9	45.6	17.5
Former drinker	27.1	50.8	22.1
Never drinker	13.4	18.8	67.9[b]
Binge drinking In past 30 days			
Yes	54.5	35.7	09.9[b]
No	26.1	45.1	28.7[b]

[a]Binge drinking was defined as five or more drinks on one occasion
[b]Values shown are from weighted analyses. Due to rounding, some row totals will not sum to exactly 100
Reprinted from Bobo and Husten [2], with permission from SAMHSA

restrain them from doing so. In the early 1990s, 90% of alcohol abusers were regular smokers [2]. Recent data shows a decline in smoking prevalence of this population. Studies from 1996 to 1997 reported that tobacco use rates in alcohol treatment patients slightly declined from 75% to 71%, respectively [2].

Those who are dependent on alcohol are three times more likely than those in the general population to be smokers. Likewise, those who are dependent of tobacco are four times more likely to be dependent on alcohol.

Epidemiology

Drinking and smoking prevalence rates were highest among young adults. Prevalence decreased with age. The co-use of alcohol and tobacco was also highest among the young and followed the same pattern as the prevalence. Peak rates of the co-use of the two substances by young adults ranged from 35% to 45%, at which codependence was 10%. In 1990, DiFranza and Guerrera found 83% of alcoholics smoked tobacco compared to 34% of their nonalcoholic counterparts [3]. In 2000, Bobo and Husten found that 37% of adults who drank were also smokers compared to 13% of those who were abstinent. In 2001, 21.7% of adults in the United States used tobacco and alcohol; this number represents approximately 46.2 million individuals. Men were more likely than women to use both alcohol and tobacco, 27.5% and 16.4%, respectively. American Indians/Alaskan Natives were found to be the highest users of the co-use of the two substances. Caucasians, African Americans, and Hispanics were all at the intermediate level of co-use, while Asians/Native Hawaiian/Pacific Islanders were among the lowest users [3].

Physiological Reasoning

Nicotine and alcohol affect the mesolimbic dopamine brain system. The mesolimbic dopamine system is a system in brain that mediates the rewarding and reinforcing properties of alcohol and nicotine [4]. Changes of this system may interfere with the effects of both substances. Cross-tolerance may also be

occurring; either drug may enhance the reinforcing properties of the other. There is a model that explains that the reduction of sensitivity to alcohol in smokers compared with nonsmokers may create a role for certain genes that may predispose individuals to alcohol and nicotine use. Reduction of sensitivity to one drug may cause the use of the other, which may lead to co-abuse. There are genetic studies in both humans and selectively bred strains of mice and rats that genetic factors may also determine a person's fate in using either substance. Studies concerning twins showed that identical twins, those who share 100% of their genes, compared to fraternal twins, who share 50% of their genes, are twice as likely to be alcohol or nicotine dependent if either one of the pair is dependent. This suggests that 50% of a person's liability to develop either nicotine or alcohol dependence is genetic. According to the Substance Abuse and Mental Health Services Administration of 2005, the prevalence of smoking is three times higher in alcoholics than the general public. Although the two substances are often used together, their mechanisms of action and effects are different. Nicotine binds directly to a nicotinic acetylcholine receptor in the brain, where alcohol does not bind to a specific receptor type. Alcohol is a depressant and nicotine has stimulating effects.

Neural Mechanisms

Much focus has been placed on the mesolimbic dopamine system. The origins of this pathway lie in the neurons of the ventral tegmental area (VTA), a region of the midbrain [4]. These neurons release the dopamine neurotransmitter to other brain cells, including those associated in reward, emotion, memory, and cognition. The nucleus accumbens, an area in the forebrain, has extensively been studied for its involvement in reinforcing the effects of drugs. Numerous studies have focused on the mesolimbic dopamine system regarding whether it plays a role in motivating an individual to drink or smoke. The neurons that release dopamine in the VTA have nicotinic receptors. These neurons usually receive signals from other neurons in a different brain region known as the pedunculopontine tegmental nucleus (PPT). PPT cells release acetylcholine, another neurotransmitter [4]. Acetylcholine travels to the VTA, which consequently acts on the nicotinic receptors, which in turn stimulate VTA cells to continue releasing dopamine to carious brain regions. There is evidence available that suggests nicotine's effects are caused by the stimulation of the nicotinic receptors from the VTA neurons. Injections of inhibitory agents into the VTA may reduce nicotine self-administration. Nicotine needs to interact with the nicotinic receptors in the VTA in order to produce its effects. Many studies have observed alcohol self-administration effects in the mesolimbic dopamine system as well. The results of these studies have produced numerous varying results depending on whether the agents were injecting systemically into the bloodstream, thereby distributing itself through all tissues of the body system. Studies in which these agents were injected directly into specific brain regions showed more consistent results. By directly injecting dopamine releasing agents into the nucleus accumbens, alcohol consumption increases [4]. Likewise, the injection of agents that reduce dopamine release in the nucleus accumbens reduces alcohol consumption. There are only a few studies that observed the mesolimbic dopamine system in relationship to nicotine and alcohol co-use. One study suggested that a pharmacological blockade of the nicotinic receptors in the VTA will decrease alcohol intake [4]. This study further suggests that the pleasurable effects of alcohol are also linked with the nicotinic receptors in the brain.

Additional studies have used a technique known as in vivo microdialysis. This technique allows researchers to measure the release of neurotransmitters in the specific brain regions of freely behaving animals [4]. These results also support the theory that alcohol and nicotine produce co-behavioral effects via the mesolimbic dopamine system. Observations using the in vivo microdialysis technique show that both alcohol and nicotine were stimulants to the release of dopamine by the nucleus accumbens. Direct nicotine injection to the VTA also caused the release of dopamine. The combination of

both nicotine injections and systemic alcohol injections further enhanced the effects of dopamine release. Again, blocking the nicotine receptors in the VTA puts an end to the alcohol-induced increase release of dopamine [4]. Altogether, this information suggests that the effects and interactions of nicotine and alcohol depend on the activity of the mesolimbic dopamine pathway.

Human Studies

In a double-blind placebo study, researchers distributed regular and denicotinized cigarettes to male smokers [5]. The participants were then asked to complete tasks, which progressively got more demanding, as a way to receive an alcoholic beverage. Results showed that the male smokers with regular cigarettes worked harder during tasks and drank more alcohol than their denicotinized cigarette-smoking counterparts.

Another study used mecamylamine, a nicotinic receptor inhibitor that binds the receptors and prevents nicotine from binding to these receptors. Forty-eight smokers who were also moderate alcohol consumers participated in four lab sessions [6]. The variables consisted of nicotine versus denicotinized cigarette smoke, mecamylamine versus a placebo, and ethanol versus a placebo, where alcohol was used as a between-subjects factor [6]. The data obtained from this study showed that social drinkers who were given mecamylamine experienced less of a "feel good" effect after consuming alcohol than when they normally did. Alcohol consumption often increased the rewarding effects of nicotine. These effects include the smoking satisfaction, stimulating yet calming effects, and the relief of nicotine craving [6].

Both of these studies suggest that alcohol and nicotine must interact in some way with the nicotinic receptors to induce pleasurable effects. Other studies have displayed that interactions between nicotine and alcohol are influenced by many modulating factors like age and gender.

Animal Studies

Animal studies are beneficial to this issue because they have never been exposed to these psychoactive drugs prior the studies in which they are involved. Researchers have developed various strains of mice and rats that differ in their responses to nicotine and alcohol [4]. An early study on laboratory rats showed similar results to those observed in the human studies. Rats were surgically implanted with nicotine-releasing capsules [4]. Compared to the control rats, there was a significant increase in alcohol consumption during the next few days in the test rats. Other studies also showed similar findings [4]. Researchers have replicated these findings by either using daily nicotine injections or subcutaneous nicotine capsules. These studies found that the nicotine increased alcohol consumption when the animals had to work to obtain alcohol and when they had access to an alcohol-filled bottle [4]. Mecamylamine had the same effects on the rats as they did on human subjects.

Another animal study showed how nicotine could enhance motivation in obtaining alcohol under a relapse or reinstatement procedure. Lab rats were provided a lever in which they would press to receive alcohol. When lever-pressing was established, the alcohol was removed. The lab rats continued to press the lever but eventually stopped. When the rats stopped alcohol was replaced, but the rats no longer pressed the lever. A nicotine injection caused the rats to press the lever again. This study suggests that nicotine might affect alcohol-seeking behavior by affecting its brain pathway [4]. In 2006, Le and colleagues conducted a series of experiments that tested if shared genetic factors increased vulnerability to both nicotine and alcohol. Two groups of rats were involved in this series: high alcohol intake rats or low alcohol intake rats. Both strains of rats were trained to use a lever to

receive nicotine injections. The high alcohol intake rats self-injected themselves more than the low alcohol intake rats [4].

The prevalence of smoking in alcoholics is thought to be as high as 90% compared to less than 30% of the general public [5]. Likewise, smokers are 50% more likely to drink alcohol. Insufficient data has been done on whether either substance is more prone to cause the other.

Tobacco Use After Alcohol Cessation

Though numerous studies have displayed data that encourage recovering alcoholics to quit smoking, randomized clinical trials show that individuals even those receiving intensive treatment for alcohol abuse continue to smoke long after their drinking is at a controlled level [4]. A longitudinal study of 575 smokers who completed intensive treatment was conducted in the Midwest during the year 1995. Results displayed that 92% of these individuals still smoked on a daily basis a year after treatment completion [4]. Furthermore, 49% of this group smoked on average a single pack or more of cigarettes per day.

Alcoholics Anonymous members are often advised to not quit smoking until they are confident in their abilities to remain sober while dealing with additional stress [4]. In 1995, researchers conducted a randomized trial in 12 residential treatment facilities in Iowa, Kansas, and Nebraska. Patients in half of the treatment centers received a four-part intervention that encouraged them to quit smoking. The remaining patients received the usual treatment provided by the center. Alcoholic patients who were also smokers could indeed benefit from smoking cessation counseling [4]. After a year, 43% of the patients who were encouraged to quit smoking were still abstaining from alcohol compared to the 29% of those who did not receive smoking cessation counseling [4].

Conclusion

Characteristics of these studies limit their generalization to the public populations. Selection bias is one of the concerns. Samples usually consist of those being provided treatment and local community samples. More national representatives would be required to make a public generalization about the correlation of the two substances [3]. Often cigarette smoking or smoking is used for nicotine intake. However, there are many different ways to consume nicotine and tobacco. Many of the studies were also conducted in the past; diagnostic criteria must be updated. Since there is a decline in both alcohol and tobacco consumption, current national data must be obtained in order to provide estimates of alcohol and tobacco co-use among individuals in the United States [3]. In relation to genetics being a factor to co-occurrence of tobacco and alcohol, studies are criticized in that many of the traits evaluated are common in numerous psychiatric disorders. Also, the ease and wide availability of alcohol and tobacco may contribute to the co-use of the substances [4]. Cross-tolerance is difficult to measure because both of these psychoactive drugs are commonly used together.

There is a definite correlation between alcohol and tobacco usage. However, there is not enough data to conclude the reasoning behind it. Research is only beginning to come across data that may suggest the co-occurrence of these substances. More research needs to be done pertaining to whether either substance has a greater influence than the other or whether physiological or psychological factors are more prone to enhance usage in both substances. There are too many inconsistencies in the current studies to make any conclusions. Data needs to be updated and reflect the current environment of the United States.

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Chapter 23
Alcohol, HIV/AIDS, and Liver Disease

Tamsin A. Knox, Logan Jerger, and Alice M. Tang

Key Points

- Hazardous alcohol use is increased in persons at risk for HIV infection and among those with HIV infection. Alcohol use increases the risk of HIV acquisition through risky sexual practices.
- Alcohol use is associated with decreased adherence to antiretroviral therapy resulting in HIV transmission and in progression of HIV infection to AIDS. In addition, alcohol use may promote progression of HIV disease through deleterious effects on the immune system.
- Alcohol use is associated with complications of HIV infection including cardiovascular and pulmonary conditions. Liver disease, in particular, is exacerbated by alcohol use, which promotes progression to cirrhosis, hepatocellular carcinoma, and death. These effects are more common in persons coinfected with HIV and chronic hepatitis C or B virus.
- Intervention studies to reduce alcohol use in populations with HIV or at risk of HIV are clearly important, but studies have had variable results.
- There may be no safe level of alcohol use in HIV infection.

Keywords HIV • Liver fibrosis • Sexual transmission of HIV • HIV acquisition • Africa • Men who have sex with men • Antiretroviral therapy for HIV • Adherence to antiretroviral therapy • Immune function • Survival • Hepatitis C virus • Liver disease • Hepatocellular carcinoma • Cardiovascular disease • Intervention studies

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Importance of Alcohol Use in HIV/AIDS

Globally, there are over 33 million persons living with HIV/AIDS resulting in 1.8 million deaths annually. While the rate of HIV transmission is slowing, it is estimated that 2.6 million new infections occur yearly [1]. In the United States, there are approximately 1.2 million living with HIV/AIDS, with 50,000 new HIV infections and 17,000 deaths from the disease annually [2]. For those who can obtain effective antiretroviral therapy (ART), HIV/AIDS has become a chronic disease with life expectancies over 30 years [3]. Research in the last 10 years has revealed the importance of alcohol in the HIV/AIDS epidemic. Alcohol use, in moderate or hazardous amounts, has been associated with increased acquisition of HIV infection, progression of HIV infection, deleterious effects on HIV treatment, and acceleration in the comorbidities of HIV infection [4–9]. Yet alcohol remains the "forgotten drug" of the HIV/AIDS epidemic [10].

Alcohol has a complex relationship with HIV acquisition. Risky sexual behaviors, among heterosexuals or among men who have sex with men (MSM), that promote HIV transmission are increased in the setting of alcohol These include increased frequency of sexual encounters with new or anonymous partners and reduced condom use [11, 12]. Attention to the locations and clientele where alcohol is served [13] has led to the development of an "ecological epidemiology" of the interplay of multiple risk factors around HIV transmission [14].

Once infected with HIV, alcohol use is associated with progression of HIV infection from asymptomatic infection, to symptomatic AIDS with declining immune function measured by low CD4 T-cell counts (<200 cells/mm [3]) in the blood, to death from wasting or an opportunistic infection. Again, the relationship between alcohol use and progression of HIV infection is multifaceted. Hazardous drinking has been associated with delayed testing and treatment for HIV infection [12, 15, 16], poor adherence to ART therapy [6, 17], and increased HIV viral replication and shedding [18–20]. Simian immunodeficiency virus (SIV) infection in monkey models has confirmed findings that regular intake of alcohol leads to more rapid progression of disease, weight loss, and death [21–24].

Alcohol use also complicates the care of persons with HIV infection. Not only is adherence to ART decreased, but drug interactions between alcohol and specific ART medications may increase the toxicity of therapy [25]. HIV infection has numerous comorbidities including coexisting infections such as chronic viral hepatitis or tuberculosis as well as progressive organ dysfunction involving the liver, cardiovascular system, neurological dysfunction, or pulmonary disease. Concurrent alcohol use may have a deleterious effect on any of these conditions [26–30]. Thus, the management of alcohol misuse is central to control and treatment of HIV/AIDS. This chapter summarizes recent research on the effects of alcohol on HIV infection.

Epidemiology of Alcohol Use in HIV/AIDS

Epidemiologic studies of alcohol use in HIV infection inconsistently define alcohol intake and problem drinking. Many studies categorize alcohol intake as "none," "moderate" drinking (ranging from any alcohol intake to daily intake over the period studied), and "hazardous" drinking (including regular daily intake or binge drinking and may or may not include a diagnosed alcohol disorder). In addition, the studies screening for alcohol disorders use different criteria including the CAGE questions, AUDIT questionnaire, self-reported drinking, or a physician's report of an alcohol disorder [31]. Thus, varying methodology and study population selection will greatly influence the results from studies of alcohol use in HIV.

Acquisition of HIV

Alcohol use, whether moderate or hazardous, daily or binge drinking pattern, increases the risk of acquiring HIV [12, 32]. Drinking alcohol is associated with an increased number of sexual encounters with new, anonymous, or high-risk partners [11, 12, 33]. Alcohol use has also been shown to increase the risk of having unprotected intercourse as well as of acquiring a sexually transmitted disease, which in itself, predisposes to HIV infection through open sores [12, 34–38]. Stein et al. found that hazardous drinkers were 5.6 times more likely to have multiple partners and/or unprotected sex than nonhazardous drinkers [39].

The importance of alcohol as a risk factor for HIV infection has been demonstrated in all at-risk groups including heterosexual men [4], MSM [37, 38, 40, 41], adolescents [34, 40], women [42, 43], and drug users [44–46]. Stueve showed that urban adolescents who use alcohol engage in high-risk sexual behaviors including multiple partners and unprotected sex, predisposing them to HIV infection at an early age [34]. Women may be particularly affected by alcohol use since even if they themselves abstain, they are at increased risk of HIV based on the alcohol intake of their male partner promoting sexual violence and coercion [43, 47].

The association of alcohol use with HIV transmission has been well documented by a number of studies in sub-Saharan Africa [11, 32, 47], which has one of the highest burdens of HIV infection and comprises over half of the persons infected with HIV worldwide [1]. Alcohol use is higher in men and women at risk for HIV and is associated with increased sexual risk practices in Africa [48]. Even low amounts of alcohol use in women (e.g., one drink in the last month) were associated with higher risk of HIV infection [49]. In a meta-analysis of 11 studies from Africa, the odds ratio of having HIV was 1.57 for drinkers and 2.04 for problem drinkers compared to nondrinkers, when controlled for other HIV risk factors [32]. Kalichman has shown that strategies for HIV risk reduction in these settings work best through interventions targeted at decreasing alcohol use [50].

Similarly, in India, risk behaviors favoring the spread of HIV are rare among men in household sampling studies (<4%) but high (70%) among men surveyed in wine shops (street shops selling liquor) [51]. Other studies have confirmed this association, which is particularly important in India where 80% of HIV is ascribed to heterosexual transmission [52]. While few women in India drink alcohol (compared to men), women may be at risk due to their husband's or male partners' drinking habits [53, 54]. In the Yunnan province of China, where the epidemiology of HIV has been well studied, spread of HIV has begun to shift from intravenous drug use (IDU) to sexual transmission [55]. This suggests that alcohol use may also play an important role in the spread of HIV in China, but there are no data on this at present.

Social locale where alcohol is served such as bars, gay bars, beer halls, and bath houses may be a nidus of HIV transmission since persons frequenting these establishments may have a higher prevalence of HIV infection and sexually transmitted infections (STI), and sexual encounters occur frequently among the clientele [13]. This may be particularly important in the transmission of HIV among gay men and female sex workers. Scribner et al. developed a model called "ecological epidemiology" that encompasses individual characteristics, social network, and the alcohol neighborhood to understand and study HIV transmission. For example, an individual who frequents a bar will be exposed to a group with multiple interrelated sexual partners and an increased prevalence of sexually transmitted disease and HIV [14].

Prevalence of Alcohol Use in HIV

In the US population, approximately 4% meet the DSM-IV definition for alcohol abuse and 14% have had an episode of binge drinking in the last 30 days [56, 57]. Table 23.1 contrasts this with the

Table 23.1 Prevalence of alcohol disorders in HIV

Demographic group	Prevalence	Reference	Notes
In general	4% alcohol abuse	Grant 2004 [56]	
US population	14% binge drinking in last 30 days	Naimi 2001 [57]	
In HIV	53% any alcohol	Galvan 2002 [166]	HCSUS, $n=2,864$ from 1996
	8% heavy drinkers		
	28% hazardous drinkers	Stein 2005 [39]	
	19% problem drinking	Cook 2001	Providence, RI, $n=262$
	5% heavy drinkers	Lucas 2002 [167]	Pittsburgh, $n=212$ from 1998
	5% moderate health	Conen 2009 [64]	
	risk (WHO)		Baltimore, $n=695$, 80%nonwhite
	3% severe health risk		Swiss HIV cohort study, $n=6,323$
In men			
Veterans	47% hazardous drinking	Gordon 2006 [58]	Homeless veterans, $n=881$
	46% any alcohol	Braithwaite 2005 [6]	VA population, 60% non
	9% binge drinkers	Goulet 2005 [106]	white
	24% alcohol disorder		
	20% hazardous drinkers	Conigliaro 2003 [163]	VA, $n=25,116$
	33% binge drinkers	and Justice 2006 [164]	VA, $n=881$
	17% alcohol diagnosis	Kraemer 2008 [59]	VA, $n=16,048$
MSM	41% alcoholism	Lefevre 1995 [63]	MSM, Michigan, $n=111$
	5% heavy drinkers	Kleeberger 2001 [168]	MSM in MACS, $n=539$
In women	14–24% hazardous drinking	Cook 2009 [42]	WIHS, $n=2,770$
	32–48% moderate drinking		
In Africa	14% binge drinking	Kalichman 2011 [48]	So.Africa, $n=529$ men in STD clinic

MSM men who have sex with men, *WHO* world health organization definition of "moderate health risk" from alcohol consumption, *VA* veteran's association, *MACS* multicenter AIDS cohort study, *WIHS* women's interagency HIV study, *STD* sexually transmitted disease

prevalence of alcohol use among populations with HIV. There are wide apparent differences in rates of alcohol use and hazardous alcohol use due to the populations surveyed, the definitions of "problem" alcohol use even in the same cohort, and the methods used to determine alcohol intake.

In general, the prevalence of alcohol use disorders is several fold higher among populations with HIV infection compared to the general US population. Some of the highest prevalence rates from problem drinking are among US veterans and homeless veterans [6, 58]. Among the Veterans Administration (VA) population, hazardous drinking patterns are found more frequently in African-Americans (26%) than in whites (18%, $p<0.001$) [59]. Cook et al. determined that the prevalence of moderate and hazardous drinking among women with HIV infection was also higher than in the general US population [42, 56, 57]. Other characteristics were associated with hazardous drinking patterns such as lower education, unemployment, nonwhite race, depression, and drug use. In both this cohort and in a VA cohort, hazardous alcohol use was associated with hepatitis C virus (HCV) infection [42, 60]. Among veterans with HCV infection, 35% were hazardous drinkers compared with 12% hazardous drinkers among matched controls without HCV infection [60]. The increased alcohol use among IDU and the high correlation of IDU and HCV infection likely explain this finding [46, 61].

Alcohol Use Over Time

Alcohol intake appears to decline over time in persons with HIV infection as it does in noninfected persons with medical illness [62]. Lefevre et al. examined alcohol intake in a group of 111 HIV-positive patients of a university hospital clinic, mostly MSM. In surveys repeated every 6 months for a mean follow-up of 30 months, the frequency of drinking decreased from 6.4 to 3.9 drinks/week ($p < 0.001$) [63]. In the Swiss HIV Cohort Study, lower alcohol use was found in those who had been on ART for longer periods of time [64]. Cook analyzed data from the Women's Interagency HIV study (WIHS) on 2,770 HIV-positive women followed for 11 years [42]. There was a slight, approximately 5%, decrease in hazardous drinking over time but no change in the overall amount of drinking, possibly as some switched categories from hazardous to nonhazardous drinking. However, there was a significant decrease in alcohol consumption among women who were coinfected with hepatitis C and HIV from 31% with hazardous drinking patterns in 1995 to 10% in 2006.

Alcohol and HIV Progression

Alcohol has been implicated in accelerating the progression of HIV disease through a number of mechanisms. Persons drinking alcohol heavily delay testing for HIV and have less connection with and retention in the health-care system [12, 15, 16], delaying the initiation of ART. Thus, heavy alcohol use predisposes persons to late presentation in the course of infection, with high HIV viral loads, low CD4 counts, and opportunistic infections, and promotes continued spread of HIV [45, 65].

Adherence

One of the central ways alcohol intake adversely affects HIV disease is by decreasing adherence to ART. Adherence to ART is key to suppression of HIV replication, prevention of developing drug resistance, and long-term survival [66]. This has been well documented among all subgroups with HIV infection [6, 64, 65, 67–71]. While there are few studies of adherence in developing countries, one study from India confirms the association of alcohol use and risk of nonadherence or discontinuation of ART medications [72]. Convincingly, there is a dose–response relationship between alcohol intake and adherence, with higher amounts of alcohol or more hazardous drinking being associated with poorer measures of adherence. Samet et al. found that the amount of alcohol consumption was the strongest predictor of adherence with highest levels of adherence being found in those abstinent from alcohol compared to moderate use or at-risk use [70]. Chander et al., studying nearly 2,000 HIV-infected persons receiving care at Johns Hopkins Hospital in Baltimore, Maryland, found that adherence was 22% lower in moderate alcohol users and 54% lower in hazardous alcohol users compared to no alcohol use. Adherence was further decreased by 68% with concurrent drug use [65].

There may be several reasons for lower adherence in persons who use alcohol. Drinking pattern affects the likelihood of noncompliance. Braithwaite et al., studying 2,700 members of The Veterans Administration Aging Cohort Study (VACS), found that abstainers missed ART on 2% of days. Non-binge drinkers missed medication on 4% of drinking days and post-drinking days but only on 2% of nondrinking days. Binge drinkers, in contrast, missed ART on 11% of drinking days, 5.5% of post-drinking days, and 4% on nondrinking days [6]. Therefore, while medication adherence was lower on drinking days for binge and non-binge drinkers, missing medications was increased twofold among binge drinkers on days they were either not drinking or post-drinking. This suggests that nonadherence was

also due to factors not directly related to alcohol but related to characteristics common among binge drinkers [6]. Sankar et al. studied beliefs about alcohol and ART medication interactions in a group of African-American patients treated for HIV [71]. Over three quarters of those surveyed felt that "alcohol and ART do not mix"; one-third attributed this to alcohol making ART ineffective and another third felt that alcohol made ART more toxic. In this study, participants reported purposely skipping ART doses when they drank, with light drinkers skipping 64% of the times when they drank and moderate drinkers 55% of the times. However, heavy drinkers skipped ART only 29% of the time when they drank and reported that they felt no ill effects from drinking and taking ART [71]. Thus, medication adherence is determined by amount of alcohol intake, drinking pattern (binge or non-binge drinking), and beliefs about the safety of alcohol combined with ART. Issues of medication adherence and alcohol are further discussed in Chap. 18 and in a meta-analysis by Hendershot [17].

Immune Function

Alcoholics have increased susceptibility to bacterial infections including tuberculosis, pneumonia, and sepsis [73]. In vitro studies have shown that alcohol impacts several areas of immune function, acting largely as an immunosuppressant. Alcohol decreases T-cell proliferation reducing CD4, CD8, and natural killer (NK) cell numbers [7] and reduces CD8 cell responses to bacteria [74]. Cell-mediated immune responses are decreased [75], and myeloid dendritic cells, which are involved in antigen presentation to the immune system, are decreased in number and function with chronic alcohol ingestion [76, 77]. Alcohol increases expression of pro-inflammatory cytokines such as TNF-alpha [78] which may enhance immune dysfunction.

Experiments by Bagasra et al. on human peripheral blood mononuclear cells (PBMC) have shown that cells from healthy persons who are infected in vitro with HIV-1 have higher levels of HIV replication when harvested after alcohol consumption [19]. Enhanced HIV replication was associated with a concurrent inhibition of CD8 cells by alcohol [18].

SIV infection, a macaque model for HIV, has produced evidence of the effect of alcohol on immune function and HIV replication. In rhesus macaques inoculated with SIV infection, SIV replication was 31- to 85-fold higher in monkeys with chronic alcohol ingestion compared to controls [21]. SIV replication persisted in the central nervous system of alcohol-fed monkeys but was undetectable in control monkeys. Poonia et al. proposed that the mechanism of alcohol's effect on SIV replication is through its effect on intestinal lymphocytes since the small intestine is one of the most lymphocyte-rich organs. Alcohol-fed monkeys had lower numbers of CD8 cells (before and after SIV infection) and higher numbers of CD4 cells in the small intestine after SIV infection. They suggested that the 1–2 $\log_{10}$ increase in SIV replication in alcohol-fed monkeys occurs because of the increase in number of CD4 cells susceptible to SIV infection in the small intestine and reduction in CD8 cells which may control SIV replication [22]. Chronic alcohol ingestion also altered the course of HIV infection with alcohol-fed monkeys having lower CD4 cell counts, lower caloric intake, higher TNF-alpha expression, and a more rapid progression to end-stage SIV disease (mean 374 days compared to 900 days in controls) [23, 24].

HIV Progression and Survival

Alcohol use has been shown to affect HIV progression and survival. In the pre-HAART era, alcohol use was not associated with progression to AIDS [79–81]. However, two well-controlled, longitudinal

studies since the introduction of combination ART have shown that alcohol is associated with HIV disease progression. Samet et al. studied alcohol use in 595 participants in the MACS cohort over 7 years [82]. Heavy alcohol use was associated with a lower mean CD4 cell count (by ~50 cells/mL) but not a decline in CD4 percentage or HIV viral load when adjusted for adherence. Baum et al. studied 231 HIV-positive persons followed for 2.5 years [5]. Frequent alcohol users of ≥ 2 drinks/day were almost 3 times more likely to develop a CD4 count ≤ 200 cells/mL, which is an AIDS-defining event. This effect was particularly marked in alcohol users not on ART whose risk of developing a CD4 count ≤ 200 cells/mL was nearly 8 times nondrinkers. In this study, alcohol use was associated with higher HIV viral load in those on ART but not in those without ART. These results suggest that the effect of alcohol on HIV viral load is mediated through adherence. However, the effect of alcohol in lowering absolute CD4 count rather than percentage could be influenced by the splenomegaly and secondary lymphopenia seen with alcoholism and chronic viral hepatitis [83]. Moderate to heavy alcohol use has also been associated with increased HIV viral shedding in the female genital tract after controlling for plasma viral load [20] suggesting that alcohol may affect HIV transmission by physiological as well as behavioral risk factors.

The VACS study has provided models for estimating the effect of alcohol on survival in HIV infection. Using data on ART adherence in the VACS cohort, Braithwaite et al. developed a model simulating survival based on levels of alcohol consumption (nondrinkers, hazardous drinkers consuming ≥ 5 drinks on drinking days, and nonhazardous drinkers) [84]. The model predicted decreased survival by >1 year in nonhazardous drinkers drinking at least once a week, 3.3 years in nonhazardous drinkers drinking daily, and up to 6.4 years in hazardous drinkers drinking daily. However, the VACS index, subsequently developed to predict decreases in life expectancy based on HIV and non-HIV characteristics, does not include a separate variable for alcohol or drug abuse beyond adjusting for severity of liver disease and coexisting HCV infection [85]. In addition, a longitudinal study of changes in physical function with age in the same cohort did not show an effect of alcohol [86]. Further longitudinal studies in this cohort and others should define the impact of alcohol use on survival in HIV.

Liver Disease and Other Harmful Sequelae of Alcohol in HIV

Persons with HIV infection are particularly vulnerable to the effects of alcohol. The detrimental effects of alcohol on the immune system have been covered above, and the effects of alcohol on general health and nutrition are covered in other chapters in this book. Persons with HIV infection are at risk of poor nutritional status, and even a 3% weight loss has been associated with increased mortality [87–90]. Thus, further changes in nutritional status due to alcohol use, particularly lower body weight or micronutrient deficiencies, would exacerbate the nutritional effects of HIV [91, 92].

Liver Disease

Approximately one-third of persons with HIV infection are coinfected with HCV, and approximately 10% have evidence of chronic hepatitis B virus (HBV) infection [93]. The prevalence of HCV coinfection increases to almost 90% in those who acquired HIV from IDU. Persons with coinfection with chronic hepatitis have accelerated liver fibrosis leading to cirrhosis [9, 94]. In a study of liver histology of IDU who had acquired HCV infection, those with concurrent HIV infection developed cirrhosis in a mean of 6.9 years after infection compared to 23.2 years among HCV

mono-infected persons ($p < 0.001$) [95]. Persons with coinfection also have an increased risk of death from end-stage liver disease [96–99]. They are also at higher risk for drug-induced hepatotoxicity from ART [100, 101] which may be related to altered cytochrome metabolism with progressive liver disease [102]. Other metabolic abnormalities are more common in coinfected persons including hyperglycemia, diabetes, and bacterial translocation from the small intestine to the portal system, predisposing coinfected chronic inflammation and progressive liver disease [103–105].

Hazardous alcohol use is increased in some populations with coinfection, particularly IDUs [64, 106]. Alcohol use further exacerbates the effect of coinfection on liver disease. Alcohol use of >50 g/day is associated with increased HCV replication [107, 108] and progressive liver fibrosis assessed by serum markers [109], transient elastometry [110] or by liver biopsy [9, 111–113]. Death from end-stage liver disease is also more common in coinfected persons who use alcohol [29, 30, 114, 115]. The incidence of, as well as deaths related to, hepatocellular carcinoma is also increased in those with coinfection who drink alcohol [30, 116]. Only one study did not find an association of alcohol use and an HCV-related severe event (including decompensated cirrhosis, hepatocellular carcinoma, or death) [117], but in this cohort, only 10% consumed >30 g of alcohol daily.

Alcohol use also contributes to metabolic abnormalities in coinfected persons. It is associated with higher rates of liver steatosis [110] and drug-induced liver disease [25, 118]. The association of alcohol use with hepatocellular carcinoma is also discussed in Chap. 32.

The adverse effects of alcohol in coinfection argue strongly for intervention. Hazardous alcohol use is a common reason for coinfected persons not receiving treatment for HCV infection, where treatment rates may be as low as 7% [106, 119–122]. Fortunately, alcohol use seems to decrease with interventions after HCV diagnosis in some populations [123, 124]. Treatment of chronic viral hepatitis whether due to HCV or HBV infection slows the progression of liver fibrosis [125, 126] and reduces the incidence of drug-induced liver disease [127]. Treatment outcomes with pegylated interferon and ribavirin [128, 129] and with the new protease inhibitors for HCV infection should continue to improve as more coinfected persons are being enrolled in treatment [130].

Cardiovascular Disease

Persons with HIV infection have an increased risk of cardiovascular disease, particularly accelerated atherosclerosis and myocardial infarction [131–133]. Cardiovascular disease is likely due to a combination of additional risk factors found in HIV infection [26] including (1) chronic inflammation from HIV viral replication and subsequent immunodeficiency [134], (2) the effect of chronic inflammation on serum lipid levels [133], (3) the metabolic effects of certain classes of antiretroviral medications [131, 133], (4) increased prevalence of insulin resistance [135], and (5) increased translocation of bacteria across the small intestine into the bloodstream as a result of immunodeficiency [136]. Persons with HIV infection have been shown to have more rapid progression of atherosclerosis measured by intermediate markers such as carotid intima–media thickness, and this has correlated with mortality [134, 137, 138].

Alcohol use further increases the risk of cardiovascular disease in HIV infection. Freiberg et al., studying the VACS Cohort, found that the risk of cardiovascular disease was increased (OR 1.55, 95% CI 1.07–2.23) in HIV-infected men with alcohol abuse or dependence, when controlled for cardiac risk factors, ART use, and CD4 count [8]. Furthermore, HCV infection may have an independent effect in increasing the risk of cardiovascular disease (OR 4.7, 95% CI 1.7–12.7) although alcohol use does not seem to affect this relationship [139]. Chapters 24 and 25 explore further the relationship of alcohol and cardiovascular disease.

Pulmonary Disease

Alcohol and HIV infection are both risk factors for pulmonary diseases. Alcoholics have increased prevalence of oropharyngeal colonization by pathogenic bacteria and an increased risk of aspiration [140]. In addition, they have impaired pulmonary immune function leading to a higher incidence of pneumonia [27, 140]. Studies have shown that alcohol use is a risk factor for the development of pneumonia in the absence of HIV, as well as more severe, multilobar pneumonia and more virulent pathogens including *Candida*, gram-negative bacteria, and *Staphylococcus aureus* infections. This, in turn, leads to longer hospitalizations and increased mortality related to alcohol use [141]. The risk for adult respiratory distress syndrome (ARDS), which has a mortality of 40–60% [142], is increased three- to fourfold in those with heavy alcohol intake [143, 144].

Similarly, persons with HIV infection are at an increased risk of community-acquired pneumonias, including unusual pathogens such as *Pseudomonas aeruginosa [*145]. HIV infection is also associated with pulmonary opportunistic infections, such as *Pneumocystis [*146]. While both alcohol use and HIV infection have an increased risk of pneumonia and tuberculosis, there are no studies to date that demonstrate the interaction of these risk factors for acute pulmonary disease [27]. There is suggestive literature that depletions in zinc levels or pulmonary glutathione stores may mediate impaired host defense [27].

Chronic lung disease in alcoholics is largely related to associated tobacco use [27]. However, persons with HIV infection have an increased risk of emphysema, lung cancer, and pulmonary hypertension, independent of smoking, and this is particularly evident in those with poorly controlled HIV infection [147].

Intervention Studies on Alcohol in HIV

The adverse effects of alcohol use on HIV are evident, and interventions to mitigate alcohol use among HIV-infected individuals are needed. To date, clinical studies and a few randomized controlled trials (RCTs) assessing the effectiveness of interventions have shown mixed results. In this section, we will briefly review the types of interventions that have been evaluated and discuss results from a few published trials. Interested readers can refer to recent review articles for more complete reviews of the literature [13, 148, 149].

Many types of alcohol interventions have been tested among hazardous alcohol users with and without HIV infection. These include brief interventions as well as more intensive behavioral, social network, and medication interventions. Brief interventions, also referred to as brief motivational interviews, are typically a single session discussing the patients' alcohol use. Studies employing this type of intervention often involve exploration of the pros and cons of a patient's alcohol use, self-assessment of the patient's alcohol consumption severity, and a more formal assessment of the patient's alcohol consumption as compared to the general population [150]. More extensive behavioral interventions have also been investigated, including cognitive-behavioral therapy, motivation enhancement, or 12-step programs. Each of these behavioral interventions is directly aimed at investigating personal motivation behind alcohol consumption and developing personal behavior modification strategies [151]. These interventions typically require multiple sessions. In addition to individualized plans and programs for those with increased alcohol consumption, social network and structural interventions which target larger populations and communities have also been evaluated. Social network interventions have most commonly focused on employing influential community leaders to change specific behaviors or promote health-conscious decisions. These studies, often referred to as Popular Opinion Leader (POL) or peer-based model interventions, may be particularly effective in communities that are difficult for outside researchers to impact [152]. Alternatively, structural interventions, which may

include political and legal action, may also be effective in altering individuals' behavior and environment. Lastly, medications, such as disulfiram, naltrexone, and acamprosate, have been shown to decrease alcohol consumption via physiologic effects, including decreasing cravings or causing adverse reactions when alcohol is consumed [149].

In addition to the type of alcohol intervention, there are several other factors to consider when evaluating results from clinical trials of alcohol interventions. The first is the setting in which the interventions are conducted. Interventions have been conducted in various settings including primary care clinics [153, 154], hospital inpatient settings [155], emergent care settings [156], and social settings or drinking venues (places where alcohol is served) [13]. A second important factor to consider is the population being targeted, which may vary depending on severity of alcohol use (dependent vs. nondependent drinkers), geographic region, and cultural practices around drinking. A third factor to consider is the outcome that is being targeted. For example, previous trials have examined the effects of alcohol interventions on decreasing alcohol consumption, improving adherence to antiretroviral medication and/or reducing sexual risk behaviors. The combination of the type of intervention, the setting in which the intervention is implemented, the population that is being targeted, and the expected outcomes of the trial will all contribute to the success or failure of an intervention.

The published literature on RCTs of alcohol interventions among populations affected by HIV reflects the various combinations of factors described above. For example, one study targeting MSM in the USA with alcohol use disorders combined two types of interventions (motivational interviewing and peer-group education/support strategies) and examined the effects on reducing at-risk drinking and sexual risk behaviors [157]. In this study, individuals receiving the combined intervention reported significantly lower number of days of drinking and number of heavy drinking days per 30-day period compared to control participants. Another study tested the effects of a brief theory-based behavioral HIV–alcohol risk-reduction intervention on sexual risk behaviors in men and women recruited from informal drinking establishments in a suburban township of Capetown, South Africa [50]. The authors reported significant reductions in unprotected intercourse, increased use of condoms, and less use of alcohol before sex in the intervention group compared to controls, with the largest impacts among lighter drinkers. These two studies illustrate the success of individual counseling interventions for reducing risk behaviors around alcohol consumption among persons at risk for or living with HIV.

Other interventions among individuals with HIV who consume alcohol have targeted the outcome of antiretroviral medication adherence. In two specific studies [151, 158], motivational interviews and cognitive-behavioral skills training were not effective in improving long-term medication adherence. Given the importance of adherence to ART to controlling HIV infection, more research is needed to develop novel interventions targeting this outcome.

Interventions directed at alcohol-serving establishments have had mixed results. Studies have focused on popular opinion leader (POL) models, in which community-defined opinion leaders are identified and trained to help shift social norms and behaviors toward safer sexual practices [152]. This type of intervention in gay bars in several US cities significantly reduced episodes of risky sexual behavior compared to control bars [152, 159, 160]; however, when this intervention was adapted for testing in several international settings, the findings were negative in that comparable reductions in risky sexual behaviors and incidence of sexually transmitted infections were seen in both intervention and control communities [161]. Another study testing the effects of a peer-based intervention on reducing episodes of unprotected sex with non-wife partners in beer halls in Zimbabwe found no difference compared to controls [162].

In summary, interventions involving varied counseling approaches directed at decreasing alcohol consumption and/or risky sexual behavior appear promising in specific settings. Other areas of investigation, such as interventions aimed at improving ART adherence among alcohol users or use of medications for alcohol dependence (such as naltrexone) in HIV-infected populations, need further research. More intervention studies will help to generalize findings across different contexts and help to improve health outcomes and minimize the effects of alcohol on persons living with HIV.

Is Alcohol Use Harmful in HIV?

In this chapter, we have examined the prevalence of hazardous alcohol use in HIV which is much higher than found in the general US population. Alcohol use and frequenting venues where alcohol is consumed has been shown to be an important risk factor for the acquisition of HIV infection. Understanding the complex interrelationships between individual characteristics and venues should improve our approach to prevention [12, 14]. The effect of alcohol on adherence to ART is well documented. There are also good laboratory models, particularly with SIV infection in macaques, to show that chronic alcohol use accelerates the progression of disease. Finally, alcohol use has deleterious effects on health, particularly related to progression of liver disease in persons with HIV/HCV coinfection.

Health-care providers may underestimate the extent of hazardous drinking among their HIV patients. A study in the VA population showed that the sensitivity for health-care providers' ability to diagnose hazardous drinking was only 22% [163]. Thus far, trials of interventions to reduce hazardous drinking in populations affected by HIV have shown mixed results. The underdiagnosis of hazardous alcohol use and lack of proven, effective treatment strategies raise the question of whether there is any "safe" level of alcohol intake in HIV. Justice et al. examined the relationship of medical illness related to alcohol use in veterans with HIV infection [164]. For diseases associated with alcohol use (HCV infection, hypertension, diabetes, chronic obstructive lung disease, and certain infections), there was a linear relationship between alcohol intake category (none, moderate, hazardous) and the disease. This suggests that there may be no "safe" level of alcohol intake for HIV-infected persons [165]. More aggressive screening and treatment of alcohol-related disorders is clearly warranted to prevent HIV transmission and to improve treatment and outcomes of persons with HIV infection [84].

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Chapter 24
Nutritional Status, Socioeconomic Factors, Alcohol, and Cataracts

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Key Points

- Diet and socioeconomic conditions and lifestyle, particularly of the habits of drinking alcoholic beverages and smoking, play an important role in etiology of cataract.
- Cataract has more prevalence in both the classes of society, and it is influenced by age, female gender, carotenoid intake, and affliction with diabetes.

Keywords Nutritional etiology of cataracts • Lifestyle • Alcoholic beverages • Interrelationships

Introduction

The etiology of cataracts is still not well understood. Apart from the influences of clinical conditions, genetic predispositions, diet, and socioeconomic conditions also play an important role in precipitation of cataract. The interrelationships between nutritional statuses, socioeconomic conditions, and lifestyle-related factors such as a habit of drinking alcoholic beverages or smoking and cataract seem to be complex. This review is based upon 68 such studies, reported during 1988–2011, representing various sociocultural backgrounds of the world. It is an attempt to understand this complexity and to evolve a model through assessment of reported studies on the nutritional and lifestyle-related etiology of cataract in variety of socioeconomic backgrounds especially for the role of alcohol in its aggravation.

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Significance of Cataracts and Associated Disorders

Cataract is an eye disorder with multiple etiologies, some of which are common to other noncommunicable diseases. Prevalence of cataract need not be necessarily linked to diabetes since cataract can be present independent of diabetes. But, still, cataract has been considered as one of the secondary consequences of diabetes. Oxidative damage to the eye lens is considered to be a principal mechanism in the progress of cataract [1, 2]. Reactive oxygen species have also been suggested to be the major contributory factor in other complications of diabetes mellitus [3–7]. Presence of hyperglycemia and the duration of diabetes increase the risk of development of cataract [8]. Besides, insulin resistance, abnormal lipid profile, oxidative modification of lipoproteins, and increased blood pressure, commonly observed among diabetics, further increase the vulnerability in precipitating eye disorders mainly sugar-induced cataract [9].

There is a possibility that the combination of cataract and diabetes be more hazardous in terms of micronutrient and antioxidant status as compared to diabetes mellitus or cataract alone. To test this hypothesis, type 2 diabetic patients (D, N = 76), nondiabetic cataract patients (NDC, N = 100), diabetic cataract patients (DC, N = 53), and age sex-matched healthy controls (H, N = 90) of age between 50 and 70 years from Pune, India, were investigated [10]. Plasma TBARS and fasting glucose were significantly higher in DC patients than NDC ($p < 0.05$), D and H groups. Lens TBARS were comparable between NDC and DC (5.5 and 5.08 nm/g lens). Further, DC men showed higher value of glycosylated hemoglobin (Hb $A1_c$) than men from D group.

To further understand which type of diabetes poses higher risk of cataract, Kiatsayompoo et al. [11] studied 151 young diabetics (age at first visit < or = 35 years). They were classified as noninsulin-dependent diabetes mellitus (NIDDM) (38.4%), malnutrition-related diabetes mellitus (MRDM) (36.4%), insulin-dependent diabetes mellitus (IDDM) (9.9%), secondary diabetes mellitus (2.6%), and unclassified category (12.6%). MRDM was further classified into two groups: 22.5% were fibrocalculous pancreatic diabetes (FCPD), and 13.9% were protein-deficient pancreatic diabetes (PDPD). Farming occupation ($p = 0.001$), abdominal pain ($p = 0.005$), male sex ($p = 0.0015$), and cataracts ($p = 0.02$) were statistically more common in MRDM compared to NIDDM and IDDM taken together.

Metabolic syndrome is a combination of medical disorders that, when occur together, increase the risk of developing cardiovascular disease and diabetes. In one cross-sectional study [12] on 2,794 Malay adults from Singapore, with the age group of 40–80 years, cataract prevalence increased with higher quartiles of blood glucose, systolic BP, and other metabolic syndrome components (P trend < 0.0001). The odds ratio of having cataract became 4.73 when both high BP and diabetes were present (OR [95% CI] = 4.73 [2.16–10.34]) (Table 24.1).

Although cataracts are known to be associated with systemic diseases such as diabetes mellitus, its association with syndromes such as Cohen syndrome, Degos disease, and Dubowitz syndrome, and neurologic disorders such as Wilson disease has also been reported [14].

Further, presence of cataract in diseases such as cystic fibrosis, atopic dermatitis, Alzheimer's disease, and mitochondrial cytopathy has been found. The basic science research has supported the

Table 24.1 The IDF consensus worldwide definition of the metabolic syndrome considers presence of any two of the following

Raised triglycerides: above 150 mg/dL (1.7 mmol/L)
Reduced HDL cholesterol: below 40 mg/dL (1.03 mmol/L) in males Below 50 mg/dL (1.29 mmol/L) in females
Raised blood pressure: systolic BP > 130 or diastolic BP > 85 mmHg
Raised fasting plasma glucose: (FPG) > 100 mg/dL (5.6 mmol/L)

Based on data from Ref. [13]

clinical hypotheses about the role of estrogens and protein condensation in cataract. Although oxidative stress continues to be the leading proposed mechanism of cataractogenesis, genetic mechanisms are gaining increasing popularity [15].

C-reactive protein (CRP) is a known marker of systemic inflammation. To examine whether systemic inflammation is associated with cataract, Schaumberg et al. [16] analyzed plasma CRP levels in baseline blood specimens from 543 men who later developed cardiovascular disease and 543 who did not. Baseline CRP was significantly higher among men who later developed cataract than levels among those who remained free of cataract, $P = 0.02$ (median 1.53 vs. 1.23 mg/L).

A large sample study named as Salisbury Eye Evaluation Project was carried out on cohort of 2,520 persons, aged 65–84 years, for the 2-year risk of death associated with different types of lens opacities and also to assess if lens opacity can be a marker for health status [17]. Nuclear opacity, particularly severe nuclear opacity, and mixed opacities including nuclear opacity were found to be significant predictors of mortality independent of body mass index, comorbid conditions, smoking, age, race, and sex (mixed nuclear: odds ratio, 2.23; 95% confidence interval, 1.26–3.95).

Nutritional Status and Cataracts

BMI and WHR

Cataract being considered as a serious health problem worldwide, its linkages with nutritional status and lifestyle have been of interest to researchers and clinicians. Body mass index (BMI) (calculated as weight in kilograms divided by the square of height in meters) is a measure of nutritional status, and values of BMI above 30 are considered as indicator of obesity, a long-term consequence of overnutrition. Since obesity has now been linked with a number of noncommunicable diseases, a measurement of simple index like BMI has gained importance. It is known to differ among the various diseases, potentially due to etiologic causes, which can lead to bias in estimating the effects of other risk factors. The relationship between BMI and disease must be identified to control for this potential bias in epidemiological investigations.

To investigate the association between BMI and cataract in a metropolitan Asian elderly population, a total of 2,045 subjects aged 65 years in Shihpai, Taipei, participated and 1,361(66.6%) completed the survey. Of the subjects, 806 were diagnosed as having age-related cataracts. With a BMI of less than 21.3 as a reference point (odds ratio [OR], 1.00), a U-shaped relationship between BMI and nuclear opacity was demonstrated. A reverse U-shaped relationship was shown for cortical opacity. Thus, results were indicative of the fact that BMI is an independent risk factor for nuclear and cortical opacities but in reverse direction to each other [18].

The data from a large hospital-based case-control study was used to analyze the difference in BMI by diagnosis, separately in males (n = 20,011) and females (n = 9,083) admitted to the hospital between 1977 and 1992 [19]. Although some associations between BMI and disease differed between the sexes, in general, fractures and diseases of the respiratory tract were associated with the lowest BMI and arthritis, cataract/glaucoma, and endometrial cancer with the highest BMI.

Debra et al. [20] examined associations of anthropometric measurements like height, waist-to-hip ratio (WHR), and BMI, with cataract in a prospective 14 year follow-up study comprising of 20,271 participants. The proportional hazards regression models adjusted for many known or suspected risk factors of cataract used in the study revealed that BMI, height, and abdominal adiposity were independent risk factors for cataract and suggest that prevention of obesity and beneficial lifestyle changes resulting in weight loss and reduction of central obesity would lessen the incidence and costs of cataract.

Dietary Habits

Age-related cataract is a major health problem in aged individuals. Although there are studies of diet and cataract risk with focus on specific nutrients or healthy eating indices, studies on special dietary groups such as vegetarians are scanty. Appleby et al. [21] have used Cox proportional hazards regression model on data of dietary and lifestyle characteristics of 27,670 self-reported nondiabetic participants aged ≥40 years. There was a strong relation between cataract risk and diet group, with a progressive decrease in risk of cataract in high meat eaters to low meat eaters, fish eaters (participants who ate fish but not meat), vegetarians, and vegans. Associations between cataract risk and intakes of selected nutrients and foods generally reflected the strong association with diet group.

The study by Ojofeitimi et al. [22] was conducted on 62 subjects with 31 cataract patients and 31 controls. A structured questionnaire was used to collect information on smoking and alcohol consumption and dietary habits. The percentage of individuals with adequate intakes of fruits and vegetables was higher for controls than patients. Vitamin supplement usage was also higher in controls than patients.

The relationship between cataract and diet was studied in a case-control study conducted in northern Italy on 207 cataract patients and 706 controls [23]. Alcohol, coffee, decaffeinated coffee, tea, and cola intakes were not associated with cataract extraction. Among food items, reduced ORs for cataract extraction (highest tertile of intake compared to the lowest), with a significant inverse trend in risk, were found for intake of meat, cheese, cruciferae, spinach, tomatoes, peppers, citrus fruit, and melon. A significant increase in risk was found for the highest intake of butter, total fat, and salt. Among micronutrients, lower ORs for cataract extraction were found for intake of calcium, folic acid, and vitamin E, while estimated intakes of methionine, retinol, beta-carotene, and vitamins A, C, and D were not associated.

Intake of Micronutrients

To examine the effect of alpha-tocopherol (50 mg per day) and beta-carotene (20 mg per day) supplementation on the incidence of age-related cataract extraction, a randomized double blind, placebo-controlled, 2 × 2 factorial trial was conducted in south western Finland on population of 28,934 male smokers with 50–69 years of age at the start [24]. Follow-up continued for 5–8 years (median 5.7 years) with a total of 159,199 person-years. Neither alpha-tocopherol (relative risk, RR, 0.91, 95% confidence intervals, CI, 0.74, 1.11) nor beta-carotene (RR 0.97, 95% CI 0.79, 1.19) supplementation affected the incidence of cataract surgery.

Oxidation of lens proteins plays a central role in the formation of age-related cataracts, suggesting that dietary antioxidants may play a role in prevention. However, the relation between specific antioxidants and risk of cataract remains uncertain. In a prospective cohort of registered female nurses aged 45–71 years (N = 761762 person-years of follow-up), after controlling age, smoking, and other potential cataract risk factors, those with the highest intake of lutein and zeaxanthin had a 22% decreased risk of cataract extraction compared with those in the lowest quintile (relative risk: 0.78; 95% CI: 0.63, 0.95; *P* for trend = 0.04) [25].

Dietary carotenoids act as antioxidants and considered to reduce the risk of cataracts possibly by preventing oxidative stress within the lens. In a prospective study [26], US male health professionals (n = 36,644, 45–75 years of age) were included for a detailed dietary questionnaire to assess intake of carotenoids and other nutrients. During 8 years of follow-up, 840 cases of senile cataract extraction were documented. A modestly lower risk of cataract extraction was observed only with higher intakes of lutein and zeaxanthin but no other carotenoids. Among specific foods high in carotenoids, broccoli

and spinach were most consistently associated with a lower risk of cataract. Other studies have also suggested an inverse relationship between dietary or serum lutein and risk for age-related macular degeneration and cataracts [27].

Our study on blood levels of micronutrients as well as oxidative stress estimated previously on 140 cataract patients and 100 controls indicated that subnormal status of micronutrients coupled with higher oxidative stress directly influenced the solubility of lens proteins, which in turn affected the lens opacity [28]. Intakes of micronutrients in these subjects based on food frequency questionnaire were also estimated during one of our studies [29].

In a separate study, data collection on type 2 diabetic patients (D = 76) was undertaken and compared with nondiabetic cataract patients (NDC = 100), diabetic cataract patients (DC = 53), and age sex-matched healthy controls (H = 90) of 50–70 years aged Indians. Subnormal status of ascorbic acid, beta-carotene, thiamine, and ceruloplasmin was elicited for all the four study groups. Prevalence of poor riboflavin status was 30–36% among all patients and 15–22.5% among controls. Synergism of diabetes and cataract coupled with gender bias and influence of socioeconomic factors seems to worsen the health status and lens opacity, especially in the DC group [10].

To investigate the association of antioxidant vitamins (vitamin C, vitamin E, vitamin A, beta-carotene, alpha-carotene, beta-cryptoxanthin, lycopene, zeaxanthin, and lutein) and minerals (zinc and selenium) and risk of cataract in a Mediterranean population, a case-control study was conducted. Data on their diet using Food Frequency Questionnaire (FFQ) and other information for 343 cataract patients and 334 age/sex-matched controls aged 55–74 years were collected from an ophthalmic outreach clinic in Valencia, Spain. Blood levels of vitamin C above 49 micromol/L were associated with a 64% reduced odds for cataract ($P < 0.0001$). Dietary intake of vitamins C and E and selenium were marginally associated with decreased odds ($P = 0.09$, $P = 0.09$, $P = 0.07$, respectively), whereas moderately high levels of blood lycopene (>0.30 micromol/L) were associated with a 46% increased odds of cataract ($P = 0.04$). The results supported a protective role of vitamin C on the aging lens [30].

Socioeconomic Risk Factors for Development of Cataract

Among the socioeconomic factors, the main risk factors for cortical cataract development include female gender and sunlight exposure. Other possible risk factors for nuclear cataract include tobacco chewing, cigarette smoking, and alcoholism. Cumulative effect of other environmental factors such as X-ray irradiation, steroids, drugs, toxins, and metals might also trigger cataractogenesis. [31]

Female Gender

Several epidemiological cross-sectional data have shown an increased prevalence of cataract in women compared with men. The female gender is generally associated with increased age-adjusted risk of cataract. The cause of the gender differences in cataract occurrence, though questionable, could partly be attributed to hormonal differences between women and men. Postmenopausal estrogen deficiency could also be another possible reason [32]. The Blue Mountains Eye Study examined 2,072 women, aged 49 years or older, during 1992–1994, of whom 1,343 (74.0% of survivors) were reexamined after 5 years. Information on reproductive factors and use of hormone replacement therapy was collected using an interview method. It was observed that women who had ever used hormone replacement therapy had a decreased incidence of cortical cataract (odds ratio = 0.7, 95% confidence interval: 0.4, 1.0). Older age at menarche was associated with an increased incidence of cataract surgery (odds

ratio = 2.6, 95% confidence interval: 1.2, 5.7) and a significant trend for increasing incidence of nuclear cataract ($p = 0.04$). There was also a significant trend for decreasing incidence of cataract surgery with increasing duration of reproductive years ($p = 0.009$). These epidemiologic data provided some evidence that estrogen may play a protective role in reducing the incidence of age-related cataract and cataract surgery [33].

In another large population-based Australian Blue Mountain Study involving 2,072 women, it was shown that late age at menarche was associated with increased prevalence of all three types of cataract, but there were no associations with age at menopause, number of children, or use of the oral contraceptive pill. Among all women, there was no association between hormone replacement therapy (HRT) and cataract [34]. Further data from the Beaver Dam Eye Study, on women through 81 years of age, evaluated a possible association between estrogen and lens opacities. It was found that early age of menarche, current and longer duration of estrogen therapy, as well as use of the oral contraceptive pill were protective for nuclear cataract. Estrogen and HRT may play a protective role in reducing the incidence of age-related cataract and cataract surgery [35].

In another study, to determine the association between HRT and the incidence of cataract extraction, a total of 30,861 postmenopausal women, participating in the Swedish Mammography Cohort, age 49–83 years were asked to complete a self-administered questionnaire in 1997 about hormone status, HRT, and lifestyle factors. In multivariate adjusted analysis, ever use of HRT was associated with a 14% increased risk of cataract extraction (rate ratio [RR], 1.14; 95% confidence interval [CI], 1.07–1.21) compared with those who never used HRT. Current use of HRT was associated with an 18% increased risk of cataract extraction (RR, 1.18; 95% CI, 1.10–1.26). Further, for women drinking on average >1 drink of alcohol per day, current HRT users had a 42% increased risk (RR, 1.42; 95% CI, 1.11–1.80) for cataract extraction, compared with women who neither used HRT nor alcohol [36].

A cross-sectional survey was conducted by our group on 140 Indian cataract patients with age 50–70 years and 100 age- and sex-matched healthy controls from both the socioeconomic classes of the society. The results showed a strong gender bias with higher number of women developing cataracts. Within the afflicted women, the affluent group was found to be relatively less vulnerable than the low-income group owing to dietary and lifestyle patterns [27].

In a database of 16,000 entries on cataract surgeries collected over 5 year period, the prevalence of cataract extractions and its gender distribution as risk factor was analyzed. Female gender showed an increased age-adjusted rate of cataract surgical prevalence. The total prevalence for cataract surgeries for males and females separately was found to be 2.7 and 3.7/1,000 population showing a female preponderance [37].

The Beijing Eye Study, conducted on 3,251 individuals in 2006 across 5-year incidence of cataract (16.82%), was found to be significantly associated with higher age ($P < 0.001$) and female gender ($P < 0.001$) [38].

Another Chinese study on 4,439 participants found that females have a shallower anterior chamber, a narrower anterior chamber angle, and a higher prevalence of dry eye, a cause, making them vulnerable for developing cataracts [39].

Smoking

The linkage of cigarette smoking with risk of cataract is well established. Of the observational evidences, heavy smokers (15 cigarettes/day or more) have thrice the risk of cataract compared to nonsmokers. Smoking is thought to increase oxidative stress in the lens thereby increasing risk of cataract. The increase in free radicals could also be attributed to the presence of tobacco smoke that directly damage lens proteins and the fiber cell membrane in the lens. Besides, heavy metals such as lead and cadmium present in tobacco can also accumulate in the lens, causing toxic effects. Studies have

shown only a temporal relationship and a partial reversible effect when smoking is withdrawn. Many researchers have shown that intake of certain antioxidants decreases incidence of cataract. The self-reported data by the smokers remain a major limitation for such studies. However, passive smoking and cataract were not found to be associated [40].

A recent study investigated the effect of smoking cessation on cataract in US men and women. Findings suggested that any healing from damage due to cigarette smoking occurs at a very modest pace, and this emphasizes the importance of never starting to smoke or quitting early in life. Compared with current smokers, former smokers who had quit smoking 25 or more years previously had a 20% lower risk of cataract extraction. However, risk among past smokers did not decrease to the level seen among never smokers [41].

In the Beijing Eye Study conducted in 2006 in 3,251 men and women, the 5-year incidence of cataract (16.82%) was significantly associated with rural region ($P < 0.001$) and smoking ($P < 0.001$). [16]. A population-based, cross-sectional study in an urban community in the Blue Mountains surveyed 49 and 97-year-old 3,654 participants to investigate the associations between tobacco smoking and cataract. Smoking history was recorded through questionnaire. Smoking was associated with a higher prevalence of nuclear and posterior subcapsular cataracts. The association between pipe smoking and nuclear cataract (adjusted OR, 3.1; 95% CI, 1.5–8.2) was stronger than the association with cigarette smoking [42]. However, in one of the studies conducted by Phillips et al. (1996), smoking was not found to be a risk factor for cataractogenesis [43]. A case-control study of cataract in Oxfordshire explored the risks and benefits associated with a variety of drugs. Steroids coupled with heavy smoking and beer drinking were associated with a raised risk [44].

The Singapore Malay Eye Study investigated 2,927 participants with gradable lens photographs, of which 1,338 had cataract. After adjusting for age, sex, body mass index, hypertension, and diabetes, current smokers had a higher prevalence of nuclear cataract (odds ratio [OR], 2.06), cortical cataract (OR, 1.33), posterior subcapsular cataract (OR, 1.39), or any cataract (OR, 1.48). These associations were not seen in the Blue Mountains Eye Study. Among men, 43.5% currently smoked compared with only 3.2% of women. The population attributed risk of nuclear cataract due to smoking was estimated to be 17.6% in men. Smoking was associated with cataract in Malay persons, with one in six nuclear cataract cases in men attributable to smoking. Smoking–cataract associations were stronger in Malay than in white persons [45].

A population-based longitudinal epidemiologic study conducted on 4,926 Beaver dam residents investigated the association of socioeconomic and lifestyle factors with incidence of age-related cataracts. After adjustment for age and sex, smoking was found to be directly related to the 10-year cumulative incidence of nuclear cataract. It was also seen that history of multivitamin did not alter the relationships of smoking to the incidence of cataracts [46].

A cross-sectional survey was conducted by our group on 140 Indian cataract patients with age 50–70 years and 100 age- and sex-matched healthy controls from both the socioeconomic classes of the society. Eighty percent of the rural patients were addicted to tobacco. Significant differences were also noted between urban smokers and urban nonsmokers for their plasma antioxidant status and soluble to total proteins ratio of lens [28].

In a population-based cross-sectional epidemiologic study conducted in Andhra Pradesh, India, a total of 7,416 subjects were interviewed, and each underwent a detailed dilated ocular evaluation by trained professionals. Increasing age was significantly associated with all cataract types and history of prior cataract surgery and/or total cataract. Consistent with other studies, tobacco smoking was strongly associated with a higher prevalence of nuclear and cortical cataracts and history of prior cataract surgery in this population. A significantly higher prevalence of nuclear, cortical cataract and history of prior cataract surgery and/or total cataract were found among cigarette smokers. A dose–response relationship was seen with respect to cigarette and cigar smoking. After adjustment, compared with never smokers, cigarette smokers who smoked heavily (>14 packs) had a significantly higher prevalence of nuclear cataract (OR = 1.65; 95% CI: 1.10–2.59) and cortical cataract (OR = 2.11; 95%

CI: 1.38–3.24). Nuclear cataract was significantly higher in cigar smokers (adjusted OR = 1.55; 95% CI: 1.16–2.01) and in cigar smokers who smoked heavily (>21 person-years of smoking; OR = 1.50; 95% CI: 1.10–1.95), compared with never smokers [47].

A hospital-based case-control study, conducted on the Nepal–India border, surveyed 206 women patients, aged 35–75 years, with confirmed cataracts and 203 controls for use of cooking fuel. A standardized questionnaire was administered to all participants. Logistic regression analysis involved adjustment for age, literacy, residential area, ventilation, type of lighting, incense use, and working outside. Compared with using a clean-burning-fuel stove (biogas, LPG, or kerosene), the adjusted odds ratio (OR) for using a fueled solid-fuel stove was 1.23 [95% confidence interval (CI) 0.44–3.42], whereas use of an unfueled solid-fuel stove had an OR of 1.90 (95% CI 1.00–3.61). Lack of kitchen ventilation was an independent risk factor for cataract (OR 1.96; 95% CI 1.25–3.07). This study provided confirmatory evidence that use of solid fuel in unfueled indoor stoves is associated with increased risk of cataract in women who do the cooking [48].

Sunlight Exposure

Too much unfiltered sunlight can harm our eyes by damaging the lens and even the retina. There are reports that UV-B rays of sunlight increase the oxidative damage to lens and induce cataractogenesis. Aging eyes are more susceptible to UV damage, since the levels of free UV filters acting as photosensitizers decrease with age [31].

Findings by our group based on data of 140 senile cataract patients and 100 healthy controls revealed higher sunlight exposure in the previous years as a major risk factor for cataract. Blood antioxidant vitamin levels and parameters of oxidative stress were also analyzed. Multiple regression analysis of lens opacity and solubility of lens proteins indicated the influence of sunlight exposure for predisposition of cataract [28].

A frequency-matched case-control study of 343 cases and 334 controls was conducted at a primary health-care center in a small town near Valencia, Spain. All cases had cataract in at least one eye based on the LOCS II while controls had no opacities in either eye. Blood antioxidant vitamin levels were also analyzed. Logistic regression models and exploratory analyses suggested a positive association between years of outdoor exposure at younger ages and risk of affliction with nuclear cataract later in life [49].

The relationships between exposure to sunlight and to the UVB component of light and the prevalence of lens opacities were examined in the Beaver Dam Eye Study. After adjusting for other risk factors, men who had higher levels of average annual ambient UVB light were 1.36 times more likely to have more severe cortical opacities than men with lower levels. However, UVB exposure was not found to be associated with nuclear sclerosis or posterior subcapsular opacities in men. Moreover, no associations with UVB exposure were found for women, who were less likely to be exposed [50]. Finally, a review of 25 different studies by WHO has indicated association of UVB exposure with cataract prevalence [51].

Age

Age-induced protein modifications, oxidation, conformational changes, aggregation, and decrease in chaperon activity together could pose a higher risk of cataractogenesis among the elderly. The cumulative increase in risk factor increase in age is believed to be the highest risk factor for cataract [52].

The protection imparted by antioxidants declines with increase in age. Simultaneously, usage of steroids increases the risk of developing cataracts in older people [53].

Lifestyle and Education

Usually, higher level of education is associated with lower risk of cataract due to awareness about causative factors. Uneducated or people with little education are ignorant about the hazardous effects of tobacco usage, smoking, alcoholism, sunlight, and also nutritious diet. This makes them more vulnerable to cataractogenesis. Findings by our group based on data of 140 senile cataract patients and 100 healthy controls revealed that in affluent patients, there was a delay in the onset of cataracts by almost 10 years as compared to rural patients who had a more compromised lifestyle with very little or no education [27].

The Singapore Malay Eye Study [54] conducted survey on 1,338 cataract patients. After adjusting for age, sex, body mass index, hypertension, and diabetes, current smokers had a higher prevalence of nuclear cataract (odds ratio [OR], 2.06; 95% confidence interval [CI], 1.46–2.98), cortical cataract (OR, 1.33; 95% CI, 1.02–1.74), posterior subcapsular cataract (OR, 1.39; 95% CI, 1.02–1.91), or any cataract (OR, 1.48; 95% CI, 1.10–1.99). These associations were not seen in the Blue Mountains Eye Study. Primary or lower education (OR, 1.67; 95% CI, 1.06–2.64) and low monthly income (OR, 1.43; 95% CI, 1.09–1.87) were both associated with nuclear cataract, while small-sized public housing was associated with posterior subcapsular cataract (OR, 1.70; 95% CI, 1.28–2.25) [52]. The Beijing Eye Study on 3,251 subjects indicated significant association between the incidence of nuclear cataract and rural region ($P < 0.001$) [55].

To investigate the association of socioeconomic and lifestyle factors with incidence of age-related cataracts, income, education, occupation, smoking, alcohol, caffeine, and multivitamin use were considered. After adjustment for age and sex, income (or education) was inversely related to the 10-year cumulative incidence of nuclear cataract. None of the factors were significantly associated with incident cortical or posterior subcapsular cataract. Incident nuclear cataract was associated with income and smoking 10 years earlier. There was no significant lifestyle exposures associated with incident cortical and posterior subcapsular cataract [56]. Findings by our group based on data of 140 senile cataract patients and 100 healthy controls revealed that all the affluent patients were literate. On the contrary, 80% rural patients were uneducated and had various addictions [28].

Data on the level of education available for 3,221 subjects, in a population-based Beijing Eye Study, had 1,484 subjects living in the rural region with an age range of 45–89 years. The participants underwent an interview including questions concerning their educational level and a detailed ophthalmic examination. In a multivariate analysis, a higher level of education was significantly associated with myopic refractive error, higher best-corrected visual acuity, lower degree of nuclear cataract, and lower prevalence of angle-closure glaucoma [57].

Alcohol Intake and Cataract

Alcohol consumption being an important lifestyle factor, its association with eye diseases need to be separately investigated. Many epidemiologic studies have assessed the relationship between alcohol drinking and cataract. Chronic alcoholism has also been linked to increased risk of cataract by many researchers. Consumption of hard liquor and wine is associated with nuclear opacities [58–60]. However, the findings on the association between cataract and alcohol consumption are inconsistent. Several prospective cohort studies have not found this association [61].

In a follow-up study of surgical cases of posterior subcapsular cataracts, 238 cases and controls were interviewed. Current alcohol intake and usual and maximum weekly consumption were assessed. Fifty-seven percent of the cases and 56% of the controls were nondrinkers, 22% of the cases and 36% of the controls had an average of seven or fewer drinks per week, and 17% of the cases and 8% of the controls had more than seven drinks per week. A matched pair analysis controlling for other known risk factors showed an increased risk associated with heavy alcohol use. Heavy drinkers were more likely to be cases than were nondrinkers (odds ratio, 4.6; $P<.05$), and light drinkers were not at an increased risk. Results suggested that heavy alcohol consumption may increase the risk of posterior subcapsular cataract [62].

In a population-based prospective cohort study of 3,654 persons aged 49+ years, an interviewer-administered questionnaire was used to collect information on alcohol consumption. It was seen that long-term risk of nuclear, cortical, and posterior subcapsular cataract was not associated with alcohol consumption. However, after adjusting for age, gender, smoking, diabetes, myopia, socioeconomic status, and steroid use, total alcohol consumption of over 2 standard drinks per day was associated with a significantly increased likelihood of cataract surgery. A U-shaped association of alcohol consumption with the long-term risk of cataract surgery was found in this older cohort. Moderate consumption was associated with 50% lower cataract surgery incidence, compared either to abstinence or heavy alcohol consumption [63].

In another population-based, prospective cohort on 30,861 postmenopausal women of which 4,324 were cataract patients, a self-administered questionnaire about hormone status, HRT, and lifestyle factors was completed. It was seen that among women drinking on average >1 drink of alcohol per day, current HRT users had a 42% increased risk (RR, 1.42; 95% CI, 1.11–1.80) for cataract extraction, compared with women who neither used HRT nor alcohol. Results indicated that postmenopausal women using HRT for a long period of time may be at an increased risk for cataract extraction, especially those drinking >1 alcoholic drink daily [36].

In the Beijing Eye Study, 4,439 subjects (age 40+ years) gave information on alcohol consumption of whom 549 (13.3%) consumed either beer or wine. In multivariate analysis, alcohol consumption was significantly associated with the systemic parameters of lower age ($P=0.001$), male gender ($P<0.001$), rural region ($P<0.001$), lower level of education ($P=0.01$), and smoking ($P<0.001$). Alcohol consumption was not a significant risk factor for the prevalence of age-related macular degeneration ($P=0.24$), dry eye ($P=0.86$), cortical cataract ($P=0.67$), subcapsular posterior cataract ($P=0.62$), or nuclear cataract ($P=0.76$). When adjusted for the systemic parameters of age, gender, rural/urban region, level of education, and smoking, self-reported moderate consumption of alcohol did not have a significant effect on the prevalence of major ocular diseases [64].

The study by Ojofeitimi et al. [20] was conducted on 62 subjects with 31 cataracts patients and 31 controls. A structured questionnaire was used to collect information on smoking and alcohol consumption habits. There was a strong negative association between past history of smoking, alcohol consumption, and cataract.

Findings by our group based on data of 140 senile cataract patients and 100 healthy controls revealed that among men, 67% of rural patients and 40% of urban patients were alcoholic. Further, significant differences were noted between alcoholics and nonalcoholics, for their plasma levels of oxidative stress and total antioxidant status as well as soluble to total proteins ratio of lens ($P<0.01$) [65].

The relationship between cataract and diet was studied in a case-control study conducted in northern Italy on 207 cataract patients and 706 controls [21]. Alcohol, coffee, decaffeinated coffee, tea, and cola intakes were not associated with cataract extraction.

The data from a large hospital-based case-control study was used to analyze the difference in BMI by diagnosis, separately in males (n = 20,011) and females (n = 9,083) admitted to the hospital between 1977 and 1992 [18]. Potential disease risk factors, including alcohol use, smoking, and education, showed a strongly negative age and a strongly positive association with BMI in females, but little or no association was found between BMI and these factors in males.

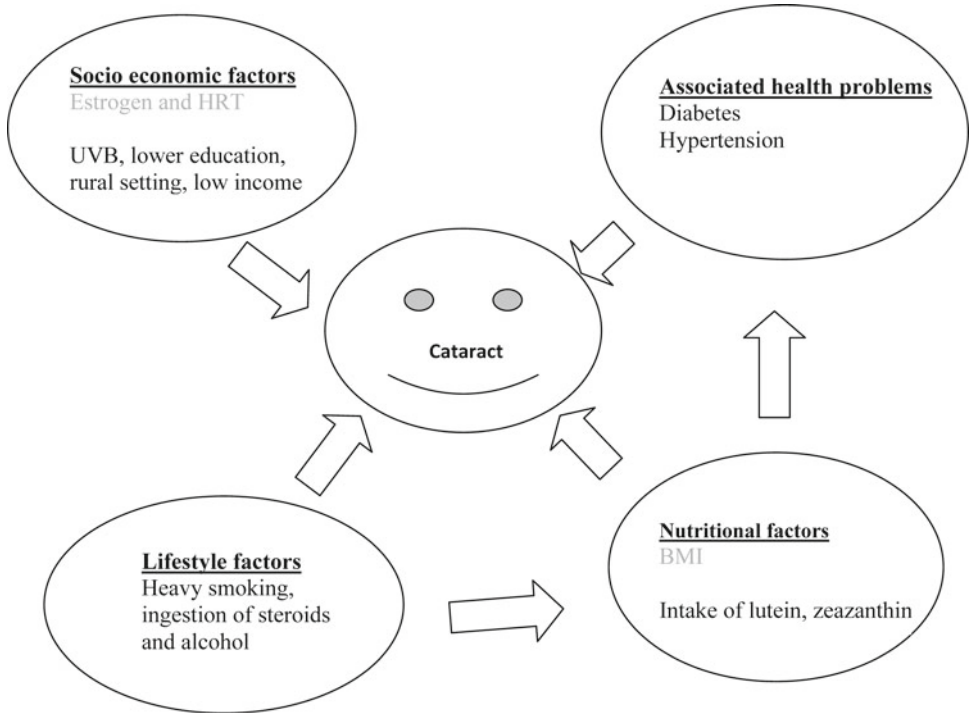

Fig. 24.1 Interrelationship between various factors influencing cataract (blue protective and red aggravating factors)

In a study by Appleby et al. [20], database of dietary and lifestyle characteristics of 27,670 self-reported nondiabetic participants aged ≥40 years was analyzed by Cox proportional hazards regression method. Results indicated that alcohol intake, BMI, physical activity, education, socioeconomic status, and dietary supplement use were not associated with cataract risk.

Cataract can be divided into three subtypes which are cortical, nuclear, and posterior subcapsular and also mixed type. The review of literature from the last decade thus brings out the fact that cataract has more prevalence in both the classes of society, influenced by age, female gender, carotenoid intake, affliction with diabetes, and lifestyle, particularly of the habits of consuming alcoholic beverages and smoking. As shown in Fig. 24.1, the risk of cataract increases with higher BMI and a compromised plasma status of antioxidant micronutrients. Since these factors are modifiable to some extent by implementing changes in lifestyle, the observations may prove important to help retard cataractogenesis to some extent.

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Chapter 25
Alcohol Intake and High Blood Pressure

Amy Z. Fan and Yueren Zhou

Key Points

- Cross-sectional, longitudinal, and interventional trial data provided relatively consistent support that excessive consumption of alcohol increases both the level of blood pressure and the subsequent incidence of hypertension.
- Proposed mechanisms include nitric oxide depletion, activation of the sympathetic nervous system, insulin resistance, HPA stimulation with increase in serum cortisol level, altered calcium-magnesium balance, and changes in the renin-angiotensin-aldosterone system.
- Preventive counseling for alcohol use should be integrated into primary care. The public should be aware of the hypertension risk associated with excessive alcohol consumption.

Keywords Blood pressure • Ethanol • Drinking pattern • Lifestyles • Mechanism • Prevention

Introduction

Hypertension remains an important public health issue. According to the National Center for Health Statistics [1], roughly one out of three US adults has high blood pressure, a primary or contributing cause of 326,000 deaths in America in 2006 [2]. Alcohol intake has long been known to be associated directly with high blood pressure, probably as early as 1915 [3, 4]. This association has been identified across gender, age, and racial and ethnic groups. J-shaped linear or threshold associations between alcohol consumption and high blood pressure have been reported. Mechanisms on how alcohol might alter blood pressure have been proposed. This chapter will attempt to summarize some of these key issues of relevance.

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Epidemiological Evidence

While there is no doubt that heavy alcohol intake is closely associated with increased risk of hypertension for both men and women, the effect of light-to-moderate alcohol usage on incident hypertension is controversial and the effects appear to be different in men and women. Sesso et al. [5] reported that light-to-moderate alcohol consumption decreased hypertension risk in women but increased risk of hypertension in men (defined as new physician diagnosis, antihypertensive treatment, reported systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg). The threshold above which alcohol became deleterious (for hypertension risk) emerged at ≥ 4 drinks per day in women versus a moderate level of ≥ 1 drink per day in men. This cohort study followed 28,848 women from the Women's Health Study for 10.9 years and 13,455 men from the Physicians' Health Study for 21.8 years. All participants were free of baseline hypertension, cardiovascular disease, and cancer at study entry. A similar J-shaped association in women and linear relationship in men was observed in an earlier cross-sectional study [6] where 45,448 women and 38,499 men were involved. However, another cross-sectional study was conducted where 2,301 women and 2,482 men [7] showed systolic and diastolic BP in both men and women to be positively and significantly related to alcohol consumption independent of the potential confounders including age, obesity, cigarette smoking, regular exercise, education, and gonadal hormone use in women. The regression analysis indicated that an average of 30 ml of alcohol per day would produce a 2–6 mmHg increase in systolic BP. In a population-based study in Spain [8], a total of 2,383 Spanish men and 2,535 Spanish women were examined in two cross-sectional surveys that took place in 1994–1995 and 1999–2000. Researchers found that total alcohol consumption, regardless of beverage type, was significantly associated with higher systolic and diastolic pressures in men but not in women. A meta-analysis [9] which included 15 randomized control trials showed that "overall, alcohol reduction was associated with a significant reduction in mean (95% confidence interval) systolic and diastolic blood pressures of −3.31 mmHg (−2.52 to −4.10 mmHg) and −2.04 mmHg (−1.49 to −2.58 mmHg), respectively." The results of epidemiologic studies suggest that up to 33% of high blood pressure in men and up to 8% of high blood pressure in women is attributable to alcohol consumption [10].

Mechanism of Alcohol-Related Hypertension

Genetic Influence

The mechanism of how alcohol intake might increase blood pressure has not been well established. Some links between alcohol intake and genetics have been made. Aldehyde dehydrogenase 2 (ALDH2) encodes a major enzyme involved in alcohol metabolism. Chen et al. found that ALDH2 *2*2 homozygotes experience adverse symptoms when drinking alcohol and drink considerably less alcohol than wild-type *1*1 homozygotes or heterozygotes. Consequently, 2*2 homozygotes had lower risk of hypertension and lower levels of blood pressure. It is concluded that this polymorphism influences the risk of hypertension by affecting individual's alcohol drinking behavior.

Biochemical Mechanism

Proposed mechanisms [11] include nitric oxide depletion, activation of the sympathetic nervous system, insulin resistance, HPA stimulation with increase in serum cortisol level, altered calcium-magnesium balance, and changes in the renin-angiotensin-aldosterone system. Nitric oxide is a

known potent vasodilator. Previous studies [12, 13] suggested that blood pressure elevation might be related to the reduction of NO production from endothelial cells after chronic high-dose alcohol consumption. In an animal study conducted by Husain et al. [14], significant blood pressure elevation was observed in rats treated with high-dose alcohol for 12 weeks. Meanwhile, plasma nitric oxide (NO) levels in those rats were found to be depleted significantly after weeks of alcohol treatment, suggesting an endothelial injury. Some reports [15, 16] suggested alcohol can raise blood pressure by activating the sympathetic nervous system. For instance, Randin et al. [17] found that alcohol intake doubled the rate of sympathetic-nerve discharge and caused sympathoexcitatory and pressor effects that may be related to blood pressure increase. Recently, Zilkens et al. [18] proposed that endothlin-1 (ET-1), a potent vasoconstrictor, may play a role in blood pressure elevation caused by alcohol. Additionally, animal data demonstrated a positive relationship between alcohol intake and the level of ET-1. It has also been suggested that polyphenols that are present in wine may inhibit the synthesis of ET-1 and therefore explain the reduction in blood pressure after wine consumption that was observed in some studies. Interestingly, an increase in endothelium-dependent NO production induced by polyphenols in wine has also been associated with a vasorelaxation effect caused by wine in animal experiments [19] but was not replicated in human subjects [18]. Central serotonergic (5-HT) neurotransmission is a new candidate for the alcohol and blood pressure association. A study led by Balldin et al. [20] showed an inverse relation between 5-HT neurotransmission and blood pressure in alcohol-dependent individuals whereas previous data suggested only an inverse relation in healthy individuals. These findings indicate a possible association between blood pressure regulation and central 5-HT neurotransmission.

Interaction with Other Cardiovascular Risk Factors for Hypertension

Other lifestyle factors can influence the susceptibility of alcohol-induced high blood pressure. Smoking and drinking often coexist in one individual [21]; smoking for daily drinkers can exacerbate blood pressure profile [22]; the effect was more pronounced in men than in women. Dietary factors interact with alcohol intake. For example, alcohol intake is associated with high-sodium intake, and low-carbohydrate and high-protein food intake [23, 24]; sodium intake and meat-related diets are all associated with risk of hypertension [10, 25, 26]. Vigorous physical activity (PA) was positively associated with alcohol use in individuals under 50 years of age but not in individuals over 50 years of age [27]. This concurrence may offset the beneficial effects of physical activity. Alcohol consumption also interacts with psychological stress [28, 29]; the combined effects on blood pressure are largely unknown.

In addition, moderate drinkers usually possess better socioeconomic status than nondrinkers and heavy drinkers. That might confound the association between alcohol use and blood pressure. The confounding may favor the “beneficial” effect of alcohol consumption and may contribute to the findings in previous studies that moderate drinking is beneficial [30].

Drinking in excess of the dietary guidelines was associated with an increased risk of impaired fasting glucose/diabetes mellitus, hypertriglyceridemia, and abdominal obesity, which are all related to higher risk of hypertension [31]. Alcohol consumption is also associated with hormone change. Reichman et al. [32] demonstrated in a controlled-diet study that after three consecutive months of two daily drinks, the levels of several hormones including estrone, estriol, and estradiol increased in premenopausal women. Oral estrogen administration in postmenopausal women and oral contraceptive use in premenopausal women may induce hypertension [33]. Thus, alcohol-induced estrogen change may also contribute to alcohol-related blood pressure alteration.

Limitations of Current Epidemiologic Studies

Current studies on the relationship of alcohol consumption and blood pressure are limited to some extent. Many epidemiological studies of alcohol consumption and increased risk of high blood pressure are observational. These epidemiological studies have in general reported lower blood pressure among moderate drinkers than nondrinkers while overlooking many other factors. Nondrinkers are a heterogeneous group consisting of former drinkers, lifelong abstainers, and irregular abstainers who may have preexisting health problems. A population-based survey revealed that of the 30 cardiovascular disease (CVD)-associated factors or groups of factors that were assessed, 27 (90%) were significantly more prevalent among nondrinkers than among moderate drinkers. It is concluded that some or all of the "protective" effects of moderate alcohol consumption on blood pressure or CVD may be attributed to residual or unmeasured confounding [34]. Therefore, if the analyses were performed with the lowest level of drinking as the reference group, the spurious J- or U-shaped relationships would disappear and the alcohol-blood pressure association would most likely become linear in both women and men.

The reports regarding beverage-specific (wine, beer, or liquor) associations with hypertension risk are inconsistent [8, 35]. It has been suggested that wine might be protective against hypertension due to its relatively high potassium content [36]. Wine is believed to contain components which confer favorable effects for counteracting the atherosclerotic process [37]. In addition, wine drinking was more favorable to women than men in terms of cardiovascular risk profile [8]. However, wine drinkers tend to have "healthier" drinking patterns and lifestyles and thus mitigate the harm of alcohol to a great extent [38]. Women tend to have "healthier" drinking patterns than men. It is difficult to discern whether the relative advantage of wine drinkers should be attributed to biological benefits or favorable drinking pattern and lifestyles.

Previous epidemiological studies usually did not distinguish drinking patterns such as binge drinking versus steady drinking, how the alcohol was consumed (with or without food, etc.), and when the blood pressure measurements were taken. A British study [39] showed that between weekend drinkers and moderate daily drinkers who consume similar amounts of alcohol per week, weekend drinkers tend to have higher daily blood pressure than moderate daily drinkers, suggesting that drinking pattern can influence the effects of alcohol on blood pressure. A small open randomized cross-over trial (n=26) conducted among centrally obese, hypertensive subjects in Brazil indicated that ingestion of 250 ml of red wine, together with the noon meal resulted in reduction of the postprandial blood pressure among these individuals [40]. The timing of blood pressure measurements is also important. In a review article including nine controlled studies, McFadden et al. [41] found that the blood pressure readings reached a nadir in 4 h after exposure and peaked after 10 h. Future studies should take into account these factors that may influence the magnitude and direction of blood pressure changes after alcohol intake.

Drinking behaviors and drinking patterns change over lifetime [42, 43]. Studies with assessment of alcohol consumption at study-entry-only are inadequate because the study design assumes no change of drinking behavior over time. A life course approach [43] should be used to ascertain detailed information on drinking quantity, frequency, beverage type, drinking years, abstinence years, and other characteristics of drinking history. Acute and chronic effects of alcohol consumption on blood pressure can thus be differentiated. It is a more appropriate approach to investigate alcohol effects on any health outcome than conventional approaches.

Alcohol Consumption in Primary Prevention of Hypertension

Despite the methodological limitations of previous studies, cross-sectional and longitudinal data provided relatively consistent support that excessive consumption of alcohol is associated with increases of both the level of blood pressure and the subsequent incidence of hypertension [35].

Government and health officials have set guidelines regarding alcohol use that identify excessive drinking. US Dietary Guidelines stated that "If alcohol is consumed, it should be consumed in moderation – up to one drink per day for women and two drinks per day for men – and only by adults of legal drinking age" [44]. American Heart Association (AHA) discouraged people from heavy drinking in general. The National Institute on Alcohol Abuse and Alcoholism (NIAAA) [45] sets the low-risk drinking limits as no more than four drinks on any day and fourteen drinks per week for men and no more than three drinks on any day and seven drinks per week for women. Canadian low-risk guidelines also posted maximum weekly consumption limits [46]. Given that risk and frequency of binge drinking increases with their frequency of drinking, drinking frequency should be considered in any dietary guidelines [47]. On the other hand, it is likely that there is no threshold that is safe in terms of hypertension risk. Fan's study among current drinkers showed that the association between alcohol consumption and risk of hypertension is linear [31]. The benefits of moderate drinking may be spurious if more and more epidemiologic studies adopt new analytic strategies [31, 34, 48]. In addition, any discussion on benefits of alcohol consumption should take into account other health and societal effects, including sexual and other risks associated with excessive drinking, motor vehicle crashes, and lost productivity. Alcohol screening and preventive counseling for alcohol use should be integrated into primary care and healthy lifestyle interventions.

Disclaimer The findings and conclusions in this chapter are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

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Chapter 26
Alcohol and Dyslipidemia

Indrajit Chowdhury

Key Points

- Dyslipidemia is a major cause of cardiovascular disease.
- Chronic alcohol abuse affects almost every organ system resulting in serious illness such as neurological problems, liver disease, impaired heart function, and inflammation of the pancreas through its oxidation products that affect lipid metabolism.
- Recent molecular studies on PPAR-α, AMPK and SREBP shed new lights for the understanding of alcohol-related dyslipidemia.

Keywords Alcohol • Dyslipidemia • Cardiovascular diseases • Low-density lipoprotein (LDL) Cholesterol • High-density lipoprotein (HDL) cholesterol • Triglycerides (TGs) • Hypertriglyceridemia (HT)

Introduction

Cardiovascular diseases (CVDs) are the world's largest killers, claiming 17.1 million lives a year [1]. Dyslipidemia is a major cause of cardiovascular disease [2–4]. An unhealthy diet and physical inactivity increase the risk of dyslipidemia and promote CVDs including heart attack and stroke. Alcohol is widely used as a part of our diet/daily life as low or empty calories drink without beneficial nutrients such as vitamins and minerals except red wine. Chronic alcohol abuse primarily affects almost every organ system resulting in serious illness such as neurological problems, liver disease, impaired heart function, and inflammation of the pancreas [5, 6]. Alcohol liver disease remains one of the most common causes of chronic liver disease worldwide and is usually accompanied by hepatitis, cirrhosis, and/or hepatocellular cancer [7]. Moreover, alcohol induces severe hypertriglyceridemia (HT) alone or in combination with other defects such as a genetic disturbance in lipid metabolism. We reviewed recent literatures to provide readers a better understanding of the alcohol and lipid metabolism and their regulatory pathways.

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Definition of Lipoproteins

Lipoproteins are a complex of lipids and protein assembly to transport lipids in blood [8]. The lipoprotein particles have an outer shell of hydrophilic groups of phospholipids, which renders the particle soluble in water; a core of fats called lipid, including cholesterol; and a surface apoproteins (Apo) molecule that allows tissues to recognize and take up the molecules to make them soluble in the salt water-based blood pool. Lipoproteins are characterized by their density and size. In order of density and size, largest to smallest lipoproteins are chylomicrons, very low-density lipoprotein (VLDL), intermediate-density lipoproteins (IDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL), which transport cholesterol and triglycerides (TG/triacylglycerol/TAG/triacylglyceride) within the water-based bloodstream. TG-fats and cholesterol esters are carried internally and shielded from the water by the phospholipid monolayer and the Apo. TG is an ester derived from glycerol and three fatty acids (FA).

Definition of Dyslipidemia

Dyslipidemia is a disorder of lipoprotein metabolism, including lipoprotein overproduction or deficiency. Dyslipidemia may be manifested by elevated LDL cholesterol (LDL-c; more than 100 mg/dL optimal level), elevated TGs (higher than 150 mg/dL or 1.7 mmol/l), or low HDL cholesterol (HDL-c less than 40 mg/dL or 1.02 mmol/l in males and less than 50 mg/dL or 1.04 mmol/l in females) [9]. In addition to elevated LDL-c, atherogenic dyslipidemia (elevated TG and low HDL-c) is increasingly being recognized as an independent risk factor for coronary heart disease (CHD) [10, 11]. There are differences between the sexes in the lipid profile that may have clinical implications [12]. For normal adults, total cholesterol (TC) level of less than 200 mg/dL is desirable. About one third of elderly men and one half of elderly women have cholesterol levels >240 mg/dL [13]. Dyslipidemia can be caused by genetic [14] and/or environmental factors including diet, obesity, physical inactivity, drugs, excessive alcohol consumption [15–18].

Chronic Alcohol Consumption and Dyslipidemia

Alcohol is a volatile and water-soluble liquid that oxidizes easily in our body. When ingested, alcohol passes from the stomach into the small intestine, where it is rapidly absorbed into the blood and quickly distributed throughout the body; affects the central nervous system and other parts of the body including cardiovascular system, digestive system, etc.; and causes physiological disturbance. On an average, ethanol accounts for half an alcoholic's caloric intake as a substantial source of energy, with 7.1 kcal (29.7 kJ) per gram, a value that exceeds the energy content of carbohydrates or proteins. In general, about 92–98% of alcohol is metabolized by our body and the rest (1–5%) is excreted as urine, sweat, or evaporates through breathing.

Prevalence of dyslipidemia is high and increases even in younger people with chronic alcohol abuse. A great portion of the impact of chronic ethanol drinks on cardiovascular health is through the effects on lipid metabolism [19, 20]. Chronic ethanol intake (over about 60 g or 4 drinks per day) raises HDL-c, LDL-c, TG, and total cholesterol levels [21]. Moreover, chronic alcohol intake displaces normal nutrients and causes secondary malnutrition through malabsorption. The malabsorption can be caused by gastrointestinal complications including pancreatic insufficiency and impaired hepatic metabolism of nutrients [22]. The malnutrition include deficiencies of folate, thiamine, and other vitamins [22–24]. This alters metabolic rate by increasing esterification of the accumulated fatty

acids to TGs, TG-rich lipoproteins, phospholipids, and cholesterol esters in the liver; stimulates lipolysis in fatty tissue, which results in a higher supply of fatty acids to the liver [25, 26]; and promotes accumulation of fat in the liver mainly by stimulation of ethanol for fatty acids as the major hepatic fuel [27, 28]. Moreover, there are strong sex differences in the alcohol-induced lipid abnormalities and in the vulnerability to alcoholic liver disease [29]. Furthermore, inhibition of the catabolism of cholesterol to bile salt contributes to the hepatic accumulation of cholesterol and causes hypercholesterolemia. Even in the absence of obesity or diabetes mellitus, excessive alcohol intake causes severe HT, although obese alcohol users are more at risk of hyperlipidemia [30] and prone to develop extremely high TG levels [31].

With excessive alcohol intake, the levels of TG increase dramatically with the highest values in the combination of obesity and diabetes mellitus and act as a prominent factor in the occurrence of severe HT (an elevated synthesis of TG-rich lipoproteins including chylomicrons and VLDL). Although dyslipidemia is often asymptomatic, patients with severe HT (~11.3 mmol/l or 1,000 mg/dl) and TG (above 2.2 mmol/l or 200 mg/dl) levels with chronic alcohol abuse are generally considered to be at increased risk for liver or spleen enlargement and acute pancreatitis [32, 33]. Increased lipoprotein production aggravates liver injury and liver steatosis (the abnormal retention of lipids within a cell). These accumulated lipids in liver or adipocytes are disposed of in part as serum lipoprotein, resulting in moderate hyperlipidemia. However, when HT exceeds 11.3 mmol/l or 1,000 mg/dl, the presence of chylomicrons may be responsible for the milky creamy aspect of the serum's supernatant. Ultimately, all these events promote dyslipidemia [34] and enhance the early stages of alcoholic cirrhosis (replacement of liver tissue by fibrosis, scar tissue, and regenerative nodules). Thus, chronic alcoholic abuse contributes to alteration of lipids (secondary dyslipidemia) and early stage of alcoholic cirrhosis.

However, in alcohol-induced atherogenic dyslipidemia, elevated triglycerides are not necessarily accompanied by low HDL-c. Epidemiological studies have shown that alcohol intake is significantly associated with increase HDL-c in a dose-dependent manner [35, 36]. HDL-c was alleged to be an important mediator in favoring cardioprotective effects [37]. The HDL-c levels have linear relationship with alcohol intake and can even be used to identify chronic alcohol drinkers [36]. Higher HDL-c levels in the chronic alcoholic drinkers have positive correlation with liver enzyme concentrations, especially serum glutamic oxaloacetic transaminase (SGOT) [38]. Studies among Korean population [39] suggested a significant direct dose–response relation of the odds ratios between alcohol consumption and metabolic syndrome in both the high and low HDL-c groups. These studies indicate that the increase of HDL-c may not proportionally be translated into cardiovascular benefit. Therefore, the benefit of moderate drinking solely based on increased HDL-c is questionable.

Metabolism of Alcohol and Its Effect on Lipid Oxidization System

A significant progress has been made in understanding the molecular effects of chronic ethanol drinking in the development of fatty liver and dyslipidemia.

Acetaldehyde Formation and Nicotinamide Adenine Dinucleotide (NAD)/NADH Ratio

A major proportion of alcohol is metabolized to acetaldehyde by the alcohol dehydrogenase (ADH) pathway in hepatocytes (liver) and then converted to acetate (Fig. 26.1). Both reactions release hydrogen atoms that reduce nicotinamide adenine dinucleotide (NAD) to its reduced form (NADH) in liver cells. NADH, in turn, participates in many essential biochemical reactions in the cell through

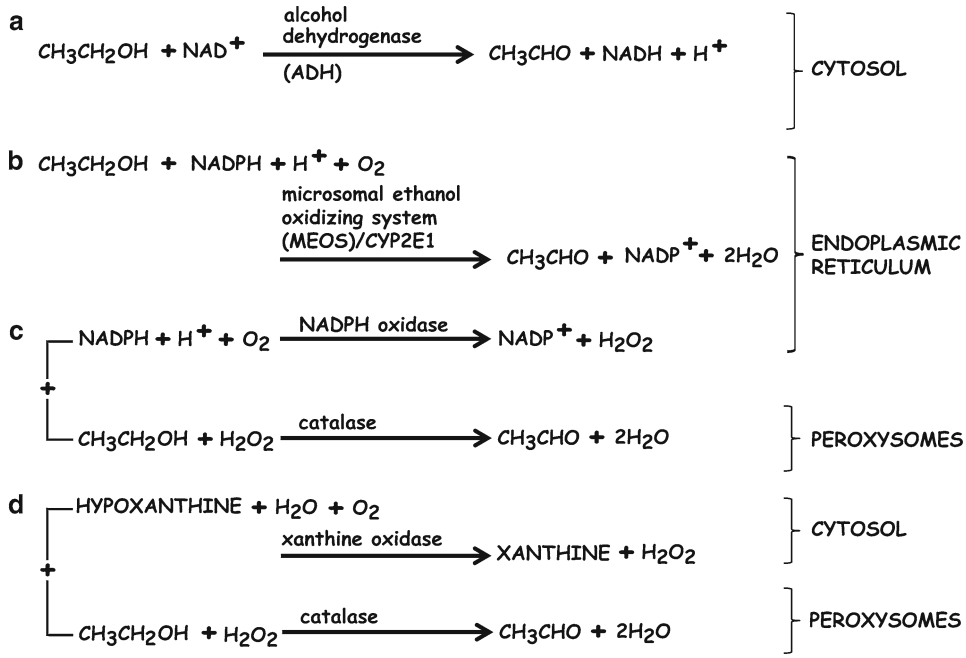

Fig. 26.1 A schematic representation of ethanol oxidation through alcohol dehydrogenase (ADH), microsomal ethanol-oxidizing system (MEOS), catalase, xanthine oxidase, nicotinamide adenine dinucleotide (NAD^+), and nicotinamide adenine dinucleotide phosphate (NADPH) in liver (for details, see the text)

transferring its hydrogen to other molecules. For proper functioning of the cell, the ratio of NAD to NADH must be tightly controlled. When alcohol metabolism generates excess amounts of NADH and the cell can no longer maintain the normal NAD/NADH ratio, then it causes a number of metabolic disorders including inhibition of the Krebs cycle and oxidation of fatty acids and the formation of abnormally high levels of lactic acids. The high levels of lactic acids reduce the capacity of the kidney to excrete uric acid and exacerbate gout, a condition that causes extremely painful swelling of certain joints. The inhibition of fatty acid oxidation favors steatosis and hyperlipidemia. In addition, with long-term ethanol consumption, the acetaldehyde produced by the oxidation of ethanol has toxic effects by inhibiting the repair of alkylated nucleoproteins [40], decreasing the activity of key enzymes, and markedly reducing oxygen use in mitochondria [41]. The impaired oxidation capacity of the mitochondria may, in turn, interfere with the oxidation of acetaldehyde [41, 42], leading to a vicious circle of progressive acetaldehyde accumulation and greater mitochondrial injury. Moreover, acetaldehyde promotes cell death by depleting the concentration of glutathione (GSH), inducing lipid peroxidation, and increasing the toxic effect of free radicals. Through binding to the tubulin of microtubules, acetaldehyde blocks the secretion of proteins and enhances protein, lipid, water, and electrolytes in the hepatocytes to enlarge as "balloon," a hallmark of alcoholic liver disease [43]. Acetaldehyde–protein adducts, with the carboxyl-terminal propeptide of procollagen, promotes collagen production [44] and also acts as neoantigens which stimulate an immune response [45, 46]. Lipid peroxidation products such as 4-hydroxynonenal stimulate fibrosis through decreased feedback inhibition of collagen synthesis [44].

Microsomal Ethanol-Oxidizing System (MEOS)

MEOS pathway plays a key role in the ethanol metabolism [47, 48] (Fig. 26.1). MEOS has several enzymes including cytochrome P450, which exists in several isoforms [49]. The most important for alcohol metabolism is cytochrome P450 2E1 (CYP2E1). The *CYP2E1* gene is located to chromosome 7 in rat [50] and chromosome 10 in human [51]. Chronic alcohol drinkers have four to ten times higher concentrations of both hepatic CYP2E1 protein and mRNA in Kupffer cells [52–54]. Enhanced CYP2E1 expression during chronic alcoholic consumption causes liver injury including alcoholic steatosis and alcoholic steatohepatitis [47, 48]. CYP2E1 also contributes to the defense mechanism of our body against the penetration of toxic xenobiotics [55]. Moreover, CYP2E1 mediates certain processes in the metabolism of fatty acids and ketones (acetone). Like alcohol, acetone stimulates CYP2E1 activity, and act as both an inducer and a substrate for CYP2E1 [56, 57]. It has demonstrated that acetone is actively metabolized by microsomal acetone monooxygenase in rats [58], rabbits [59], and human beings [60] and identified as CYP2E1. Moreover, CYP2E1 participates in fatty acid ω-1 and ω-2 hydroxylations [61–63]. The CYP4A subfamily catalyzes ω-hydroxylation at the terminal carbon of fatty acids. Ethanol drinking increases the activity of CYP4A1 [64]. Enhanced CYP2E1 activity in response to chronic alcohol consumption contributes to the hepatic disposition of nonesterified fatty acids and development of alcoholic liver disease called steatohepatitis, an inflammation with concurrent fat accumulation in the liver.

Alcohol-induce enhanced activation of the MEOS promotes alcoholic liver disease through other mechanisms as well. Alcohol breakdown by CYP2E1 generates several types of highly reactive oxygen-containing molecules called reactive oxygen species (ROSs). Increased ROSs damage liver cells by inactivating essential enzymes, altering the breakdown of fat molecules, and causing oxidative stress. These ROS effects are exacerbated if the body's normal defense systems such as glutathione (GSH) and vitamin E (α-tocopherol) are impaired. Alcohol and its metabolic products such as acetaldehyde have been shown to reduce the levels of both GSH and vitamin E in the liver. Patients with cirrhosis have reduced amounts of vitamin E in the liver. Thus, excess alcohol metabolism causes lipid peroxidation largely through increased ROSs and reduced GSH [65].

Metabolism of Triglycerides (TG)

Chronic heavy alcohol consumption directly affects the TG metabolism in liver, muscle (myocytes), adipose tissues (adipocytes), and pancreatic and intestinal cells (Fig. 26.2). The metabolic energy in our body is mainly derived from TGs, which constitute 15–20% of total body weight and provide 9 kcal/g TG [8]. However, the preferred and the first source of energy to be used is glucose (4 kcal/g), followed by TG. When glucose is not used for energy production and glucose storage is saturated, then all of the excess glucose is shifted toward the synthesis of free fatty acids (FFA) and TG [8]. Ethanol in doses >30 g/d augments the TG level [66] through FFAs delivery to the adipocytes following the hydrolysis of TG by lipoprotein lipase (LPL) in the TG-rich lipoproteins (TRL) at the surface of endothelial cells [67]. After crossing the endothelial cells and entering the adipocytes, the FFAs are activated and incorporated into TG, a process referred to as "fatty acid trapping" [22]. The final step in this process is the addition of a fatty acid CoA to diacylglycerol (DAG) through the action of diacylglycerol acyltransferase (DGAT) [22].

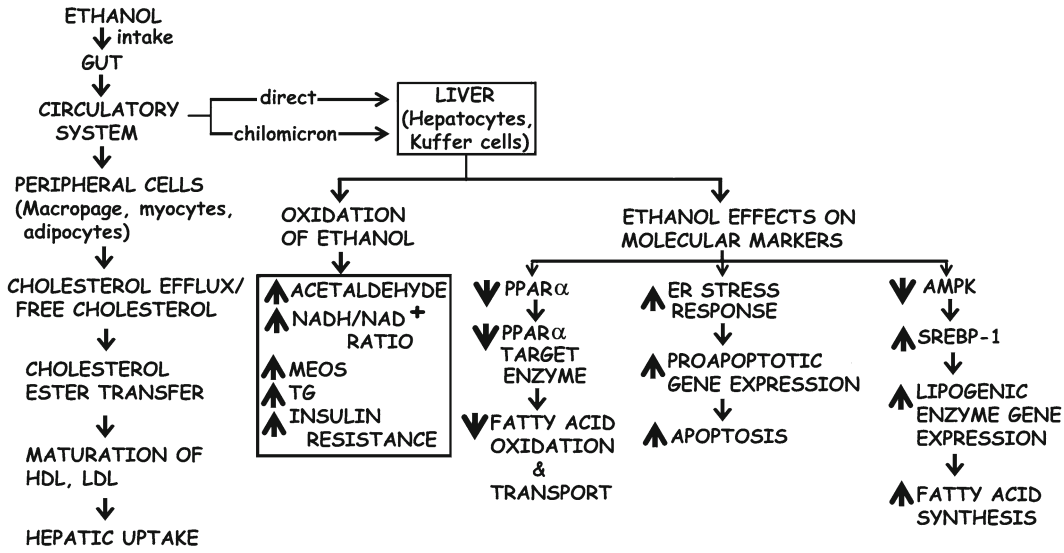

Fig. 26.2 A schematic representation of ethanol ingestion, absorption, transcriptional control lipid metabolic genes, and molecular markers (for details, see the text)

Insulin Resistance

Insulin plays an important role in TG metabolism and FFA production in association with alcohol consumption by affecting multiple tissues and organ systems including liver, adipose tissues, pancreas, intestine, myocytes, etc. The main function of insulin in the liver is the control of endogenous glucose production (EGP), which is the sum of gluconeogenesis (GNG, the formation of glucose from non-glucose precursors), and glycogenolysis (GL, the formation of glucose from the hydrolysis of glycogen) [22, 68]. Insulin normally decreases cholesterol synthesis and inhibits apoB secretions from liver, increases lipoprotein lipase (LPL) activity, and stimulates the formation of TG. Excessive caloric intake through alcoholic consumption leads to adipocyte hypertrophy and increases visceral adipose tissue. Adipose tissue is an endocrine organ that secretes many cytokines and adipokines. The phagocytic cells of liver, the Kupffer cells, are also a major source of cytokines and reactive oxygen radicals [69]. Oxidative stress caused by chronic alcohol consumption promotes proinflammatory cytokines including tumor necrosis factor (TNF)-α; interleukin (IL)-1, IL-4, and IL-6; monocyte chemotactic protein (MCP)-1; interferon (IFN)-γ; and nitric oxide synthase (NOS)-1 in the Kupffer cells and adipocytes [67]. These cytokines promote inflammation, insulin resistance, and dyslipidemia. For detailed mechanism of action of insulin in dyslipidemia, see relevant chapters of this book.

Molecular Markers of Chronic Alcohol Consumption and Their Role on Dyslipidemia

Recent studies have revealed that chronic ethanol intake inhibits mitochondrial fatty acid-oxidizing dehydrogenases through inhibition of peroxisome proliferator-activated receptor-α (PPAR-α) and AMP-activated protein kinase (AMPK), and upregulates transcription factor sterol regulatory element-binding protein (SREBP)-1c (Fig. 26.2).

Peroxisome Proliferator-Activated Receptor-α (PPAR-α)

PPAR-α, a nuclear hormone receptor, is involved in regulating fatty acid oxidation and transport. When PPAR-α is activated, it binds as a heterodimer with retinoid X receptor (RXR) to peroxisome proliferator response element genes that involve in the fatty acid oxidation pathways [70]. PPAR-α is activated in both fatty acid oxidation and export and thereby protects against the accumulation of TG, improves the enzymatic defenses against oxidative stress, reduces the apoptotic response, and prevents fat accumulation [70]. Chronic ethanol consumption decreases the binding of PPAR-α/RXR to DNA and expression of several PPAR-α-regulated genes through posttranslational modification of PPAR-α or RXR [71]. These effects are mediated by acetaldehyde as blocking aldehyde dehydrogenase (ALDH) increases the effects, whereas blocking ADH prevents it [70, 72, 73].

AMP-Activated Protein Kinase (AMPK)

Chronic alcohol intake directly inhibits AMPK. AMPK is a master regulator of metabolism that senses cellular stresses such as oxidative stress and reduced energy charge, increases the activity of the major energy-generating pathways such as glycolysis and fatty acid oxidation, and downregulates energy-demanding processes through fatty acid, cholesterol, and protein synthesis [70]. Activation of AMPK increases fatty acid oxidation and inhibits its synthesis, whereas inhibition of AMPK blocks fatty acid oxidation and promotes fatty acid synthesis [70]. The key regulator of this switch is malonyl-CoA that promotes the uptake of long-chain acyl-CoA in mitochondria. Thus, it regulates lipid synthesis both directly through sterol regulatory element-binding protein (SREBP)-1c and indirectly through phosphorylation and inhibition of acetyl-CoA carboxylase (ACC) and 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase, a downstream targets of SREBP-1 and SREBP-2, respectively [70]. AMPK directly inhibits SREBP-1c by decreasing its stability [70, 74–76]. In addition, AMPK system suppresses activation of HSL and facilitates a balance between the amount of FFA release from TG by HSL [67]. Otherwise, excess FFA in adipocytes will be recycled back into TG in presence of ATP. Moreover, adiponectin activates AMPK through increasing oxidation of FFA and insulin sensitivity [67].

Sterol Regulatory Element-Binding Protein-1 (SREBP-1)

SREBPs are transcription factors regulating fatty acid, TG, and cholesterol synthesis [70]. SREBPs are bound as precursors to the endoplasmic reticulum (ER) and nuclear envelope. SREBPs are activated and released by SREBP cleavage-activating protein (SCAP) and translocated to the nucleus, where they bind to sterol response elements and activate transcription [70]. Chronic ethanol consumption upregulates the SREBP-1c expression and affects fatty acid synthase (FAS), stearoyl-CoA desaturase (SCD), malic enzyme (ME), ATP citrate lyase (ACL), ACC, and ultimately enhances synthesis of fatty acids [77, 78]. The activity of SREBP-1 is controlled by several different pathways including AMPK. Moreover, SREBP-1 induces lipopolysaccharide (LPS) and TNF-α levels in the liver [79].

Fatty Acid Binding Protein Type 2

The chronic alcohol consumption increases esterification of FFA to TG and plays a key role in the intestinal fatty acid binding protein (FABP)-2 gene expressions. FABP-2 is a member of a family of

more than 20 FABP genes [80] that only express in the intestinal epithelial cells and promotes the transport of hydrophobic FFA from plasma membrane to ER. A common polymorphism in the FABP-2 gene, Ala54Thr, promotes insulin resistance and increases dietary fat absorption with higher plasma FFA and TG and affects insulin action in the hepatocytes and skeletal muscle cells [80].

Conclusions

Chronic alcohol consumption causes alcoholic fatty liver and hyperlipidemia through its oxidation products that affects hepatic lipid metabolism. An early target of ethanol toxicity is mitochondrial fatty acid oxidation. Acetaldehyde and ROSs have been incriminated in the pathogenesis of the mitochondrial injury. Microsomal changes offset deleterious accumulation of fatty acids, leading to enhance formation of triacylglycerols, which are partly secreted into the plasma and partly accumulate in the liver. However, this compensatory mechanism fades with progression of the liver injury. Increased production of toxic metabolites exacerbates the lesions and promotes fibrogenesis. The early presence of these changes confers to the fatty liver a worse prognosis than previously thought. Alcoholic hyperlipidemia results primarily from increased hepatic secretion of VLDL and secondarily from impairment in the removal of triacylglycerol-rich lipoproteins from the plasma. Hyperlipidemia tends to disappear because of enhanced lipolytic activity and aggravation of the liver injury. Recent molecular studies on PPAR-α, AMPK, and SREBP shed new lights for the understanding of alcohol-related dyslipidemia.

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Chapter 27
Dietary Antioxidants in Chronic Alcoholic Pancreatitis

Mirosław Jarosz and Ewa Rychlik

Key Points

- Alcohol is the most common etiological factor of chronic pancreatitis. It is the cause of 60–80% cases of this disease.
- Probably, oxidative stress plays very important role in the pathogenesis of chronic pancreatitis and development of its complications.
- Antioxidants are important elements in combating the oxidative stress. The higher antioxidative potential of the body increases its capability of destroying free oxygen radicals.
- The use of antioxidants (especially vitamins C and E, carotenoids) has beneficial influence on the course of chronic pancreatitis. They are effective in reducing pain and frequency of acute pancreatitis episodes and can improve the external and internal secretive function of the pancreas.

Keywords CP • Alcohol • Oxidative stress • Antioxidants

Introduction

Chronic pancreatitis (CP) is defined as a disease which involves progressive, irreversible destruction of glandular tissue and its replacement by fibrous connective tissue. Clinical symptoms of those changes are abdominal pains and destruction of the external (steatorrhoea) and internal secretive function of the pancreas (diabetes), developing over varying time periods [1, 2]. The life expectancy among CP patients is decidedly shorter than the average for the whole population. Only about 50% of the patients live for 20 years after occurrence of the first clinical symptoms. The prognosis is better for patients who have stopped drinking alcohol.

Epidemiological studies show that occurrence of the disease is probably more frequent than assumed earlier [3–5]. In Japanese nationwide survey in 2002, the overall prevalence of chronic pancreatitis was calculated to be 35.5 per 100,000 and it increased from 28.5 per 100,000 in 1994 [4, 5]. The most common type of CP was alcoholic pancreatitis (67.5%). CP affects men more often than women.

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In Japan, the prevalence was approximately two times more in males than in females (43.9 vs. 22.4 per 100,000) [6].

Most cases of chronic pancreatitis require hospitalization due to the presence of pain as well as to the emergence of other complications.

The etiopathogenesis of chronic pancreatitis has still not been sufficiently explained, and the treatment of this disease is a difficult task. This is why many medical centres in the world are involved in research aimed at explaining its causes, development mechanisms and complications, as well in search for new methods of treatment.

The main cause of the disease is alcohol [1, 3, 7]. Studies raise increasingly often the importance of oxidative stress in the pathogenesis of chronic pancreatitis and development of its complications, such as, among others, cysts and abscesses in the pancreas, diabetes and others. There are hypotheses that disturbances in the oxidants–antioxidants system lead to damage to pancreatic cells caused by excess of free radicals, which facilitates development of CP and emergence of its complications [8].

A few research centres in the world have undertaken attempts to apply antioxidants in treatment of this disease. The effectiveness of this therapy is difficult to assess both from the methodological viewpoint and with regard to selection of the antioxidants and their doses. Nevertheless, benefits following from the use of antioxidants have been shown in a few papers [9–12].

Etiological Factors of CP

The most common etiological factor of CP is alcohol. According to various authors, it is the cause of 60–80% cases of this disease [3, 7, 13–18]. However, authors of the largest epidemiologic study on CP from the United States have observed that the current etiologic profile of CP patients evaluated at the US referral centres and the proportion of patients in whom alcohol was identified as the sole or contributing cause of CP was much lower (44.5%) than expected [19].

Alcohol can lead to the onset of pancreatitis in a number of ways [20–22]. It can have a direct toxic effect on the pancreas and cause mechanical obstruction of pancreatic ducts and pancreatic autodigestion. Alcohol also affects the production, rheological properties and the flow of pancreatic juice and in this way leads to pathological alterations. Ethanol is metabolized in two different ways: oxidative and non-oxidative one. The major products of the oxidative metabolism are acetaldehyde and the formation of reactive oxygen species. The non-oxidative metabolic pathway of ethanol is characterized by its esterification with production of fatty acid ethyl esters. The above-mentioned products of ethanol metabolism can have a number of toxic effects on pancreatic acinar cells.

The amount of alcohol needed for development of the disease varies significantly depending on the studied population [7, 23, 24]. In the studies conducted in the European countries, the average dose of alcohol consumed before diagnosis of the disease was 150 ±89 g/day, while in Brazil it was as much as 397 ±286 g/day, ranging from 80 to 1,664 g/day. The period of consuming alcohol ranged from 4–7 years to 44 years, amounting on average to 18–19 years.

The meta-analysis by Corrao et al. [25], investigating alcohol consumption and the risks of selected diseases, has demonstrated strong direct trends in the risk of chronic pancreatitis. A significantly increased risk has been found starting from the lowest dose of alcohol considered (25 g/day, which corresponds to about two drinks per day).

The meta-analysis by Irving et al. [26] has found a monotonic dose–response relationship between alcohol consumption and the risk of pancreatitis. A completely novel finding in that study was the existence of a threshold effect between alcohol intake and the risk of pancreatitis. The threshold of alcohol intake associated with the risk of pancreatitis was about 4 drinks daily, where a drink was equivalent to 12 g.

The increasing incidence of CP in recent years in Japan may be closely related to the gradually increasing alcohol consumption [5].

Women are threatened with developing CP when consuming smaller amounts of alcohol. The studies conducted by Sarles et al. [27] imply that for women, the risk of developing CP starts already in the case of consuming 20 g of pure ethanol per day for a few years.

It has also been shown that smoking is a significant coexisting risk factor for CP [19, 28–31]. Some papers have even shown that this is a risk factor independent of alcohol. This risk increases with the number of cigarettes smoked. The mechanism through which addiction to nicotine leads to damaging the pancreas is unknown. Tobacco smoking has been found to inhibit secretion of bicarbonates by the pancreas and to decrease the concentrations of trypsin and $\alpha(\text{alpha})_1$-antitrypsin in the serum. In the light of the new hypotheses regarding the pathogenesis of CP, it is also possible that by causing deficiency of antioxidation vitamins (especially vitamins C and E) in the body, it is conducive to disturbances in the oxidative balance, which is probably one of the mechanisms leading to development of this disease [32].

Another toxico-metabolic factor of CP risk mentioned by researchers is hypercalcaemia occurring in the course of hyperparathyroidism and hyperlipidemy [1, 3, 33]. While hyperparathyroidism is an accepted and documented but rare etiological factor of CP risk [21], hyperlipidemy (hypertriglyceridemy > 500 mg/dl) raises essential controversies, and its connection with CP needs to be better documented [33, 34].

A significant group of risk factors leading to development of CP are cases of recurring and severe episodes of acute pancreatitis (AP), complicated by pancreatic necrosis. This group included cases of AP with different aetiologies, like biliary lithiasis or alcohol. Researchers have collected part of clinical and pathological evidence from tests on animals indicating possible connection between recurring and severe forms of acute pancreatitis and chronic pancreatitis [35–37]. Disseminated necrosis or severe cases of diffuse necrosis are assumed to induce fibrosis near the pancreas lobules, which results in narrowing the ducts [36, 38, 39]. This can result in hampering the outflow of pancreatic juice, which is conducive to precipitation of proteins and creation of deposits. This theory, presented by Klöpell and Amman, seems to explain in what way alcohol-generated AP can lead in many cases to chronic pancreatitis.

Part of CP cases is connected with pathology in the pancreas head which hampers the outflow of pancreatic juice [40, 41]. This form of CP is characterized by uniform widening of the Wirsung's duct behind the obstacle and uniform, disseminated fibrosis behind the obstacle, together with absence of deposits and calcifications in the pancreas [27, 40, 42, 43]. The most common causes of obstructions hampering the outflow include a slowly growing tumour in the Vater's papilla and cancer in the pancreas head, a cyst pressing on the duct, a post-injury scar narrowing down the duct and bipartite pancreas [27, 44]. In this form of the disease, surgical removal of the obstruction results in clinical improvement and absence of disease progress (except for the pancreas cancer) [27, 45–48].

An important group of risk factors are genetic factors which lead to development of hereditary pancreatitis [49, 50]. This form is inherited in an autosomal way, dominant with incomplete penetration. The factor responsible for developing this form of the disease is a mutation within the PRSS1 gene, coding the cationic trypsinogen and located on the long arm of chromosome 7 (7q35) [51–55]. This results in synthesis of protrypsinogen with a changed structure. This enzyme is also easily activated inside acinar cells of the pancreas, which results in their damage and development of inflammatory changes. The disease develops most often in young people. Its progress can be partly prevented by imposing an absolute ban on alcohol drinking and tobacco smoking. Covering the above-mentioned patients with an appropriate care is of essential importance due to the greatly increased risk that they develop pancreas cancer, estimated at about 40% [56]. The risk increases even more if the disease has been inherited from the father. Besides, this risk is also connected with the time of clinical symptoms occurrence [57, 58]. In part of patients, genetic mutations are also found in various other forms of CP, including those with alcoholic aetiology [1, 59–62].

There are also reports on autoimmunological factors which allegedly lead to development of a pathological image characteristic for CP [1, 63, 64]. Isolated autoimmunological CP has been

distinguished, as well as CP occurring in the course of nonspecific inflammatory diseases of intestines (ulcerative colitis, Crohn's disease) and of primary biliary cirrhosis [65]. These forms are characterized by increased levels of biochemical exponents of cholestase and G class immunoglobulines in the blood serum, diffuse or segmental, irregular narrowing of the main pancreatic duct, as well as a positive response to treatment with corticosteroids [66].

In part of the cases, termed idiopathic CP, no etiological factor of the disease can be established [67]. However, studies have shown that part of the patients (25% with idiopathic CP) exhibit genetic mutations (SPINK 1 mutations), which probably determine development of the disease. Over the last period, which has seen identification of many environmental and genetic factors leading to development of CP, this form of the disease had been diagnosed less and less often.

Pathogenesis of CP

The pathogenesis of chronic pancreatitis has not been sufficiently explained yet. Most probably, there are a lot of factors and mechanisms which play an important role in development of the disease, including also genetic mutations in part of CP patients [1, 51, 59, 60].

There are two characteristic phenomena occurring in this disease that can help explain the pathogenesis of CP. The first of them is hypersecretion of enzymatic proteins without simultaneous increase in secretion of biocarbonates [68, 69]. The second are inflammatory changes discovered in a histopathological examination, present between pancreatic alveoli [70].

The first phenomenon explains in which way deposits may form in minor pancreatic ducts. They are probably a consequence of increased concentration of proteins, which undergo precipitation and then calcification in minor pancreatic ducts [69, 71]. They damage the ducts, forming scar-like strictures hampering the outflow of juice, which leads to atrophy and fibrosis of the pancreatic tissue. A factor that plays an important role in preventing formation of deposits in pancreatic ducts is lithostathine, which is synthetized and secreted by acinar cells [72]. It slows down precipitation of calcium carbonate and hence formation of deposits [73]. A lowered level of lithostathine is observed among CP patients, but its lowered level is also noted among alcoholics. Most probably, lithostathine is only one of many factors which play some role in hampering formation of deposits.

Another probable factor playing a role in the CP pathogenesis is ischaemia [74, 75]. The increased pressure in the minor ducts observed in CP compared to healthy individuals leads to reducing flexibility of the gland and hampering the flow through minor pancreatic ducts. Tests on animals have proved that ischaemia may lead to changes characteristic for CP.

Disturbances in the Oxidation–Antioxidation Balance Among CP Patients

The possible role of disturbances in the oxidants–antioxidants balance in pancreas diseases has come to attention of the researchers in the 1980s. At that time, the free radical theory was proposed as an essential component in the etiopathogenesis of acute and chronic pancreatitis. A hypothesis was put forward that the pathway of generating free radicals is the same in all kinds of damage to the pancreas (as the only type of reaction to damage) [8, 74, 76, 77]. However, there is no experimental model of chronic pancreatitis due to the relatively short life of test animals. It has been shown that probably deficiency of antioxidants and surplus of polyunsaturated fatty acids, in presence of existing induction of the P450 cytochrome enzymatic system by xenobiotics, facilitates peroxidation of lipids, which can be important in pathogenesis of damage to pancreatic cells.

Free radicals are single atoms, groups of atoms or chemical molecules having a non-coupled (single) electron on the last orbit, which is the cause of their high activity [78, 79]. The factor responsible for creation of free radicals is first of all endogenous oxygen metabolism. The most dangerous radicals are those resulting from oxygen reduction. An oxygen molecule can be reduced to a supraperoxide anion, hydrogen peroxide and hydroxyl radical. The supraperoxide anion is a radical having special importance for biological membranes, while the hydroxyl radical is the most reactive one. A hydroxyl radical can be created during reduction of hydrogen peroxide with participation of iron or copper ions. As other oxygen derivatives can also exhibit unfavourable action, a frequently used term is reactive oxygen species, which is broader than oxygenic free radicals. The former term includes also activated singleton oxygen and hydrogen peroxide.

Oxygenic organisms have developed complicated mechanisms of defence against toxic oxygen derivatives in the course of evolution [80, 81]. Normally, as much as 95% of oxygen in the sequential reaction chain (with single electron passage) is transferred to water. The remaining 5% is deactivated by the so-called endogenous wipers, i.e. antioxidants. The cells engage natural antioxidation defence mechanisms, such as catalase, glutathione reductase, supraperoxide dismutase, glutathione, α-tocopherol or ascorbic acid.

Special sensitivity to the action of free radicals characterizes lipid components of biological membranes (side chains of fatty acids), which can be damaged in the lipid peroxidation process [82, 83]. A free radical when acquiring a hydrogen atom destabilizes the side chain, which allows action of lipophilic radicals and leads to damaging the cell structure. This can cause instability of cellular membranes, change in their permeability and disturbances in their functions and cross-membrane transport. Free radicals and reactive oxygen species can also cause conversion of proteins, which results in changing their structures and functions. This process leads to modifications in amino acids and enzymatic proteins, oxidation of thiol groups and denaturation of protein. Free radicals also cause DNA restructuring, which can lead to mutations.

If the balance between free radicals and antioxidants which neutralize them is preserved, there is no threat to health. The threat appears when there is a surplus of radicals, and antioxidants are unable to deactivate them fast enough. Then, we have to do with the so-called oxidative stress. Oxidative stress can cause damage to, organic compounds, cellular organelles, whole cells, tissues, body organs, systems and finally death [78, 84, 85].

In the course of chronic pancreatitis, the pancreas structure is damaged in a progressive and irreversible way. Free oxygen radicals may probably play a material role in progressive damage to the pancreas parenchyma (Fig. 27.1). Consumption of alcohol causes growth in the number of free radicals in various mechanisms [8, 74]. The radicals cause damage to acinar cells as well as stimulate fibrinogenesis and damage blood vessel endothelium.

Characteristics of Antioxidants

Important elements in combating the oxidative stress are antioxidants [86, 87]. They are a group of chemical compounds which possess the capability of neutralizing free radicals created under the influence of UV radiation, operation of hormones, environmental pollution, stress, consumption of certain foods, addictions and as a result of ageing processes. Antioxidants are substances which prevent cell damage.

Antioxidants are divided into endogenous ones, i.e. enzymes present in each cell, which include supraperoxide dismutase, catalase, glutathione reductase and peroxidase, and exogenous ones, which are delivered to the body with food or in the form of supplements (vitamins A, C, E; coenzyme Q_{10}; carotenoids; xantophiles; selenium; phenolic acids; flavonoids; zinc; manganese) [88, 89].

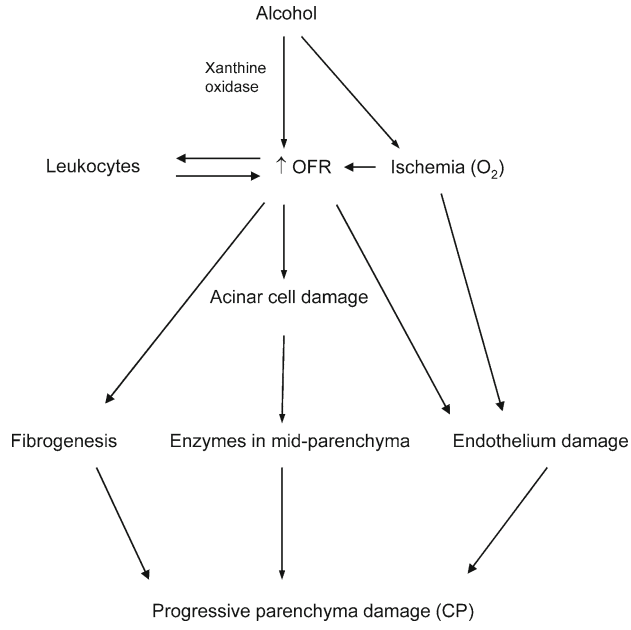

Fig. 27.1 Free radicals in CP pathogenesis. *OFR* oxygen free radicals (Based on data from Ref. [8])

Vitamin A

The most common form of vitamin A is retinol, which consists of a β(beta)-ionone ring and a polyene chain connected to that ring [86, 88]. Next to a free alcohol form, it occurs also in an esterified form. The activity of vitamin A, though lower than of retinol, is also exhibited by retinoic acid and retinal. In the human body, this vitamin can be created out of some carotenoids termed provitamins A, with the most important of them being β(beta)-carotene.

Vitamin A occurs solely in products of animal origins [88, 89]. Its most important source is sea fish liver oil – e.g. cod liver oil. It is also present in butter, milk, full fat milk products and egg yolks. Many countries enrich with vitamin A certain food products, e.g. margarine. Carotenoids occur mainly in plant products. Among the plant products rich in β(beta)-carotene, we should mention carrots, pumpkins and green leafy vegetables and among fruits apricots, cherries, plums and oranges. Vitamin A and carotenoids are absorbed together with food fats, but this process is six times slower for β(beta)-carotene than for retinol.

The antioxidative properties of vitamin A and carotenoids follow from the presence in their molecules of a coupled system of C=C bonds in the side chain [86, 87]. Thanks to it, these compounds effectively extinguish singleton oxygen and neutralize free radicals created during peroxidation of lipids. Vitamin A operates both in the first and the second line of defence against reactive oxygen species in prevention processes and in free radical reactions at the termination stage.

Vitamin E

This name is used for 4 tocopherols and 4 tocotrienols which contain the ring system of chroman with an attached isoprene side chain [86, 90]. The full activity of vitamin E is exhibited by α(alpha)-tocopherol.

The most valuable natural source of this vitamin is wheat sprouts oil [88, 89]. It is also present in complete cereal grains (especially wheat and corn grains), nuts and green leafy vegetables. Cold pressed oils contain much more vitamin E than refined ones since the process of their refinement itself destroys as much as 75% of the natural vitamin. Absorption of vitamin E is facilitated by presence of fats in the food.

The antioxidative activity of vitamin E is due to the phenol group OH^- connected to the ring system [86, 87, 90]. Thanks to its strong antioxidative properties, this vitamin is considered as one of the main compounds protecting the body against oxidative stress. It can participate in the first line of defence against reactive oxygen species, effectively extinguishing singleton oxygen. This prevents reaction of the latter with remainders of polyunsaturated fatty acids contained in phospholipids of cellular membranes and slows down the peroxidation reaction and generation of their radicals. In the second line of defence, vitamin E reacts speedily with free peroxide radicals of lipids and deactivates them, breaking at the same time their production, and slows down the sequence of free radical chain reactions damaging the cells.

Selenium

It is classified among trace elements [87, 89]. Particularly large amounts of selenium are found in the offal, especially kidneys, as well as in fish and seafood. Other sources of this element are wholemeal cereal products, pulses, mushrooms and garlic. Absorption of selenium taken in with food differs depending on its form. The best absorbable form is selenium from L-selenium methionine, which occurs in vegetables.

Selenium is a component of enzymes which protect cells against the harmful action of free radicals [91]. It is connected with the operation of glutathione peroxidase (GSH-Px) – the enzyme which reduces the speed of peroxidation processes in cells by decomposing peroxides and in this way protects cellular membranes against damage by free radicals.

Vitamin E and selenium operate in a synergistic way [90–92]. Vitamin E reduces the demand for selenium, preventing the loss of this microelement by the body as well as maintaining it in an active form. Selenium and α(alpha)-tocopherol complement each other in reactions destroying lipid peroxides. Moreover, selenium is needed for correct functioning of the pancreas, which is necessary for digesting lipids, and hence indirectly vitamins soluble in fats.

Vitamin C

Despite the use of the name "ascorbic acid", it is not an acid but a compound related to hexoses [86, 89]. Its biosynthesis is one of the paths of glucose transformations, leading to creation of γ(gamma)-lactone of the L-gulonic acid. Vitamin C is the enol form of its dehydrated form.

The human body lacks the principal enzyme for biosynthesis of vitamin C – L-gulonic oxidase, and so the body must receive it with food [88, 89]. The source of vitamin C are first of all plant products. Especially large amounts of this vitamin are contained in citrus fruit, blackcurrants, grapes, apples, raspberries, strawberries, cranberries as well as horseradish and tomatoes. Slightly lower amounts are present in green leafy vegetables and potatoes.

Ascorbic acid exhibits strong antioxidative properties [87, 89]. The coupled pair of its oxidized and reduced forms creates an oxido-reductive system capable of reducing reactive oxygen species which are toxic for cells, such as singleton and molecular oxygen, or hydroxyl radicals. Its immunoprotective influence neutralizes the ionizing action of extracellular, phagocyte-derived $MPO/H_2O_2/J$

system: myeloperoxidase, the phagocyte granularity enzyme, which together with H_2O_2 and cofactors (such as iodides, chlorides, bromides, cyanides) forms a strongly oxidizing system affecting destructively both pathogens and host cells.

Other Antioxidants

The largest group of antioxidants are *phenolic compounds* [93, 94]. The most important classes of polyphenols are phenolic acids and flavonoids, encompassing flavones, flavonoles, isoflavones and chalcones. Large amounts of polyphenols are present in apples, onions, broccoli, blueberries, olives, lettuce, red wine and chocolate. An especially valuable product rich in antioxidants is tea, particularly green tea. These compounds exhibit the capability of capturing peroxide anions as well as lipid- and hydroxyl-free radicals. Over the last years, attention has been drawn to resveratrol – an antioxidant occurring in red grapes peel and red wine, which actively prevents oxidation of the LDL cholesterol fraction and exhibits detoxifying properties.

Another substance mentioned among important antioxidants coming from food is *coenzyme* Q_{10} [95, 96]. The human body is capable of producing it, but not always, in amounts sufficient for correct functioning. This compound occurs especially in fat fish and seafood. Its rich sources are also meat and offal, and small amounts can be also found in fresh fruit and vegetables. Coenzyme Q_{10} plays an important role in oxidation-reduction mechanisms. It is capable of capturing free radicals and also acts indirectly by intensifying transition of tocopherol from the oxidized form to the reduced form.

Basic Principles of CP Treatment

The basis for treatment of CP is eliminating the causative factor [97]. In case of calcifying CP, this will be an absolute ban on alcohol consumption and tobacco smoking. Continued alcohol drinking has been proved to increase not only the frequency and intensity of abdominal pains but also the frequency of complications occurrence [38]. Moreover, it is also a contraindication for surgery in severe forms of the disease since surgery does not give any greater results then. This is a very important problem in medical practice. The patient should have a schedule of regular control visits (at best, with persons close to the patient, the family) and appropriate psychotherapy planned for him/her. Some patients require treatment in outpatients' clinics or wards for therapy of addictions. Conduct by choice will also include surgical removal of parathyroids in case of hyperparathyroidism or parathyroid cancer. If we diagnose obstructive CP, it will most often represent indication for surgical treatment and in some cases (*odditis, papillitis stenosans*) for endoscopic treatment [98].

Conservative treatment of CP reduces in principle to treatment of the two main symptoms: pain and in the later period exo – and endocrine failure of the pancreas (i.e. the malabsorption syndrome and diabetes) [99]. An appropriate dietetic conduct is expedient [100]. It should take into consideration not only the duration of disease but also its course for a specific patient. CP involves a number of metabolic disturbances: in digestion (decreased number of acinar cells of the pancreas and their lower activity, obstruction of pancreatic and bilious ducts), in absorption (toxic influence on the mucous membrane of the small intestine) and in malnutrition (chronic pain, chronic ethanol intake, too low energy value of the diet, disturbances in digestion and absorption). Metabolic disturbances result from the above factors and limited consumption of meals, which leads to malnutrition [101].

In most CP patients, we can observe too low weight and biochemical features of malnutrition. High energy diet (most often, between 2,500 and 3,000 Kcal) is recommended to prevent patient's weight loss [101]. However, due to decreased digestive and absorptive capabilities, the daily

diet should be divided into 5–6 meals with similar energy values, whereby fats should not exceed 60–80 g/day. This follows from the fact that bigger meals, especially rich in fats, can intensify pains experienced by the patient [98].

In case of diabetes, we apply a diabetic diet and pharmacological treatment [101]. Acceptable glycaemia is between 120 and 180 mg% since the simultaneously occurring absence of glucagon can lead to severe hypoglycaemia.

The treatment and prophylactics of pain includes undertaking attempts at treatment with pancreatic preparations in the hope that this will reduce the pressure in pancreatic ducts and hence prevent pain attacks [101].

Pains of short duration (of a few hours) and occurring rather rarely are treated extemporaneously by applying ordinary analgesics. If the pains are constant and do not disappear under the influence of those drugs, it is sometimes necessary to administer narcotic analgesics for some time. Then, it is recommended to start administering buprenorphine (in the form applied under the tongue) together with psychotropic drugs, which gives a smaller number of addictions. A satisfactory but unfortunately transient (3–6 weeks) pain relief effect is obtained after applying neurolysis of the visceral plexus through Xylocaine or alcohol injections under USG or CT control [101, 102].

If no satisfactory effects of this type of treatment are obtained for the period of about 3–6 months, and in particular if disease complications occur, endoscopic or surgical treatment should be considered [101]. Such treatment is aimed at decompressing the main pancreatic duct or cyst by their joining to the small intestine. Resections, most often aimed at sparing the duodenum (various variants), are rarer. Before decision on surgical treatment is made, a series of reference examinations should be made (CAT, echoendoscopy, MRCP or ECPW) in order to obtain the best possible identification of the morphological changes responsible for the pain (Fig. 27.2).

Endoscopic methods of treating CP have been being developed for many years now, but they still raise controversies, especially with regard to long-term effects. However, in specific clinical situations, they are an alternative to surgical treatment [2, 103]. These methods include pancreatic sphincterotomy, evacuation of pancreatic deposits, breaking of stones using an extraneous electromagnetic wave and prosthetics of the Wirsung's duct. Indications for their application include stricture in Oddi's sphincter, segmental stricture in the main pancreatic duct and presence of a deposit in it or deposits within the pancreas head. Good effects are also obtained by applying endoscopic treatment of pancreatic cysts projecting themselves into the stomach and the duodenum (cystogastrostomy and cystoduodenostomy). External drainage is most often applied only extemporaneously. If the procedure gives good results, conservative treatment is continued. In case of failure or unsatisfactory effects, the patient should undergo surgical treatment.

If symptoms of malnutrition appear, at the first stage, good effects are obtained by limiting intake of fats to about 60 g per day and administering vitamins soluble in fats (A, D, E, K) together with B group vitamins and folic acid [101]. When this conduct does not lead to normalization of motions and weight and/or evacuation of fats exceeds 15 g per day, we start enzymatic substitution, most often adjusting the dosage to clinical effects. Just in rare cases of very severe pancreatic insufficiency, in order to compensate for the deficiencies in fats, it is necessary to administer mid-chain triglycerides (MCT).

Antioxidants in Treatment of CP

The effects of conservative treatment of CP obtained up to now are still unsatisfactory [101]. In this chronic, progressive disease with insufficiently explained pathogenesis, treatment reduces in principle to eliminating the action of the causative factor (i.e. in case of the most common form of calcifying CP – stopping consumption of alcohol) and symptomatic treatment (i.e. combating pain) and at a later stage of the disease also to relieving the symptoms of failure in the external secretive action and deterioration of the internal secretive function of the pancreas.

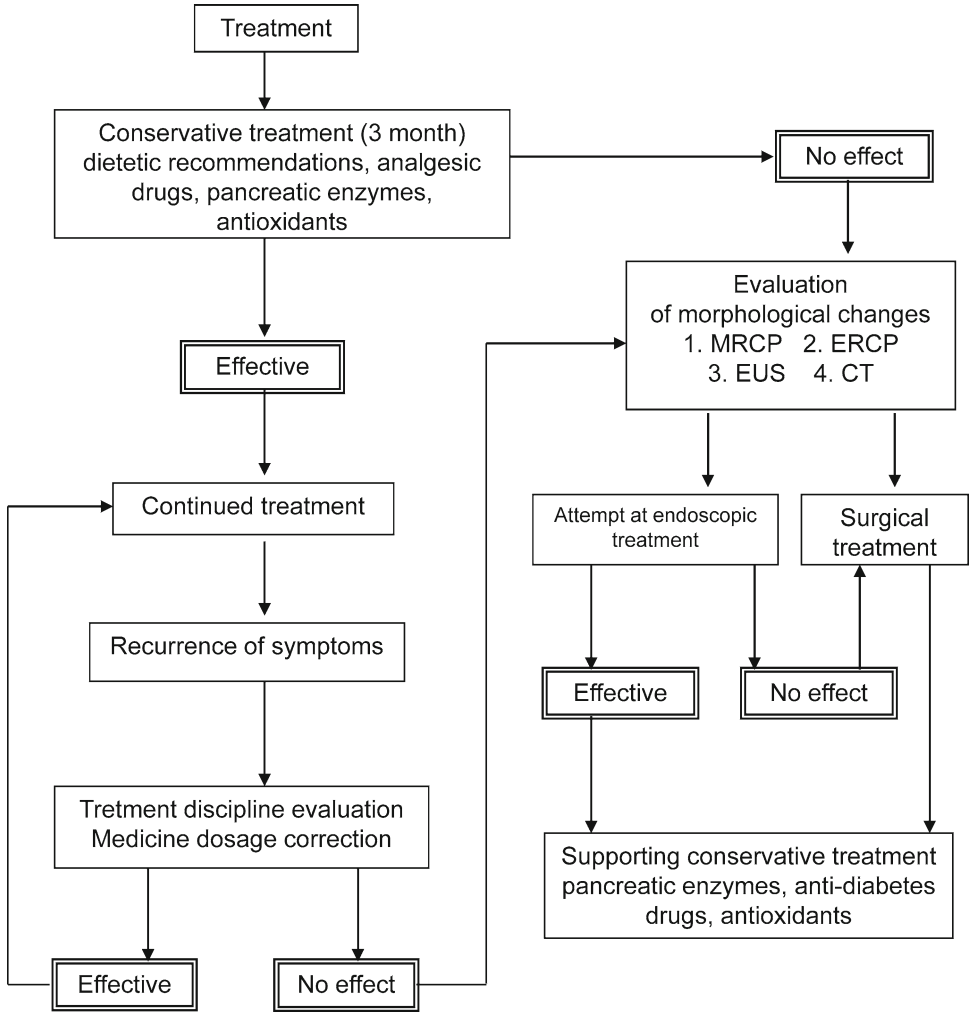

Fig. 27.2 Algorithm for the treatment procedure in chronic pancreatitis. *MRCP* magnetic resonance cholangiopancreatography, *ERCP* endoscopic retrograde cholangiopancreatography, *EUS* endoscopic ultrasound, *CT* computed tomography (Modified from Ref. [101]. With permission from Cornetis)

Studies on the use of antioxidants in treating CP have been conducted for many years now [9–12]. However, this is difficult for several reasons. First of all, it is difficult to select patients with comparable degrees of morphological changes advancement and intensification of clinical changes. The disease is rather rare, so qualification of patients to studies takes a relatively long time. Another important element is cooperation, which is often insufficient with regard to treatment discipline. A quite large group of patients are addicted to alcohol and smoking – factors which disturb the treatment and observation process and are reflected, regardless of the applied medicines, on the occurrence of clinical symptoms, morphological changes in the pancreas and results of biochemical tests. Hence, a relatively large group of patients must be excluded from the studies in order to ensure objective evaluation of the treatment results.

The basis for attempts at treatment with antioxidants were observations that an important element of the CP pathogenesis may be disturbances in the oxidation–antioxidation balance [8]. Most probably, as shown by some studies, Braganza's free radical theory is the key to explaining many biochemical changes in the blood and morphological changes in the pancreas. According to that Braganza's theory,

oxygenic free radicals released during oxidative stress in the course of AP after, e.g. experimental infusion of free fatty acids, following stimulation with secretin in presence of partial obstruction of the pancreatic duct and after ischaemia (reperfusion) are the cause for obstructing internal cellular metabolic paths, joining lysosomes with zymogen (preenzyme) inside pancreatic cells, activation of proteo-lipolytic enzymes and oxidation of fats accompanied by production of the appropriate fatty peroxides [104–106]. A consequence of this would be atrophy of glandular tissue and development of fibrous tissue, resulting in morphological changes in the major and minor pancreatic ducts and gradually increasing deterioration in the external and internal secretive function of this gland.

Evidence confirming this hypothesis has been collected, showing heightened levels of lipid peroxidation markers and lowered levels of antioxidative vitamins in chronic pancreatitis. Decreased concentrations of antioxidative vitamins and other antioxidants (selenium, methionine) among CP patients have been shown, among others, in the studies by Braganza et al. [107], Uden et al. [10], Sandilands et al. [9] and Morris-Stiff et al. [108].

Interesting observations were made by Quilliot et al. [109], who fed CP patients with tomato paste (source of lycopene). Most of them had deficiency of carotenoids. After an intervention period of 8 ± 2 months, lycopene concentration increased twice. Despite malabsorption, it was possible to increase carotenoid plasma concentration by increasing carotenoid intake.

Our own studies have confirmed that individuals with CP exhibit significantly (about twice) lower levels of vitamins C and E in the blood serum [110]. This is most probably caused by three factors: low intake of those vitamins, their utilization in pathophysiological processes, especially in the processes of neutralizing free oxygen radicals, and, among a large part of the patients, also by smoking. After applying for 6 months the standard treatment and administering additionally vitamin C (at the dosage of 2×200 mg per day) and vitamin E (at the dosage of 2×150 mg per day), increased serum concentration of those vitamins was noted compared to the correct values. Moreover, application of antioxidants helped improve the effectiveness of CP patients' treatment through reducing the pain and the frequency of AP episodes as well as improving the external and internal secretive actions of the pancreas.

The study by Kirk et al. [11] used the combination of antioxidants (selenium, β(beta)-carotene, L-methionine and vitamins C and E) in CP patients. In this trial, pain was reduced after 10 weeks of the treatment. The quality of life, physical and social functioning and health perception were also enhanced as a result of antioxidant therapy.

In 2009, a placebo-controlled double blind trial reported good results in pain relief using antioxidant supplementation on a large number of chronic pancreatitis patients ($n = 147$) [12]. In that study, consecutive patients with chronic pancreatitis were randomly assigned to groups which were given either placebo or antioxidants (selenium – 600 μg/d, ascorbic acid – 0.54 g, β(beta)-carotene – 9,000 IU, α(alpha)-tocopherol – 270 IU, methionine – 2 g) for 6 months. The reduction in the number of painful days per month was significantly higher in the antioxidant group compared to the placebo group. The reduction in the number of analgesic tablets per month was also higher in the antioxidant group. Furthermore, 32% and 13% of patients became pain-free in the antioxidant and placebo groups, respectively. Thus, the results of this study seem to confirm that antioxidant supplementation is effective in relieving pain and reducing levels of oxidative stress in patients with chronic pancreatitis.

The mentioned studies showed that serum concentrations of the above-mentioned antioxidants were higher after a period of intake, and those laboratory indices of oxidative stress markers, such as lipid peroxidation, free radical activity and total antioxidant capacity, improved after the therapy.

Conclusions

The results of numerous studies allow us to state that administering antioxidants, especially vitamins C and E, to CP patients is justified for two reasons. The first of them, not raising any essential doubts, is the fact that a decisive majority of patients exhibit a considerable deficiency of those vitamins in the

blood serum – and a chronic deficiency of those vitamins in the body may lead to many negative consequences for the health [111, 112]. Secondly, as shown by the quoted studies, the use of antioxidants has beneficial influence on the course of the disease [11, 12, 110]. Most probably, increase in the antioxidative potential of the body increases its capability of destroying free oxygen radicals, which probably play an important role in damaging the pancreas.

Among CP patients, disturbances in the oxidation–antioxidation balance depend not only on the increased production of oxidating compounds but also on the deficiency of antioxidants. Very often, a contributing factor is a very low concentration of antioxidative vitamins, caused on the one hand by their low intake and on the other hand by their higher utilization in the course of pathological processes in the pancreas and often also by coexisting tobacco smoking. Vitamins C and E seem to be very effective antioxidants occurring in large quantities in the body, and in case of vitamins C, they have regenerative capabilities [113, 114]. Application of these vitamins allowed for improving the balance in the cytochrome P450–antioxidants system.

Though explanation of the free radicals theory in the CP pathogenesis is difficult, and we still do not possess convincing evidence from experimental studies, the attempts at applying antioxidants in this disease made up to now indicate benefits of their use. However, for the time being, there are no established views which antioxidants should be best used and in what doses. Attempts at treatment with vitamins A, C and E, with vitamins A and E, and even with a selection of antioxidants (selenium, vitamins A, C and E) have been undertaken. Allopurinol has also been applied. However, we cannot carry out an objective comparative evaluation of the results due to the differences in the sizes of patient groups qualified to the studies and in the selection criteria as well as methods for evaluating treatment effectiveness. The doses of medicines applied in the studies are usually contained between those covering the daily demand and the highest safe doses.

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Chapter 28
Alcohol Consumption, Lifestyle Factors, and Type 2 Diabetes

Martin D. Stricker, Henk F.J. Hendriks, and Joline W.J. Beulens

Key Points

- The prevalence of diabetes mellitus patients is expected to rise from 220 million in 2011 to 366 million in 2030.
- Important risk factors for diabetes mellitus type 2 are obesity, physical inactivity, suboptimal dietary intake, and smoking.
- Light to moderate alcohol consumption, i.e., 10–30 g alcohol per day, is associated with a ≈30% decreased risk of type 2 diabetes. Although beverage type does not influence this association, drinking patterns do: more frequent drinking leads to greater risk reductions, while bingeing was found to increase the risk.
- Alcohol consumption varies by age, gender, and country and is related to diet and lifestyle factors. Persons meeting 3 or more other low-risk lifestyle behaviors and drink in moderation, however, still have a lower risk of DM2 compared to teetotalers.

Keywords Alcohol • Type 2 diabetes • Demographic and lifestyle factors

Introduction

In 2011, the World Health Organization estimated that 220 million people have diabetes mellitus (DM) [1], and this prevalence is expected to rise to 366 million by 2030 [2]. Already in 2000, the lifetime risk of developing DM was estimated to be 38.5% for US women and 32.8% for US men [3]. Consequently, the worldwide burden of DM is growing at a rapid pace, challenging public health settings and letting health-care costs skyrocket. The American Diabetics Association estimated an increase of direct health-care spending for DM management in the United States from $92 billion in 2002 to $138 billion in 2020 [4].

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DM is associated with several macro- and microvascular complications and an overall doubled risk of dying [1]. Besides being an important predictor for coronary artery disease and stroke, it is also among the leading causes of renal failure. Furthermore, damages to nerves and to the retina of the eye are common microvascular complications which can further lead to foot ulcers, pain, numbness, and tingling in the hands or feet, limb amputations, and visual impairment [1].

Pathogenetically, DM is characterized by elevated blood glucose levels (hyperglycemia) which manifests either because the body is unable to produce insulin (type I) or due to decreased insulin sensitivity and abnormal insulin secretion (type 2). Type 2 diabetes (DM2) accounts for 90% of all DM cases [1]and is the result of a complex interplay between genetic predisposition and exogenous factors [5]. Evidence indicates that a suboptimal lifestyle could possibly outweigh genetic susceptibility in causing DM2 [5]. Randomized controlled trials showed that lifestyle interventions can decrease the risk of developing DM2 by 58% [6, 7], and 91% of all DM2 cases in the Nurses' Health Study were attributable to an unhealthy lifestyle [8]. Finally, changes in the incidence of DM2 have occurred over a short duration of time, strengthening the argument that environmental changes rather than genetic causes are responsible for the steep increase of DM2 patients [9].

Lifestyle, Nutrition, and Incidence of Type 2 Diabetes

Considerable efforts have been made to identify lifestyle factors connected to the development of DM2. Obesity, physical inactivity, unhealthy diet, and smoking were found to be independent predictors. A study of 24,150 English adults associated the achievement of five behavioral goals, i.e., body mass index (BMI) <25 kg/m^2, physical activity >4 h/week, total fat intake <30% of total energy intake, saturated fatty acids intake <10% of total energy intake, and fiber intake ≥15 g/1,000 kcal, with the incidence of DM2 and found a significant inverse relation. None who met all five criteria developed DM2. The authors estimated that the incidence of DM2 would decline by 20% if the whole population met at least one of the goals [10]. Hu et al. and Mozaffarian et al. confirmed these findings. Participants whose BMI, fiber and fat intakes, physical activity, smoking status, and alcohol consumption were in the low-risk group had a 91% and 89%, respectively, decreased risk of developing DM2 [8, 11].

Obesity is the most important predictor of DM2, accounting for 60–90% of the risk variance [12]. The risk increase is mainly attributable to intrahepatic and intra-abdominal fat stores [13], and BMI and waist circumference (WC) were identified as independent predictors [14]. In the Health Professionals Follow-Up Study, participants in the highest quintiles of BMI (>27.2) and WC (>101.6 cm) had a 2.7 (1.9–3.7) and 4.5 (3.0–6.7), respectively, times higher risk for developing DM2 than participants in the lowest quintiles (<22.8; <86.4) in a multivariate adjusted model [15].

These findings are corroborated by the successful prevention or delay of DM2 through lifestyle interventions [6, 7, 16, 17]. One of them is the Diabetes Prevention Program (DPP), a randomized controlled trial of 3,234 nondiabetic persons at high risk for DM2 [6]. An intensive, individualized lifestyle intervention with goals of ≥7% weight reduction through a healthy low-calorie, low-fat diet and physical activity of at least 150 min/week decreased the incidence of DM2 by 58% compared to standard lifestyle recommendations after 3 years of follow-up. Further analysis revealed that this risk reduction was mainly achieved through weight loss; there was a 16% reduction in risk for every lost kilogram of weight [18]. A comparison between the 90th and the 10th group of weight loss showed a 96% decreased risk in the former group, indicating that people losing even more than 5–7% body weight and meeting dietary and physical activity goals could reduce their risk by more than 90% [18, 19].

Physical Inactivity

Although physical inactivity and weight gain are closely connected and weight loss has a greater effect on reducing DM2 risk than being physically active, they are both independent predictors [19]. This appears from results of the Nurses' Health Study in which sedentary, obese women had a 16-fold higher risk for DM2, and lean, but inactive, women still a twofold increased risk compared to physically active women with normal body weight (BMI <25) [20]. The Finnish Diabetes Prevention Study confirmed these results. In the group of people who received lifestyle intervention but failed to reduce ≥5% of the initial body weight during 1 year, physically active participants had an odds ratio of 0.2 (0.1–0.6) for DM2 compared to those who stayed sedentary [19].

Nutrition

An unhealthy diet represents another independent risk factor for DM2, which remains significant after controlling for BMI [21]. Traditionally, single nutrients such as dietary fiber, fatty acids, or sugar have been associated with the incidence of DM2 and reviews indicate that high intakes of saturated and trans fat [13], sugar-sweetened beverages [22], and low-fiber products with high glycemic indices (GI) [12, 13] are associated with an increased risk of DM2. Conversely, high consumptions of poly-unsaturated fat [13] and whole grain products [12], which contain typically lower GIs than refined cereals, lead to a reduced risk of DM2. Interestingly, risk reductions were observed to be stronger for fiber from cereals than from fruits or vegetables [12].

These findings are corroborated by the results of dietary pattern analyses, i.e., combinations of food groups and nutrients into eating patterns. A "Western" pattern consisting of high intakes of red meat, processed meat, French fries, high-fat dairy products, refined grains, and sweets and desserts was related with an increased risk of DM2 (1.59; 1.32–1.93) and a "prudent" pattern characterized by a higher consumption of vegetables, fruit, fish, poultry, and whole grains with a decreased risk of DM2 (0.84; 0.70–1.00) [23].

Smoking is the final classical lifestyle risk factor for DM2. A meta-analysis including 25 studies with a study period range from 5 to 30 years revealed a clear positive dose–response relation [24]. The pooled adjusted relative risk for current smokers was 1.44 (1.31–1.58) compared to nonsmokers, and heavy smokers (>20 cigarettes/d, RR 1.61, 1.43–1.80) had a higher risk than lighter smokers (1.29; 1.13–1.48). Moreover, former smokers (1.23; 1.14–1.33) had a lower risk than current smokers. This risk increase is believed to be independent from other risk factors. A large prospective study of 18,831 Swedish and Finnish participants confirmed that smoking remains a risk factor (1.39; 1.10–1.61) after adjustment for biological and genetic predictors [25]. Additionally, the beneficial effect of smoking cessation appears to outweigh its effect on weight gain [13].

Alcohol Consumption and Type 2 Diabetes: Is There an Association?

Besides weight, dietary intakes, physical activity, and smoking, alcohol consumption could be regarded as another lifestyle factor influencing DM2 risk. In recent decades, associations of alcohol intake on DM2 incidence have extensively been studied, and current evidence consistently shows a U-shaped relation. In 2009, Baliunas and colleagues conducted a meta-analysis which included 20 prospective cohort studies with 477,200 individuals and 12,556 incident cases of DM2 (results are presented in Fig. 28.1) [26]. Among women, consumption of 24 g alcohol per day was most protective with a risk reduction of 40% (0.52–0.69) and became deleterious at 50 g/day (1.02, 0.83–1.26),

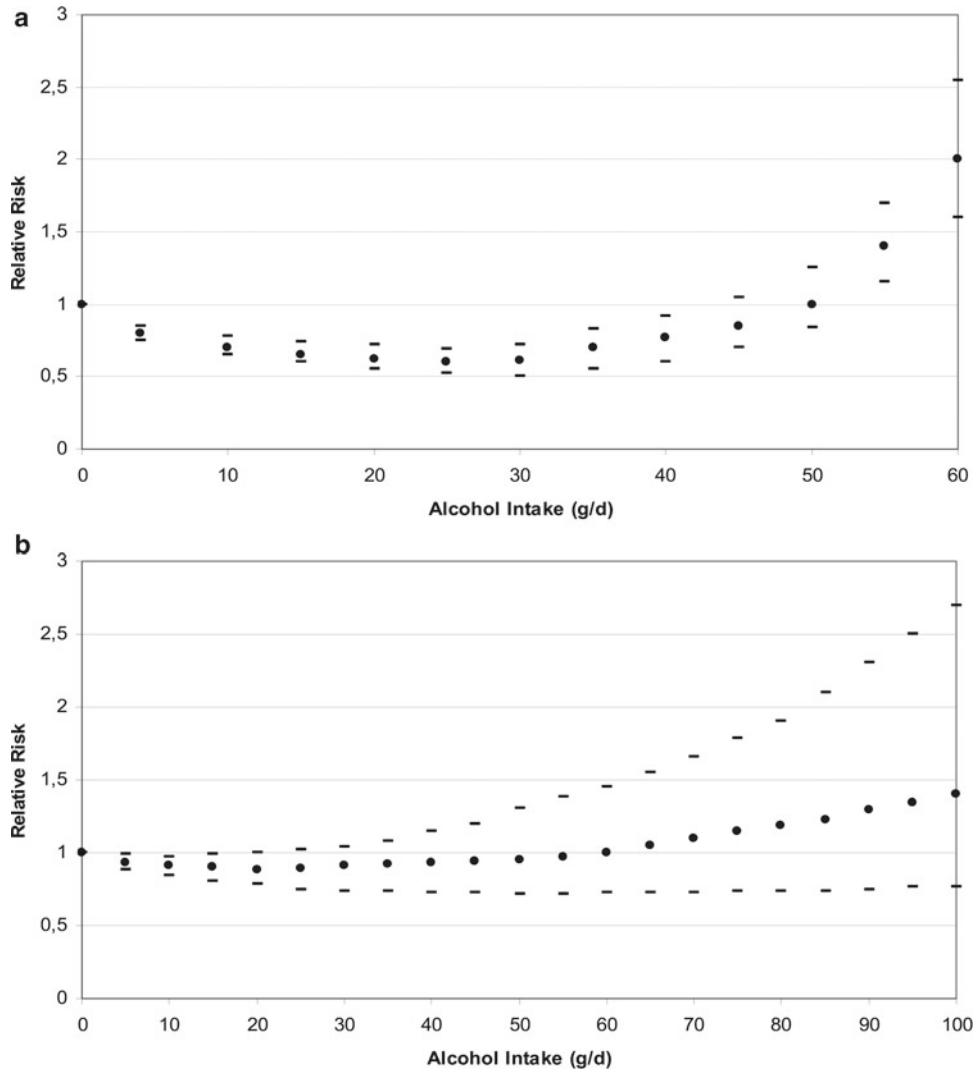

Fig. 28.1 Association of alcohol consumption with DM2 risk, pooled and fitted relative risks, and 95% confidence interval bands for women (**a**) and men (**b**) (Based on data from ref [26])

compared to lifetime abstainers. Risk reductions for men were lower compared to women, i.e., an intake of 22 g/day was optimal and related to a 13% decreased risk of DM2 (0.76–1.00), and consumption of just over 60 g/day became detrimental (1.01, 0.71–1.44).

These results are mostly in line with a previous meta-analysis, carried out by Koppes et al. in 2005 [27]. Compared to nonconsumers, low to moderate consumption led to significant inverse associations among women, i.e., intakes of ≤6, 6–12, and 12–24 g/day were associated with relative risks of 0.81 (0.75–0.88), 0.59 (0.54–0.64), and 0.55 (0.47–0.65), respectively. Among men, risk reductions were less pronounced and only moderate consumptions led to lower risks of DM2, i.e., for 6–12, 12–24, and 24–48 g/day, relative risks of 0.80 (0.71–0.90), 0.75 (0.60–0.95), and 0.71 (0.60–0.83) were found. Higher consumption (>48 g/day) was observed to be deleterious (1.06; 0.86–1.32). In contrast with the meta-analysis of Baliunas, inverse associations among men were statistically significant and much stronger (29% vs. 13%). The reason for this difference could lie in the definition

of the reference category. While Koppes used former drinkers and lifetime abstainers as reference, Baliunas used lifetime abstention, therefore addressing the sick-quitter effect. Former drinkers may have quit drinking because of health reasons and are actually more vulnerable for developing DM2. Ignoring this effect would lead to overestimation of the beneficial effects of moderate alcohol consumption [26].

In combination with these results, several limitations should be mentioned [26, 27]. First, misclassification of alcohol consumption cannot be ruled out, although the validity of alcohol intake measurements is generally good [28]. If, however, errors occurred, they are likely to be present as under-reporting. Therefore, the amount of alcohol associated with the lowest risk of DM2 would be higher in reality. Second, the presence of diabetes was ascertained in various ways in the different studies. Koppes et al. [27] found lower RR estimates for studies using self-reported DM2 status compared to studies based on population testing. Finally, most studies included in the meta-analyses were conducted in Western countries. This could possibly narrow down the generalizability of the results. The few studies performed in Asian countries, predominantly in Japan, yielded, however, comparable results.

Taken together, current evidence suggests a ≈ 30% decreased risk of DM2 for light to moderate alcohol consumption, i.e., 10–30 g ethanol per day, compared to abstention. The effect, however, seems to be stronger among women than men.

The Underlying Mechanism

It is generally accepted that DM2 develops through a combination of decreased insulin sensitivity and abnormal insulin secretion of the pancreas [13]. Obesity is linked to a chronic low-grade inflammatory state and an abnormal adipose secretion of adipocytokines such as leptin and adiponectin, which leads to impaired insulin signaling and hyperglycemia [29]. Additionally, chronic inflammation, accumulation of lipids in pancreatic islets, and hyperglycemia are believed to cause progressive failure of pancreatic β cells [13].

The mechanism by which moderate alcohol consumption intervenes into the pathogenesis of DM2 has not clearly been elucidated yet. There are various possible pathways. First, the intake of alcohol could improve insulin sensitivity through an increase of adiponectin [30–32] and leptin [33] concentrations. Although cross-sectional studies consistently show a positive association between alcohol intake and insulin sensitivity, results from intervention studies are discordant [34]. While Davies et al. [35] reported a sensitivity increase by 7.2% ($P = 0.002$) after consumption of 30 g/day and Joosten and colleagues [32] a significant decrease of insulin resistance ($P = 0.02$) for an intake of 25 g/day, other studies failed to confirm these findings [30, 36–39]. These inconsistencies could be explained by the longer duration of alcohol consumption in the studies of Davies and Joosten, i.e., 8 and 6 weeks, respectively, compared to 30 days or less, or by gender differences. Davies and Joosten included postmenopausal women, while other studies contained exclusively men [30, 36–38] or premenopausal women [39].

The anti-inflammatory properties of alcohol present another plausible pathway. Studies in mice suggest that alcohol oppresses inflammatory and increases anti-inflammatory factors by gene regulation [40], and as stated above, moderate alcohol consumption improves adiponectin levels, which is known to act as an anti-inflammatory [41]. Beulens et al. confirmed that the risk-lowering effect of moderate alcohol consumption is mediated by adiponectin; it accounted for 25–29% of the association [42]. Different markers of inflammation, e.g., C-reactive protein (CRP) and fibrinogen, have also been shown to be reduced through moderate alcohol consumption [43, 44]. A recent review, however, concluded that while associations with lower fibrinogen are consistent, other markers including CRP led to less constant results [45].

Finally, moderate alcohol consumption may decrease postprandial glucose responses. Brand-Miller et al. recently confirmed that alcohol intake alone, with or before a carbohydrate-containing

meal, reduces postprandial glycemia by up to 37% in lean healthy men and women, indifferent of the drink (beer, wine, or gin) consumed [46]. Interestingly, insulin levels were unchanged indicating an acute enhancement of glucose metabolism as underlying mechanism.

In conclusion, improvements in insulin sensitivity, a decrease of postprandial glucose responses, and anti-inflammatory properties present possible pathways how alcohol consumption lowers the risk of DM2. The mechanism, however, remains to be investigated.

Beverage Type: Is There a Difference Between Wine, Beer, and Liquor?

Despite consistent results, debates remain whether the inverse association between the consumption of alcohol and DM2 is attributable to alcohol itself or to other substances contained in alcoholic beverages. Many studies have therefore tried to disentangle effects of different alcoholic beverages, but reported inconsistent results. While some studies found significant risk reductions only for wine [47, 48] and more deleterious effects of high liquor – than beer – or wine consumption [49, 50], others observed no influence of the type of beverage. A study of male Americans showed no differences between beer, white wine, and liquor, i.e., adjusted relative risks for a 15-g increment were 0.70 (0.60–0.81), 0.74 (0.62–0.88), and 0.75 (0.66–0.84), respectively [51]. Moderate red wine consumption was also inversely associated with DM2 incidence, although not significantly (0.92; 0.77–1.09). Similarly, Wannamethee et al. reported in a study of 109,690 women adjusted relative risks of 0.53 (0.28–1.00), 0.62 (0.43–0.89), and 0.66 (0.45–0.96) for a daily consumption of 5–29.9 g of alcohol from wine, beer, or liquor, respectively, compared to abstention [50]. Finally, a study of 5,888 men and women aged ≥65 years reported comparable risk reductions for wine (0.6; 0.4–0.9), beer (0.7; 0.4–1.1), and liquor drinkers (0.6; 0.4–0.9) [52]. Inconsistencies in these results could be due to power issues, i.e., in many populations, certain beverages are less consumed than others. It has been observed that the predominantly consumed beverage type in a certain population is often most strongly associated with disease risk [53]. In any case, it is difficult to distinguish effects of different alcoholic beverages since alcoholic drinks are rarely consumed in isolation. Randomized controlled trials may therefore provide further indications. Davies and colleagues [35] found that the consumption of orange juice with ethanol improves insulin sensitivity, in comparison with pure orange juice. Secondly, Brand-Miller et al. [46] found comparable reductions of postprandial glycemia for beer, wine, and gin in a trial of Australian students. Finally, Imhof et al. [31] investigated effects of different drinks on adiponectin levels and reported no differences. Altogether, these trials indicate that the type of alcoholic beverage may not influence the association between alcohol and DM2 and that the beneficial effects of moderate consumption would consequently be ethanol-mediated.

The Influence of Drinking Patterns

The way alcohol is consumed, i.e., equally distributed over the week or primarily during the weekend (bingeing), is related to various health outcomes [26]. For this reason, drinking patterns are likely to influence associations with DM2, and indeed, it has been shown that more frequent alcohol drinking leads to greater protections of DM2. In the Health Professionals Follow-Up Study of 45,892 men, each additional drinking day per week lowered the risk of developing DM2 by 7% (3–10%), after adjustment of average daily consumption. The highest risk reduction was observed for light drinking (<1 drinks/day) on more than 5 days per week (0.48; 0.27–0.85), compared to nondrinkers [51]. These results are well in line with a study of Japanese men where light to moderate alcohol consumption on 4–7 drinking days/week was related to the highest risk reduction compared to abstention (0.74; 0.58–0.95) [54].

Wannamethee et al. further confirmed these findings among women. The authors reported that a moderate intake of alcohol (5–29.9 g/day) was associated with a lower risk when consumed more frequently (4–7 day/week) than when the same amount was taken over 1–3 day/week [50].

Conversely, bingeing, i.e., alcohol consumption of ≥210 g over 1–3 drinking days, was related to a fivefold increased risk of DM2 in men while consumption of the same amount distributed over a week did not influence the risk [48]. Similarly, binge drinking doubled the risk of DM2 among women (2.1; 1.0–4.4) in the Finnish Twin Cohort [55]. However, bingeing was not associated with DM2 in men. More studies are needed to fully understand the effect of binge drinking on the risk of DM2 and to elucidate whether there are differences between men and women.

In summary, drinking patterns seem to influence the association of alcohol intake on DM2 risk. More frequent drinking of low to moderate quantities is associated with greater risk reductions, while bingeing was found to increases the risk.

Alcohol Consumption and Demographic or Lifestyle Characteristics

Despite the consistent evidence, critics have questioned the beneficial effects of moderate alcohol consumption on disease outcome [56]. It has been argued that these associations could be confounded by healthier lifestyles or other characteristics of moderate drinkers compared to abstainers. Although most studies adjusted for such lifestyle factors, the possibility of residual confounding cannot be ruled out. This section therefore summarizes demographic characteristics and lifestyle in relation to alcohol consumption.

Beverage Preference and Drinking Patterns: Variation by Age, Gender, and Country

The "Substance Abuse and Mental Health Services Administration" surveys annually 67,500 US-American persons on their habitual drugs, alcohol, and tobacco use. Results from 2009 indicate that alcohol intake increases dramatically during adolescents and declines gradually during adulthood [57]. The percentage of current drinkers rose from 3.5% for persons aged 12 or 13 to 70.2% for those aged 21–25, before leveling off to 39.1% among people aged ≥65. These rates of alcohol consumption were modified by gender. More men than women drank alcohol on a regular basis, i.e., 57.6% of males and 46.5% of females were current drinkers. However, among female and male youths aged 12–17, alcohol consumption rates were very similar (15.1% vs. 14.3%) [57]. Regarding beverage preference, liquor and beer were the most prominent drinks among male adolescents and malt beverages, wine coolers, and wine among female teenagers in a study of 24,600 students from eight US-American states [58].

These results are well in line with a study of Russian men and women aged 45–69 [59]. Men were found to consume alcohol more frequently, with drinking at least once a week being reported by 52% of men and 9.5% of women. The annual intake of alcohol was also much higher among men, i.e., ≥3 l of pure alcohol was consumed by 41.6% of men and only 2.7% of women [59]. The European Prospective Investigation into Cancer and Nutrition (EPIC) study which included almost 36,000 persons aged 35–74 from 10 different European countries further confirmed that women drink lower quantities of alcohol [60] and that alcohol consumption decreases with age, excluding some Mediterranean countries where it was found to rise in the oldest age category [61]. Additionally, the authors concluded that women drink more slowly and more often with meals and have different preferences to men regarding to the type of alcoholic drinks [60]. Gender differences, drinking patterns, beverage preference,

and total alcohol intakes, however, differ significantly by country [60–63]. Highest alcohol consumptions were reported for eastern European countries, i.e., Lithuania consumed 17.2 l of pure alcohol per capita, followed by Latvia (16.5 l) and Slovakia (16.4 l). These countries were characterized by high consumptions of beer and spirits. Lowest recorded consumptions were observed for Bulgaria (9.4 l, wine/spirits), Slovenia (10.1, beer), and Nordic countries (10.2 l, beer). More spirits were generally consumed in eastern European countries. Moreover, the proportion of abstainers or very light drinkers was much higher among women compared to men in all countries [63].

In conclusion, alcohol consumption differs markedly by gender, age, and country. Alcohol intake peaks in young adulthood and decreases with age. Men drink higher quantities and more frequently and have different preferences compared to women regarding to the type of alcoholic drinks. Finally, eastern European countries were found to consume the highest amount of alcohol.

Alcohol Consumption in Relation to Diet and Lifestyle Factors

Alcohol intake has extensively been related to diet and lifestyle factors. Results reported, however, showed inconsistencies, possibly due to imprecise nutrient measurements, differences in assessing and categorizing alcohol intake and/or cultural differences between study populations [13, 60, 61]. Nevertheless, many interesting associations were reported. Compared to non- or lighter drinkers, heavier drinkers were observed to consume more protein and fat and less carbohydrates (in percent from energy) [61, 64–68]. However, a study on Scottish men reported an inverse association between alcohol consumption and total fat, saturated fat, and MUFA [69]. Clustering between the use of alcohol and low intakes of vegetables and fruits have further been reported [70, 71], while another study found an inverse association with an unhealthy diet [72]. Regarding to total energy intake, alcohol energy is believed to be largely additive to the normal diet [73], although results have been discordant. While one study found a decrease in total energy intake for increased alcohol consumption [74], most others reported an increase [64, 67, 75–77]. When energy from alcohol was excluded, to evaluate whether more or less energy from other nutrients is consumed, results diverged even more [64, 68, 74, 76, 77]. These differences could be due to cultural/geographical variations. The EPIC study reported higher total and nonalcohol energy intakes for heavier drinkers from Mediterranean countries, compared to abstainers, but lower energy intakes for those from Scandinavia [61]. Since energy intake is closely related to weight and alcohol is relatively energy dense, effects on BMI are expected. However, while alcohol consumption consistently showed an inverse association with BMI in women, its relationship was less consistent in men [61].

Furthermore, studies on clustering of different diet and lifestyle risk factors have consistently reported positive associations of alcohol consumption and smoking, but discordant results for physical activity [70–72]. While some studies found higher alcohol consumption to be positively related with physical activity [71, 78, 79], others reported no significant association [70, 72].

Finally, educational level and socioeconomic status (SES) were inconsistently associated with alcohol intake. While some studies found decreased educational levels along with increasing alcohol intakes in both genders [80–82], others found no significant association in women [83, 84] or in men [85]. Similarly, heavier alcohol consumption has been associated with lower SES [64, 86, 87], or with higher SES [85]. Possible explanations for these differences are different assessments and categorization of alcohol intake, educational level and SES, geographical variation, and/or different age of the study populations. The EPIC study [61] and a multinational study containing 15 European and non-European countries [88] showed both differences between men and women and between countries in the association of alcohol intake with educational level. Furthermore, the Ontario Student Drug Use Survey reported that associations vary with age, i.e., associations of higher SES with less harmful drinking were more pronounced among younger than older adolescents [89]. This is in line with a review on characteristics of binge drinkers in Europe which concluded that more pocket money or

lower alcohol prizes lead to higher binge rates among adolescents and economic stress, e.g., unemployment, and a low level of education to more binges among adults [90].

In conclusion, heavier alcohol consumers were found to have unhealthier diets and to be more often smokers and possibly physically more active compared to non- or lighter drinkers. Associations with total and nonalcohol energy intake, BMI, socioeconomic status, and education were quite inconsistent. Variations by country, sex, and age are possible explanations.

Diet and Lifestyle Factors in Relation to Beverage Preference

Associations between alcohol consumption and diet or lifestyle characteristics may, however, also depend on the preference of alcoholic beverage. Current evidence suggests an association between wine preference and healthy diet and beneficial lifestyle behaviors. The American UNC Alumni Heart Study reported that wine drinkers are less likely to smoke, but eat more fruit and vegetables and consume less red or fried meat compared to beer or spirit drinkers or those who had no preference [91]. Moreover, dietary intakes of wine drinkers contained less cholesterol, saturated fat, and more fiber. These results are well in line with those of the French MONICA study [64]. Wine drinkers were older, more physically active, and less often smokers than beer or mixed drinkers. Furthermore, a preference for wine was associated with higher intakes of vegetables, fruits, bread, eggs and milk, and soft cheese and lower consumptions of potatoes compared to beer. A Danish study [92] further corroborated these findings. Wine drinkers were observed to consume more fruit, fish, cooked vegetables, and salad and use olive oil more frequently compared to consumers of other alcoholic drinks. However, results of the Spanish SUN cohort study are only in partial agreement with these studies, reporting higher intakes of fiber and olive oil and lower consumptions of fat (only in men), dairy products, fast food, and sugared soda drinks for wine drinkers compared to other beverage alcoholic groups or abstainers [93]. By contrast, intakes of fruit, vegetables, cereals, and whole grains were not increased. According to the authors, the differences in results in comparison to the American and the Danish study could lie in the fact that wine is consumed by all social classes in Spain, whereas in other countries it is expensive and mainly purchased by individuals belonging to higher socioeconomic levels who are more likely to have healthier lifestyles. However, controlling for income and education did not alter the associations in the UNC Alumni Heart Study.

Beverage preference has further been associated with social, cognitive, and personality characteristics. Danish wine drinkers were observed to have higher IQs, higher parental educational levels, and higher socioeconomic statuses compared to non-wine consumers [94]. Beer drinking was significantly associated with lower scores on the same characteristics. It has further been confirmed that the association between wine consumption and higher IQs is irrespective of the socioeconomic status [95].

In conclusion, wine drinkers were observed to have healthier diets, beneficial lifestyle behaviors, and better social, cognitive, and personality characteristics compared to consumers of other types of alcoholic drinks and nonconsumers. These associations, however, are likely to vary by country, and therefore, more studies with different populations are needed.

Combined Effects of Alcohol Consumption with Lifestyle Behaviors on Type 2 Diabetes

We have seen that demographic and lifestyle characteristics differ significantly between drinkers and abstainers and among groups of different beverage preference. Moreover, moderate alcohol consumption has been associated with healthy dietary intakes and lifestyles [64]. The concern of critics who

question the beneficial effects of moderate alcohol consumption is therefore justified. For this reason, Joosten and colleagues examined the combined effect of alcohol consumption and lifestyle behaviors on DM2 risk in the Dutch EPIC cohort [96]. The authors defined low-risk categories of five lifestyle factors, i.e., moderate alcohol consumption, BMI <25, physical activity ≥30 min/day, current non-smoker, and healthy diet. The association of alcohol consumption with DM2 was investigated within strata of these categories. Moderate alcohol consumers meeting ≥3 other low-risk behaviors had a hazard ratio of 0.56 (0.32–1.00) for developing DM2 compared to teetotalers, also meeting ≥3 low-risk behaviors. Similar inverse relations were observed for the 2 or 1 other low-risk factor strata. This indicates that the association between moderate alcohol intake and DM2 risk is not driven by confounding due to other lifestyle characteristics [96].

Summary and Conclusion

The worldwide burden of DM is growing at a rapid pace. Experts estimated the number of patients to increase from 220 million in 2011 to 366 million in 2030. Important risk factors are physical inactivity, suboptimal dietary intake, smoking, and – most importantly – obesity, which accounts for 60–90% of the risk variance. Moderate alcohol consumption, on the other hand, has been found to have a risk-lowering effect on DM2. Current evidence suggests a ≈ 30% decreased risk for light to moderate alcohol consumption, i.e., 10–30 g of alcohol per day, compared to abstention, although the effect has been shown to be stronger among women than men. This risk reduction is not affected by the type of beverage consumed indicating that ethanol itself is responsible for the beneficial effects. Drinking patterns, on the other hand, seem to have an influence. Greater risk reductions were observed for more drinking days and bingeing was found to be associated with an increased risk.

Despite the consistent evidence, critics have questioned the beneficial effects of moderate alcohol consumption, arguing that the associations are confounded by healthier characteristics of moderate drinkers compared to abstainers. However, a study investigating the combined effects showed that moderate consumers with a low-risk profile have still a lower risk of DM2 compared to teetotalers.

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Chapter 29
Alcohol, Overweight and Obesity

Sasiwarang Goya Wannamethee

Key Points

- Alcohol is metabolized primarily by the liver and used immediately as energy or stored in the liver or in the rest of the body as fat.
- Evidence from cross-sectional and prospective studies suggests that high alcohol intake (≥ 3 drinks/day; ≥ 30 g alcohol) is associated with increased abdominal adiposity and weight gain.
- The association between alcohol and adiposity appear to be greater for abdominal adiposity (waist circumference or waist to hip ratio) than for general adiposity (BMI).
- There is no clear evidence that the effects of alcohol differ according to the type of drink and that wine protects against abdominal fat deposition.
- Wine drinkers tend to have more favourable dietary patterns and lifestyle characteristics than other drinkers.
- The effects of alcohol on adiposity may be influenced by dietary patterns, lifestyle characteristics and amount and pattern of drinking.

Keywords Alcohol intake • Body mass index • Fat distribution • Type of drink • Weight gain

Introduction

Increased body weight and, in particular, abdominal obesity is associated with increased cardiovascular disease risk [1]. In many developed countries, the average alcohol intake in those who drink is about 10–30 g/day or 3–9% of the total energy intake [2], and the efficiency of alcohol for the maintenance of metabolizable energy is the same as for carbohydrate [3]. Alcohol suppresses the oxidation of fat, favouring fat storage and can serve as a precursor for fat synthesis [4, 5]. Moderate alcohol consumers usually add alcohol to their daily energy intake rather than substituting it for food, thus increasing energy balance [5]. On the basis of this, it would seem surprising if alcohol did not contribute directly to body weight. While laboratory studies on energy and nutrient balances show that

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alcohol is a nutrient that is efficiently utilized by the body and that alcohol calories do count, the epidemiological evidence is conflicting and whether moderate amounts of alcohol is a risk factor for weight gain and obesity is still controversial [6]. Several factors have been proposed which may explain the inconsistencies between studies, including the suggestion that the effect of alcohol on adiposity is influenced by type of drink [5], whether the alcohol is consumed with meals or not [5] and the pattern and amount of drinking in the population study [7]. A review conducted in 2005 concluded that the issue of whether alcohol calories count may be dependent on the characteristic of the drinker and the amount and pattern of drinking [6]. Moreover, evidence from a number of studies suggests that in drinkers, fat is preferentially deposited in the abdominal area [5] and that alcohol may be more associated with abdominal obesity than with general obesity [8–11]. The aim of this chapter is to review the epidemiological evidence for alcohol as a risk factor for overweight and obesity with particular focus on prospective studies. The influence of type of alcohol, pattern of drinking and confounding will also be discussed.

Epidemiological Studies on Alcohol and Body Weight

Cross-Sectional Studies

In several reviews of studies of the alcohol and obesity relation, most of which are cross-sectional in nature, the association between alcohol intake and body weight has been inconsistent and has varied between men and women [2, 5, 6, 12, 13]. In men, the association between alcohol and body weight has been found to be positive or non-existent [2, 5, 6, 9, 12–16], but in women, the majority of cross-sectional studies report an inverse relationship [2, 5, 6, 8, 12, 13, 15, 17, 18].

It is not clear why alcohol may promote leanness in women although it has been suggested that the calories from alcohol are added to energy intake from other sources in men and that the energy from alcohol intake displaces sucrose in women [19]. Cross-sectional analyses are limited in assessing cause and effect. The patterns of higher obesity rates in non-drinkers compared to drinkers commonly seen in women may reflect history of dieting or current dieting to lose weight. The higher BMI levels in non-drinkers may in part be due to self-selection bias. Women who are more prone to weight gain for reasons other than alcohol may abstain from drinking because of their belief that alcohol causes weight gain.

Prospective Studies

There have been an increasing number of prospective studies on the relation between alcohol intake and weight gain in men and women and the findings have been inconsistent [20–33]. Table 29.1 summarizes the main findings from prospective studies on alcohol and weight change [20–33]. Early data from the Framingham study showed that both men and women who took up drinking or increased their alcohol intake during follow-up experienced weight gain [20]. In a study of over 12,000 Finns, heavier drinking (>75 g/week) in men and (>10 g/week) in women was associated with increased risk of weight gain (>5 kg), although the prevalence of obesity was inversely associated with alcohol intake in women [21]. This suggests that the higher BMI levels in female non-drinkers in cross-sectional studies may in part be due to self-selection bias. In a study of over 2,000 Chinese adults, alcohol was associated with a significant weight gain in men; in women, only a small but positive association was seen [22]. In the British Regional Heart Study (BRHS), a study of over 7,000 men aged 40–59 years, an examination of the association between changes in alcohol intake and body weight over 5 years showed stable heavy drinkers (>=30 g/day; 1 UK unit is approximately 10 g/alcohol) and new heavy drinkers to have the greatest weight gain and the highest prevalence of obesity [23].

Table 29.1 Summary of the prospective association between alcohol and weight gain in epidemiological studies

			Overall main findings	
Study	Subjects	Outcome	Men	Women
Framingham study (1983) [20]	5,209 men and women aged 29–62 years	20-year weight change	Positive	Positive
Nurses I Health Study (1990) [31]	31,940 non-smoking women aged 30–55 years	8-year weight gain	–	Inverse
Social Insurance Institution Finland (1991) [21]	12,669 adult Finns aged 30–64 years	5-year weight gain (≥ 5 kg)	Positive	Positive
Healthy Worker Project (1993) [26]	1,639 male and 1,913 female employees	2 years change in body weight	No association	No association
NHANES I Study (1994) [32]	7,230 US adults 25–74 years	10-year weight gain (≥ 10 kg)	Inverse	Inverse
Male firefighters (1996) [27]	438 male fire service personnel 20–58 years	7-year weight gain (≥ 5 lbs)	No association	–
American Cancer Society (1997) [28]	79,236 adults	10-year waist gain	No association	No association
Pound of Prevention Study (2000) [29]	826 women, 218 men 20–45 years	3-year weight gain (≥ 5 lbs)	No association	No association
Male athletes (2000) [30]	1,143 men aged 36–88 years	10-year weight change	No association	–
Chinese adults (2001) [22]	2,488 adults 20–45 years	8-year weight gain (>5 kg)	Positive	Positive
British Regional Heart Study (2003) [23]	7,608 men aged 40–59 years with no history of diabetes	5-year weight gain (≥ 4% body weight)	Positive	–
Nurses Health Study (2004) [24]	49,324 women aged 27–44	8-year weight change	Positive	–
The National Epidemiological Survey of Alcohol and Related Conditions (2010) [25]	43,093 men and women >18 years	2-year BMI change	Positive	No association
Women's Health Study (2010) [33]	19,220 women mean age 38.9 years	12.9-year weight gain	–	Inverse

Light and moderate drinkers showed no increase risk in weight gain compared to non-drinkers. These positive findings in heavier drinkers have been confirmed in a prospective analyses carried out in a US cohort of over 40,000 female nurses women aged 29–42 years at baseline in 1989 (Nurses II Health Study) [24]. An inverse relationship was seen between alcohol and BMI in cross-sectional analyses, but in prospective analyses, light-to-moderate drinkers (up to 30 g/day) had significantly lower risk of weight gain (>5 kg) over 8 years than non-drinkers, but heavy drinkers (>=30 g/day/3 UK units/day) had the highest risk of weight gain (>5 kg). In a recent pooled large analysis of over 40,000 men and women (the National Epidemiological Survey on Alcohol and Related Conditions), increasing frequency and intensity of alcohol use was associated with small weight gain for men but not for women [25]. The largest effect was seen in younger men (18–25 years). These prospective data support the concept of alcohol as a risk factor for overweight and obesity. However, weak positive or no association has been reported between alcohol and weight change and weight gain in five prospective studies from the USA [26–30]. In these studies, data by levels of alcohol consumption were not presented, and the average intake in these populations is not known. By contrast, in three US studies, an inverse association was seen between alcohol and weight gain [31–33]. The inverse pattern seen particularly in women may in part be due to the small number of women who drank more than two

drinks a day, the level at which alcohol appeared to have an effect on increased weight gain and/or the characteristics of the non-drinkers. It is also possible that light and moderate drinkers have better lifestyle behaviours (e.g. better diet and exercise) so that increases in alcohol consumption are accompanied by more physical activity and lower fat intake which may offset the additional energy from alcohol [6, 25]. Overall, evidence from prospective data suggest that heavier alcohol intake contributes directly to body weight and obesity as one might expect if the energy derived from alcohol consumption was added to the usual dietary calorie intake.

Intervention Studies

Intervention studies are inconclusive. In an experimental trial where 630 Kcal alcohol was added to the baseline diet, Crouse et al. [34] found that a positive association was seen between alcohol intake and weight gain only in those who were already overweight or obese. Cordain et al [35] reported that the addition of 35 g/day of wine to the daily energy requirements during a period of 6 weeks does not affect body weight or energy metabolism. This is consistent with the findings in the BRHS and Nurses II Health Study in which up to 30 g (3 UK units) was not associated with weight gain [23, 24].

Alcohol Intake and Body Fat Distribution

Evidence from a number of studies suggests that in drinkers, fat is preferentially deposited in the abdominal area [5]. In contrast to the cross-sectional relationship between alcohol and body weight, which has been found to be almost equally positive or non-existent in men and negative in women, the majority of studies report positive associations between alcohol and waist circumference in men [8–11, 36–44], and several studies report positive associations between alcohol and body fat distribution in women [8, 11, 36, 37, 40, 42, 44, 45]. Some studies have observed stronger associations between alcohol and central adiposity as measured by the WHR or WC than with BMI. In the French MONICA study, no association was seen between alcohol and BMI in men and an inverse association was seen in women [8]. However, alcohol consumption was positively associated with waist-to-hip ratio (WHR) independently of BMI in both men and women [8]. In the Italian Bollate Eye Study, alcohol was inversely associated with BMI in women with non-drinkers showing the highest BMI and light drinkers the lowest. But moderate to heavy drinking was associated with higher waist circumference (WC) than both non-drinkers and light drinkers [42]. In a large-scale European cohort of almost a quarter of a million men and women (EPIC study), lifetime alcohol use was positively associated with increased abdominal obesity (WC) and general obesity (BMI) in men. In women, alcohol was only positively associated with abdominal obesity (not general obesity) [44]. In the Uppsala study of men [10], higher alcohol intake was associated with significantly increased waist circumference but not BMI. In the BRHS, a positive relationship was seen with both central and general adiposity, but the effects as measured by the standardized regression coefficients were greater for WC and WHR than for BMI and % body fat (measures of total adiposity) [9], and the increase in percentage of men with large WC was more marked than the increase in rates of obesity as measured by BMI (Fig. 29.1). The findings of a stronger and more positive association between alcohol and central adiposity as measured by the WHR or WC than with BMI in several of these studies suggest that alcohol is more associated with abdominal obesity than with general obesity.

Prospective studies on the relationship between alcohol and fat distribution are relatively few and the findings are inconsistent. In contrast to cross-sectional studies which consistently report positive associations between alcohol and WC in men, the prospective findings on alcohol and change in fat distribution (waist to hip ratio or waist circumference) in men have been very mixed (Table 29.2). In

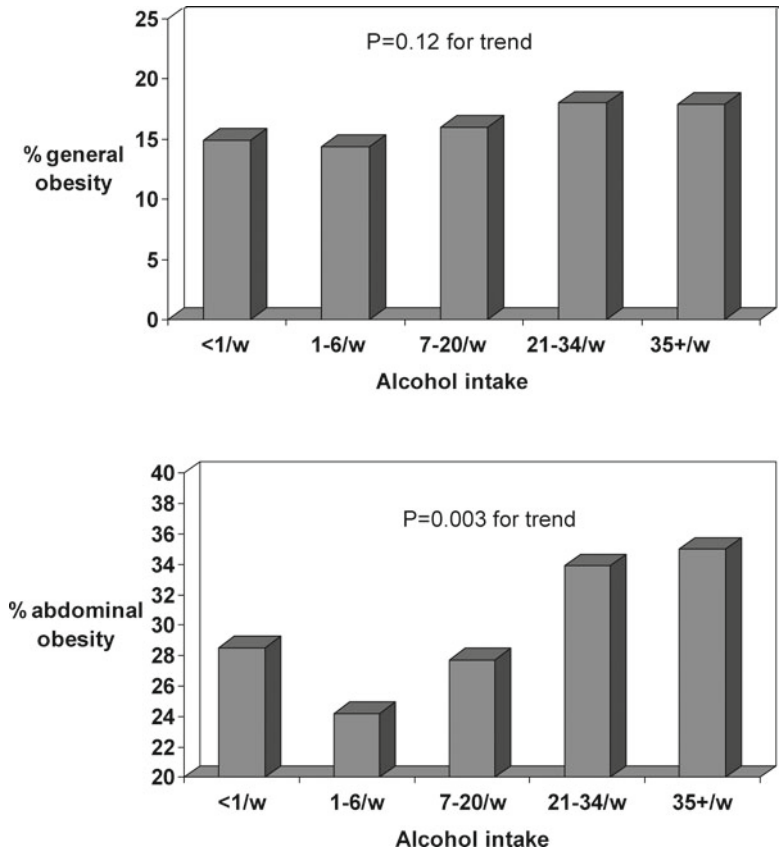

Fig. 29.1 Total weekly alcohol intake and prevalence (%) of general obesity (BMI ≥ 30 kg/m^2) and prevalence (%) of abdominal obesity (WC > 102 cm) in men aged 60–79 years (Based on data from Ref. [9])

women, the prospective findings have generally been more positive (Table 29.2). Strongest evidence that alcohol increases abdominal fat comes from the Copenhagen City Heart Study [46]. It was observed that four or more drinks/day was significantly associated with increased WHR measured 10 years later in both men and women. In the EPIC Potsdam study, heavy beer consumption was associated with increase in waist circumference in men but not in women [43]. By contrast in another study of men and women from five countries involved in the EPIC study, alcohol consumption related positively to change in WC in women but not in men [47]. In the Danish MONICA study of men and women, high intake of beer was associated with gain in WC in women but not in men [48]. In the US male Health Professional Study [49], no association was seen between alcohol and waist gain. In the Diet Cancer Health Study, an inverse association was seen between alcohol intake and major waist gain in men and women largely due to the increased odds in non- or occasional drinkers [50]. Little differences in weight gain were seen among regular drinkers (>1 drink/week).

Table 29.2 Summary of the prospective association between alcohol and central adiposity in epidemiological studies

Study	Subjects	Outcome	Overall main findings	
			Men	Women
Copenhagen City Heart Study (2003) [46]	2,916 men and 3,970 women aged 20–83 years	10-year high WC (>102 cm for men; >88 cm for women)	Positive	Positive
Health Professionals Follow-up Study (2003) [49]	16,587 men aged 40–75 years	9-year waist gain	No association	–
Danish MONICA study (2004) [48]	2,300 men and women aged 50–64 years	6-year change in WC	No association	Positive
Danish Diet, Cancer and Health Study (2008) [50]	43,545 men and women aged 50–64 years	5-year change in WC	No association	Inverse
EPIC Potsdam study (2009) [43]	12,749 women,7,876 men aged 35–65 years	8.5-year change in WC	Positive	No association
EPIC study (2010) [47]	48,631 men and women Mean age 50 years	5-year change in WC	No association	Positive

Patterns of Drinking and Adiposity

Type of Drink

It has been suggested that the type of alcohol consumed might explain the discrepant results in studies of alcohol and body weight. Alcohol is metabolized primarily by the liver and used immediately as energy or stored in the liver or in the rest of the body as fat. Since beer contains more carbohydrate and thus more usable energy per unit of ethanol than most wines or spirits, the common belief is that beer drinking promotes abdominal fat distribution [5] and that wine in contrast has no effect and may even have beneficial effects on metabolism [41, 51]. However, the relationships reported between type of drink and body weight and obesity have been unequivocal. Some studies have reported differing effects of type of beverage on body weight and fat distribution and observed no effect with wine while others have observed all alcoholic beverages to be associated with abdominal fat.

In a US study of 12,000 men and women aged 45–64 years, the WHR of those consuming more than 6 beer drinks/week was significantly greater than in non-drinkers, while in those drinking more than 6 wine drinks/week, the WHR was significantly lower than non-drinkers. The findings were regarded as supporting the popular concept of the "beer belly" [41]. By contrast, in another US study (CARDIA), beer, wine and liquor were all positively associated with WHR in white men [36]. In a study of some 3,500 French men and women aged 35–64 years drawn from three distinct geographic areas of France (MONICA centres), wine was the main source of alcohol (67% of intake). Wine and beer consumption were positively and strongly associated with WHR in women, but only poorly associated with WHR in men [8]. In the EPIC study, both beer and wine were associated with increased abdominal fat although the effect was strongest among beer drinkers [11]. Findings from the British Regional Heart Study show a strong positive relationship between alcohol intake and central obesity (WC >102 cm) in beer and spirit drinkers; no association was seen with wine drinking [9]. However, in the adjusted analyses after adjustment for lifestyle characteristic, dietary fat, time taken with meal and each of the other type of alcohol, a positive association was seen between weekly alcohol intake and mean WC for all types of drink although the effect was strongest in beer drinkers which suggests that alcohol per se rather than any alcoholic beverage consumption is associated with increased abdominal fat deposition.

However, several studies have failed to find any significant association between beer and adiposity. In the SU.VI.MAX intervention study on the effects of antioxidant supplement on chronic diseases in men and women, spirit was positively associated with BMI and WHR in both men and women, a J-shaped relationship was seen for wine, but no association was seen for beer drinking

[52]. In the Spanish national survey [15], there appeared to be a positive association between wine intake and the prevalence of obesity in women and between spirit intake and obesity in men. No significant trends of association were observed for beer or wine in men, or for beer or spirits in women. In a study of Caucasian-American and African-American men liquor drinking was associated with a greater tendency for greater central adiposity but beer drinking was unrelated [7]. In the Japanese study of male self-defence officials, abdominal obesity was associated with Japanese spirits but not with other types of alcohol [39]. In the Uppsala study, higher intake of spirits was associated with increased abdominal obesity. No association was seen with wine and beer showed a small but non-significant increase in WC [10].

Prospective studies on the effects of alcohol on weight gain and adiposity by type of drink are few and inconsistent. The Copenhagen City Heart Study reported high consumption of beer and spirits to be associated with increased waist circumference whereas moderate to high wine consumption was associated with lower WHR [46]. In the Danish MONICA study of men and women, beer and spirits were associated with increases in WC in women but not in men [48].

Influence of Drinking with Meals

It has also been postulated that the effects of alcohol on body weight and fat distribution may differ according to whether the alcohol is consumed with meals or not although data are limited. There has been suggestion that wine drinkers may take their alcohol more frequently with meals than other drinkers and consume it more slowly which in consequence turn may have lesser effect on adiposity [5]. Regular alcohol use at meals may increase total energy expenditure by potentiating normal-dietary-induced thermogenesis [5]. In the BRHS, wine drinkers were more likely to drink with meals than other drinkers, but in cross-sectional analyses, total alcohol intake (>=21 drinks/week) is positively associated with adiposity irrespective of whether the alcohol is usually drunk with meals or separately [9].

Table 29.3 Alcoholic beverage preference in men drinking at least 1 unit/week and diet and lifestyle characteristics

	Predominant type of drink		
	Beer	Wine	Spirit
N	1,037	303	198
Average no. of drinks/week	12.4	9.6	12.0
% with meal	28.5	83.7	22.5
Total non-alcohol calories (kcal)	2,126	1,928	1,990
Dietary nutrients g/day			
Total fat	75.5	66.4	70.1
Protein	25.1	26.5	24.5
Carbohydrate	284.3	264.4	268.6
Fibre	25.2	27.0	24.6
Vitamin C	78.3	87.5	76.2
Lifestyle characteristics			
Mean BMI (kg/m^2)	27.1	26.3	26.6
Mean WC (cm)	97.1	96.4	97.0
% Non-manual workers	34.7	72.0	45.1
% Inactive	32.0	27.8	32.8
% Smokers	17.4	5.0	14.4

Based on data from the British Regional Heart Study 1998–2000

Pattern of Drinking and Lifestyle Characteristics

The differences in findings between studies may be associated with unrecorded differences in lifestyle or differences in nutritional characteristics between wine, spirit and beer drinkers. Alcoholic beverage preference has found to be associated with dietary habits, social class and lifestyle factors including smoking, exercise and BMI. Table 29.3 shows the characteristics of beer, wine and spirits drinkers in men aged 60–79 years using data from the British Regional Heart Study. There is evidence that wine drinkers have healthier diets than other drinkers, are less likely to smoke, are more physically active and tend to be of higher socioeconomic status. Wine drinkers tended to drink less on average than beer or spirit drinkers and had lower total fat intake and higher intake of fibre and vitamin C, reflecting higher intake of fruits. Beer drinkers had the highest fat and carbohydrate intake. These findings are consistent with previous observations reported in France [53], the USA [54] and Denmark [55]. It is also suggested that differences in association between specific types of alcoholic beverage and fat distribution seen between studies may be due to mean consumption per day being too low to show any association [52], as it appears that a minimum amount of alcohol added to the usual food intake may be required (≥3 drinks/day) to increase body weight and fat distribution. Several studies have observed increased rates of overweight or obesity or weight gain only in those drinking 3 drinks/day or more [23, 24, 44, 56]. The lack of heavy wine drinkers and the multiple healthy lifestyle characteristics associated with light to moderate wine drinking is more likely to explain why many studies have shown no association or even inverse associations with adiposity for wine.

Mechanisms

The mechanisms involving alcohol and abdominal fat deposition are not clearly established, but endocrine changes reflected by various hormonal changes including increased cortisol secretion appear to be involved [57, 58]. These hormones are involved to a certain extent in the regulation of energy balance and affect fat-tissue enzymatic activities which may promote abdominal fat deposition [5]. Suter and colleagues note the significant positive relationship between alcohol and fat intakes and the lack of inhibitory effect of moderate alcohol intake on daily energy and fat intake. It has been suggested that alcohol consumers on a high-fat diet may experience weight gain more easily than an alcohol consumer with a lower dietary fat intake due to the metabolic effects of alcohol on suppressing fat oxidation rate leading to a positive fat balance [59]. These findings are of considerable relevance in view of the observation that alcohol intake, especially when accompanied by a high-fat diet, favours truncal obesity particularly in women [60].

Although alcohol appears to be added to the diet, light and moderate drinkers have often been shown to have significantly lower body weight and weight gain than non-drinkers. This lower weight gain in light and moderate drinkers may be due to residual confounding or it may reflect a true physiological effect of alcohol on increased basal energy expenditure and inefficient energy utilization [61, 62]. It has further been suggested that alcohol enhances weight gain in obese subjects, but not in lean subjects [34, 63]. In a recent study of 37 healthy premenopausal women aged 21–40 years, heavier subjects (mean BMI 25.2 kg/m^2) required fewer calories to maintain body weight when consuming alcohol than leaner women (mean BMI 22.6 kg/m^2). It was suggested that heavier women utilize alcohol more efficiently than lean women [63]. If these findings are confirmed, the examination of data on women's response to alcohol may require stratification by BMI, body weight or WHR for proper interpretation.

Conclusion

While metabolic studies indicate fairly unequivocally that alcohol consumption even in moderate amounts contributes to weight gain, the epidemiological evidence on the relationship between alcohol intake and body weight is conflicting. This may not be surprising, given the heterogeneity of the groups studied, the problems of assessing true alcohol intake in men and in women, and the wide range of variables affecting energy balance, such as overall diet, physical activity and ill health and selection bias. The inconsistencies between studies may be caused by incomplete control for confounding, by heterogeneity of study populations regarding alcohol consumption, the low prevalence of heavier drinking, socioeconomic factors and lifestyle characteristics or by differences in other lifestyle characteristics among drinkers which may offset the additional energy from alcohol. There is increasing evidence suggesting that higher total alcohol intake (>3 drinks/day; >30 g alcohol/day) is associated with increased adiposity and that alcohol intake may be more associated with increased abdominal fat than with general obesity. There is no clear evidence that the effects of alcohol differ according to the type of drink, and there is no convincing evidence that wine is protective against abdominal fat deposition. In many studies, the number of heavier wine drinkers (3 or more drinks/day) is very small, and this may explain the lack of positive effect in wine drinkers. Overall evidence from cross-sectional and prospective studies suggests that light-to-moderate drinking is not associated with weight gain but that higher levels (> 3 drinks/day; > 30 g alcohol/day) may contribute to weight gain and increased abdominal fat distribution in both men and women.

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Chapter 30
Nutrition: Alcohol and Anorectic and Bulimic Adolescents

Konstantina Magklara

Key Points

- Anorexia and bulimia nervosa have usually their onset in adolescence and share a common central psychopathology: the overevaluation of body shape and weight.
- Most of the medical complications of anorexia nervosa derive from the extreme low food intake and the resulting low body weight, and they usually reverse with refeeding, while in bulimia nervosa, medical complications are usually the results of the patients' purging behaviours.
- Research investigating various nutritional aspects implicated in the clinical manifestation of eating disorders, such as the role of regulators of feeding behaviours, can contribute to a better understanding of these disorders.
- The evaluation of anorectic and bulimic patients includes medical, family, psychiatric and nutritional assessment. Special considerations should be kept in mind when assessing and treating adolescent patients with an eating disorder.
- The goal of the nutritional rehabilitation of anorectic and bulimic patients is the development of an eating plan, which will enable the normalization of the eating habits. Especially in anorexia nervosa, the dietary treatment includes weight restoration, weight maintenance and development of healthy eating habits with a balanced food intake.
- Regarding alcohol, some studies report that eating and alcohol use disorders frequently co-occur, especially among patients with binging and purging behaviours, while other studies suggest that patients with restricting anorexia, as well as patients with binging and purging types of eating disorders do not use alcohol significantly more often.

Keywords Anorexia nervosa • Bulimia nervosa • Adolescents • Nutrition • Alcohol

Eating Disorders in Adolescence

Eating disorders in adolescence are characterized by an excessive preoccupation with control over body weight and food intake, overvaluation of weight and/or body shape and are accompanied by inadequate, irregular or chaotic food intake [1]. Eating disorders are more prevalent in females than

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in males [2] and usually have their onset in late adolescence, between 16 and 20 years of age. Between 2% and 4% of female adolescents and young adults develop full syndrome eating disorders (anorexia nervosa, bulimia or binge-eating disorder), while subclinical abnormal eating behaviours are estimated to affect up to 25% of adolescent women [2, 3]. Eating disorders are persistent and have a chronic course and a relatively difficult treatment [4].

Various factors are implicated in the aetiology of eating disorders. A combination of environmental – social, cultural and familial – biological and psychodevelopmental factors may increase adolescent's susceptibility, which could eventually lead in the development of eating disorders [5]. The evidence of a genetic predisposition is clear [6–8], while family studies agree that eating disorders may share common risk factors [9, 10]. Studies investigating possible pathways to eating disorders have shown that although socioeconomic status correlates with unhealthy dieting behaviours, it does not with eating-disordered behaviours [11]. Risk factors include female gender, nationality, excessive body weight, dietary restraint, body shape concerns, low or negative self-esteem, problematic intrafamilial communication (low contact, high expectations), psychiatric morbidity (especially social phobia and obsessive-compulsive disorder), history of sexual or physical abuse and a family history of eating disorders, depression or substance abuse [4, 12].

Nutrition and Anorectic Adolescents

Anorexia Nervosa

Anorexia nervosa is characterized by an excessive weight loss caused by the patient. Its causes remain unknown, but sociocultural and biological factors, as well as various psychological processes and a vulnerable personality may play a significant role [13]. Over the past years, anorexia nervosa has been reported more frequently than in the past, especially in the developed world. However, the observed increase may be not a true increase in the prevalence of the disease, but rather the result of greater help seeking or changes in diagnostic practices, which may have lead to a better detection [14]. The prevalence of anorexia nervosa is between 0.5% and 1% in adolescent girls and is estimated to occur 10–20 times more often in females than in males. In adult clinical samples, anorexia nervosa comprises 10–15% of all eating disorder cases, while the proportion in adolescent samples is a little higher, however still the least common of the eating disorder diagnoses [15]. The onset of anorexia occurs between the 10th and 30th year of age with the most common age of onset being late adolescence, between 14 and 18 years. Almost 5% of anorectic cases have their onset in their early twenties. Some studies report that the disorder occurs more frequently among adolescents from higher socioeconomic classes and it may be more prevalent in developed countries and in professions, in which a thin figure is important, such as modelling, ballet, gymnastics or figure skating.

Diagnosis

According to the classification of DSM-IV-TR [16], the following features need to be present in order to make the diagnosis of anorexia nervosa:

- Constant pursuit of weight loss and maintenance of an extremely low body weight (e.g. body weight less than 85% of the expected weight or as regards children and adolescents a body mass index below the second percentile for age).
- Overevaluation of body weight and shape, which can take the form of an intense fear of becoming fat. Self-worth is judged principally on the grounds of weight and shape and the ability to control them.
- Amenorrhea in postpubertal girls.

Within anorexia nervosa, DSM-IV-TR distinguishes between two types on the basis of the presence of binge-eating or purging behaviour (e.g. self-induced vomiting, misuse of laxatives or diuretics): restricting type and binge-eating/purging type. Anorectics with binge-eating/purging type of the disorder are more likely to have a substance abuse or borderline personality disorder and show impulse control problems, mood lability and suicidality [17, 18].

Nutritional Aspects in the Clinical Manifestation of Anorexia Nervosa

Clinical Features

Most of the clinical features of anorexia nervosa derive from the overevaluation of body shape and weight, which leads to a constant pursuit of weight loss. Many patients are preoccupied with their shape, tend to focus on parts that dissatisfy them, weigh themselves frequently and become obsessed even with minimal fluctuations of their body weight, while others avoid weighing themselves or seeing their body, which they find ugly and unacceptable. In anorexia nervosa, famine is self-imposed and not due to lack of availability of food, while most of the symptoms observed are directly related to starvation. Depressive and labile mood, irritability, poor concentration, anxiety features and obsessional symptoms, which include an obsessional thinking about food, occur frequently. Many patients develop weird eating rituals, seem constantly thinking about food, start eating very slowly or diluting food or cutting it up in small pieces in order to make it seem more and collect cookbooks or recipes, which they often prepare for their friends and family.

Recent research has tried to investigate a number of possible regulators of feeding behaviour in anorexia nervosa. For instance, evidence shows that exposure to food cues may increase eating, especially in restrained eaters. More specifically, restrained eaters seem to be more responsive to pre-eating exposure to smell and thought cues than unrestrained eaters. Self-reported desire to eat and craving for a particular food increased for restrained eaters after exposure to the smell and thought of that food, which shows that restrained eaters have a highly specific response to exposure to food cues [19]. Other researchers have focused on the response of anorectic patients to visual food stimuli. They have tried to investigate the visual ratings of liking and desire to eat various categories of food and the possible influence of the caloric or macronutrient content of food. Evidence shows that anorectic patients tend to rate their desire to eat high-calorie food significantly lower than their desire to eat low-calorie food, a fact that should be considered when designing treatment strategies [20]. There may be an initially elevated taste preference for calorie-dense foods in anorexia nervosa, while an abnormal sensory response to high-calorie food may be responsible for binge eating [21]. On the other hand, altered appetite or satiety signals may play a significant role in the development of anorexia. Neurotransmitters such as serotonin and catecholamines, peptides, such as pancreatic polypeptide and gastrin, concentration of blood glucose and insulin levels have been also implicated in the development of anorexia nervosa [22, 23].

Physical Abnormalities

Most of the medical complications which are present in anorexia nervosa are caused primarily by the patients' unduly low food intake and the resulting low body weight. The majority of them reverse with refeeding, achievement of a normal body weight and healthy eating habits. Common symptoms include cold intolerance, constipation, gastrointestinal discomfort, decreased gastric motility and delayed gastric emptying [24], hyperactivity, dizziness, headaches, poor motor control, sleep problems with early morning wakening, decreased body temperature, heart rate and basal metabolic rate (BMR)

and increase in fine body hair (lanugo) [25]. Amenorrhea results from starvation-induced hypogonadism, and in 20–30% of anorectic patients, it persists despite weight gain [25]. Reduced levels of hormones, increased liver enzymes and amylase, mild anaemia and leukopenia and EGK abnormalities are often observed. Low body weight is related to decreased bone formation and increased bone resorption, which leads to reduced bone mineral density and osteopenia. Among other variables, reduced levels of IGF-I, a nutritionally dependent endogenous bone trophic factor, and calcium intake below 600 mg per day are predictive of osteopenia [26].

Nutritional Aspects in the Treatment of Anorexia Nervosa

The treatment of anorexia nervosa includes a variety of options. The treatment setting may be outpatient, day patient or inpatient, while the therapeutical interventions offered may be pharmacological, psychological or a combination of them. The evaluation of patients with anorexia nervosa should always include medical, family, psychiatric and nutritional assessment. A detailed physical and laboratory assessment should always take place on admission to the hospital. Most of the clinical issues are presented with similar frequency in adults and adolescents. However, adolescents differ from adults both physiologically and in terms of their psychological development. The nutritional management of adolescent anorectic patients cannot be separated from their overall management, and it should take place in an inpatient, day patient or outpatient service appropriate for their age and staffed by clinicians experienced in working with adolescents. Ideally, adolescents should be treated in separate services and not within adult services. The management plan should be discussed in a comprehensive way with the patient, even when the patient's age does not allow complicated explanations and arguments. The cooperation of the patient is highly significant for a successful treatment; however, anorectic patients are typically resistant to treatments that focus on weight gain. The involvement of parents or substitute carers is essential. Their role is crucial in the management of anorectic adolescents, and they should be included in any dietary education or meal planning. The reasons for that are that parents have parental rights, they are expected to have an important role in determining food intake at home, and they can provide a developmental history, while young people are likely to ignore their nutritional needs. Some researchers support the option of a separate parental interview; however, the gains are not clear since many adolescents are very sceptical when they are excluded from a discussion focused on their own problems.

Special Considerations in the Treatment of Adolescent Patients

As shown in Table 30.1, the dietary treatment of patients with anorexia nervosa includes:

- Weight restoration
- Weight maintenance
- Development of healthy eating habits with a balanced intake of food and nutrients and a wide variety of foods

Table 30.1 Dietary goals in the treatment of adolescent patients with anorexia nervosa

Goals of dietary treatment in anorexia nervosa
1. Weight restoration
2. Weight maintenance
3. (a) Healthy eating habits
(b) Balanced intake of food and nutrients
(c) Large variety of food

Table 30.2 Special considerations, which should be kept in mind when treating adolescent anorectic patients

Special considerations in the treatment of adolescents
1. Low energy stores → problematic reliability of the BMI as an indicator of fat reserves
2. Growth retardation caused by anorexia → calculation of the BMI using predicted height for age rather than the actual height
3. Assessment of pubertal development: important when planning the target weight
4. Complication of osteopenia and the challenge to improve bone density

A number of points should be borne in mind when treating adolescents [27]. A brief description of these points is presented in Table 30.2. First of all, their energy stores are low since their stores of fat and other substances are incomplete. As a result, the medical complications are severe, even after relatively small amounts of weight loss. Although the body mass index (BMI) is widely used as an indicator of body fat stores in adults, its use in the adolescent population presents certain difficulties since it cannot always express the fat reserves of an adolescent. In adolescents, a change in BMI is not a reliable indicator of change in fat, protein or carbohydrate stores [28]. Moreover, emaciation can occur more rapidly in adolescents, who dehydrate more quickly than adults.

Secondly, when anorexia nervosa develops prior to the completion of growth, it can result in growth retardation and height reduction. This is especially evident in boys, because boys grow for 2 years longer when compared to girls. This complication can reverse with nutritional rehabilitation; however, many of these adolescents may never reach their prior to the disorder potential. As a result, weight loss will be underestimated if the assessment is based only on the BMI. It is suggested that a calculation of the BMI using predicted height for age rather than the actual height may provide more accurate information as regards the assessment of weight loss. Because BMI norms vary with age, the assessment of BMI in this age group and up to the age of 20 years should be related to BMI percentiles [29], which are available from the Child Growth Foundation. The Child Growth Foundation defines "significant underweight" as being below the second percentile. For example, on the BMI percentile chart for girls, the second percentile line gives a BMI of 15.5 kg/m^2 at age 14 years, 16.3 kg/m^2 at age 16 years, 16.9 kg/m^2 at age 18 years and 17.4 kg/m^2 at age 20 years; similar figures are provided also for boys.

Third, adolescent patients include prepubertal patients, those in pubescence, as well as postpubertal adolescents. A careful assessment of pubertal development is of highly significance and should employ the Tanner Staging Norms [30], while a pelvic ultrasonography can be very useful. When the disorder develops prior to the completion of puberty, pubertal delay may occur. Menarche is usually triggered at a weight of around 45 kg and puberty is unlikely to be completed below this weight. Weight gain results often in the resumption of menstruation, in some adolescents though amenorrhea persists. This is the reason why the relationship between weight and pubertal development should be carefully considered, when planning the target weight for adolescent patients.

Finally, another important point that should be considered when treating anorectic adolescents is the complication of osteopenia since this developmental stage is especially critical as regards the acquisition of bone mass. Adolescence and young adulthood are the time that maximum bone density is built for the rest of life (peak bone mass). Most of these adolescents will not reach their full genetic potential for bone mass, which means that they are going to have an elevated fracture risk. Weight restoration is the key to improve bone density, while a complementary prescription of calcium supplements though may be also beneficial.

Assessment of Target Weight

At the time of the admission of a patient with anorexia nervosa, an expected weight should be established. Nevertheless, the assessment of target weight presents some certain difficulties in this age

group. According to the American Psychiatric Association, a "healthy target weight" is one at which normal menstruation and ovulation are restored or one at which normal physical and sexual development resumes [31]. However, some adolescents are not presented with amenorrhea even at low weights, and others may continue to have no menstrual cycles even after weight gain. This is why target weight is often assessed as at least 90% of ideal weight for height according to standard charts [31]. NICE guidelines recommend weight gain of 0.5–1 kg per week for inpatients and 0.5 kg per week for outpatients [32]. Research evidence shows that the lower the target, the lower the weight gain. For the assessment of target weight, oestrogen levels and pelvic ultrasound can be useful. The calculation of target weight needs constant monitoring and a revision may be necessary during refeeding. Generally, it is better to identify a "target range" (2 kg) rather than a "target weight". Finally, it should be stressed that expected weight should not always be reached during inpatient treatment. However, it should remain as target of the overall treatment even after discharge from the hospital.

Weight Restoration

Average energy requirements for healthy adolescents aged 11–18 years range from 1,845 kcal to 2,110 kcal per day for girls and from 2,220 kcal to 2,755 kcal per day for boys [33]. Patients suffering from anorexia nervosa require hypercaloric diets in order to gain weight. The restoration of weight should proceed with slow steady increases in dietary intake starting at 1,200 kcal with a standard low-fat meal plan based on three meals per day. The American Psychiatric Association [31] recommends an energy intake of 70–100 kcal/kg per day. Snacks can be added once 3,000 kcal is reached. Standard plans are expected to reduce anxiety by reducing the need to choose and a choice of dietary increases can be introduced later. Food rich in calcium (like dairy based sources) and an adequate balance of proteins, vitamins and minerals (e.g. iron) need to be included. Patients in the early stages of refeeding should be monitored closely for possible biochemical, cardiovascular and fluid balance abnormalities; electrocardiographic monitoring is recommended in cases of electrolyte disturbance.

Weight Maintenance

Increased energy needs continue into this period too. Treatment to this step progresses once target weight is reached. The American Psychiatric Association suggests 40–60 kcal/kg per day during the weight maintenance period [31]. Energy requirements remain elevated for a further 6–12 months, while concentrated calorie (kcal) sources are required. It has also been demonstrated that patients with the restricting subtype of the disorder require significantly more energy than those with the binge/purging subtype [34, 35]. Moreover, energy needs are linked to activity levels. Energy requirements are assessed by multiplying BMR by an activity factor and an amount for growth. This calculation though is not always accurate.

Healthy Eating Habits

The development of healthy eating habits with food intake from all food groups is important. Eating a large variety of food within each food group should be encouraged. Participating in family meals and eating out with family and peers allows a better social interaction, which can further contribute to a successful treatment. Religious dietary restrictions and cultural practices should be respected, unless they present a threat to recovery.

Enteral Feeding

In the treatment of adolescents with anorexia nervosa, enteral feeding may be considered essential in cases that a possible medical deterioration of the patient presents a serious risk to life. Enteral feeding should be carried out by clinicians experienced in its use. The rate and volume of enteral feeding depends on the oral intake of each patient. Generally, it is safe to provide an amount equivalent to the amount of energy delivered by the current food intake of the patient, with the rate being relatively slow at first and gradually increasing, depending on tolerance. Adolescents should be encouraged to eat normally and consider enteral feeding a supplement and not a substitute to their diet. In order to help patients normalize their oral food intake, enteral feeding can be delivered during the night. When treating patients undergoing enteral feeding, serum electrolytes should be monitored carefully, in order to correct possible deficiencies as soon as possible.

Nutrition and Bulimic Adolescents

Bulimia Nervosa

Bulimia nervosa is characterized by constant attempts to restrict food intake interrupted by episodes of binge eating, during which patients typically consume 1,000–2,000 kcal. Most of these episodes are followed by compensatory behaviour in order to prevent weight gain. As a result, the weight of most bulimic patients remains in the healthy range. While anorexia nervosa is typically a disorder of adolescence, most of the patients with bulimia nervosa are in their twenties [36]. However, the onset of the disorder occurs usually during adolescence. Various studies have investigated the epidemiology of bulimia nervosa and report prevalence between 1.1% in female and 0.01% in male student athletes [37], 1.8% in female and 0.3% in male Scandinavian adolescents aged 14–16 years [38] and an incidence of less than 2% in Great Britain [39] and 4% in US female adolescents [40]. A relatively rapid growth in the prevalence of bulimia nervosa was reported in the 1970s and 1980s especially among young women with high socioeconomic status living in western industrialized countries. Today, however, bulimia nervosa is often reported in non-western countries too.

The aetiology of bulimia nervosa is similar to that of other eating disorders. Unlike anorexia nervosa though, according to recent studies, there is little evidence of heritability in bulimia nervosa [41]. Childhood and parental obesity, a history of sexual abuse, early menarche and parental alcoholism and other substance abuse disorders have been identified as independent risk factors for bulimia nervosa. Other studies about bulimia nervosa have also reported abnormal levels of a number of neurotransmitters, neuropeptides (like neuropeptide Y and peptide YY) [42] and hormones (e.g. cholecystokinin), which are linked to satiety, appetite and eating habits.

Diagnosis

According to the classification of DSM-IV-TR [16], the following features need to be present in order to make the diagnosis of bulimia nervosa:

- Like in anorexia nervosa, self-worth is judged principally on the grounds of body shape and weight.
- Episodes of binge eating at least twice a week and for 3 months. During an episode of binge eating, the patient consumes usually in less than 2 h an amount of food definitely larger than most people

would consume in this period of time. During the episode, patients have the feeling that they are not able to control the amount of food they ingest, neither can they stop the behaviour.
- Engagement in compensatory behaviours, like self-induced vomiting with or without the use of syrup of ipecac, dietary fasting, exercising to excess and misuse of laxatives, appetite suppressants, thyroid preparations or diuretics, in order to prevent weight gain.

Similar to anorexia nervosa, DSM-IV-TR distinguishes between two types of bulimia nervosa on the basis of the presence of purging behaviour (e.g. self-induced vomiting, misuse of laxatives, diuretics) during the current episode: purging type and non-purging type. The above-mentioned criteria provide the diagnosis of bulimia nervosa, as long as these behaviours do not occur only during episodes of anorexia nervosa.

Nutritional Aspects in the Clinical Manifestation of Bulimia Nervosa

Clinical Features

The majority of bulimic patients maintain a normal weight or are moderately overweight, because the results of under eating and binge eating cancel each other out. As a result, the disorder is often undetectable by appearance, and patients avoid the various physical and psychosocial complications of having a very low body weight. In bulimia nervosa, dieting typically leads to binging and the vicious circle begins, as shown in Fig. 30.1. However, in a subgroup of bulimic patients, binging proceeds and these patients tend to maintain a higher body weight. Although patients are constantly preoccupied with thoughts about the amount and quality of food they should ingest, their eating patterns and habits are usually chaotic. Most of the time bulimic patients are restricting their diet, which can lead to subsequent binge eating. The self-disappointment of losing control by eating something more or of higher caloric content than what was initially intended may also lead to a binge-eating episode. In the next stage of this vicious circle, any subjective or objective sensation of stomach fullness can lead to purging behaviours. In the beginning, purging behaviours may offer a feeling of relief, which is however typically followed by feelings of guilt and shame. These feelings, as well as the various gastrointestinal complaints, such as bloating or constipation, caused by the binge eating and purging behaviours result in a restricting type of behaviour, which completes the cyclical pattern of bulimia nervosa. The abovementioned behaviours aim primarily at controlling the total food intake of the patient. However, most of the patients use a similar pattern in order to regulate their emotions too.

Physical Abnormalities

Most of the medical conditions met in bulimia nervosa are the results of the purging behaviour of the patients. Many of the symptoms are secondary to dehydration, electrolyte abnormalities and the fact that many bulimic patients are hypometabolic [43]. Body weight is not always a good indicator of the degree of malnutrition. The conservation of energy supplies becomes essential as the disorder proceeds and the body reacts by lowering the metabolic rate. As a result, many patients with normal weight are hypometabolic. Psychological symptomatology in bulimia nervosa includes sleep disorders, irritability, impaired concentration, obsessive-compulsive symptoms, reduced libido and psychological distress or depression [44].

Nutritional abnormalities depend on the amount of restriction of food intake during the binge-free time, while purging behaviours do not completely cancel out the effects of the caloric intake during

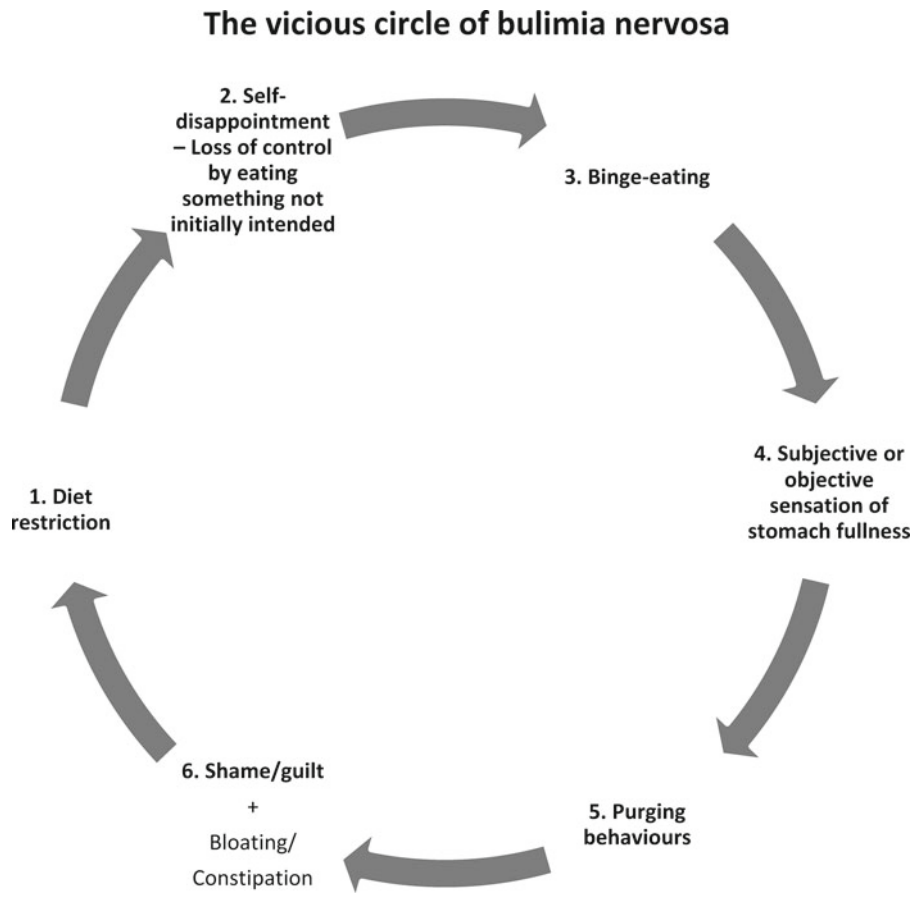

Fig. 30.1 The cyclical eating patterns in bulimia nervosa

an episode of binge eating. Bulimic patients appear often with a round face with swollen cheeks because of fluid retention and enlarged salivary glands caused by the frequent vomiting. Muscle weakness or pain, fatigue, hypotension, cardiac arrhythmias, cold intolerance, polyuria, sense of epigastric fullness and abdominal pain are often described by patients. Hypokalemia and hypochloremic alkalosis can also occur. Dental problems such as cavities or enamel erosion and loss are caused by self-induced vomiting. Chronic use of ipecac syrup can cause skeletal myopathy, electrocardiographic changes and cardiomyopathy. Bleeding of oesophagus and a stomach or oesophagus rapture are serious complications, which can occur in bulimia nervosa. Co-morbid conditions such as oesophageal reflux disease and *Helicobacter pylori* may increase the pain and the need for the patient to vomit.

Nutritional Aspects in the Treatment of Bulimia Nervosa

The treatment of bulimia nervosa includes pharmacological approaches (antidepressants like SSRIs), psychoeducational interventions, psychological methods (cognitive or dialectical behavioural, psychodynamic and interpersonal therapy) and nutritional rehabilitation. As in the treatment of anorexia nervosa, an interdisciplinary team management is essential when treating bulimic adolescents. The majority of bulimic patients are treated in an outpatient setting, while an inpatient treatment is

indicated only in severe cases. Motivating the patient, taking into account behavioural aspects and treating physical complications are the goals of the various interventions. As regards adolescent patients suffering from bulimia nervosa, their parents' involvement in psychoeducational procedures and meal planning may be beneficial.

Nutritional rehabilitation aims at developing an eating plan, which will enable the normalization of the eating habits of the patients with bulimia nervosa. The monitoring of electrolytes, vital signs and weight is necessary. Restoration of fluid and electrolyte balance and the treatment of hypokalemia with oral potassium supplements are primary goals of every intervention. Many bulimic patients may desire a weight loss at the beginning of treatment. It is highly important though to communicate to the patient that dieting and recovering from the eating disorder at the same time is not possible and that the desired weight loss may occur through a normalization of the eating habits and the elimination of binge eating.

Alcohol and Anorectic and Bulimic Adolescents

The relationship between eating disorders and alcohol use disorders attracts still considerable scientific interest since the relevant findings seem often controversial. Many studies report that eating disorders are frequently associated with co-morbid alcoholism and other substance use disorders, especially among patients in treatment [3, 45–47]. However, across the various studies, there is significant variability in the reported rates of co-morbidity. Between 20% and 40% of bulimic women also have a history of alcohol and/or drug problems, while the estimates for anorexia nervosa range from 2% to 10% [45, 48, 49]. Women with binging and purging behaviour show higher rates of co-morbid substance abuse [50], while the presence of binge-eating behaviours may predict the development of substance use disorders later in life. Among adolescents, almost one third of bulimic females report drinking alcohol or using other substances at least weekly [51].

On the other hand, recent research reports that the relationship between eating and substance use disorders is not significant or only marginally significant, when certain methodological issues are taken into account [46, 52]. Some findings indicate that the relationship between bulimia and alcohol abuse may be indirect and mediated by associations with major depressive disorder and post-traumatic stress disorder [53]. Furthermore, eating disorders co-occur not only with substance use disorders, but also with other psychiatric disorders and the frequency is not higher [46]. There is also evidence that non-purging anorexia nervosa may be not as strongly associated with substance use disorders, as are other forms of eating disorders, while some studies report that adolescents with restricting anorexia nervosa use significantly less alcohol when compared to the general adolescent population [54]. Some studies finally suggest that even adolescents with binging and purging symptoms do not use substances significantly more often, when compared to their healthy peers [54, 55].

Common Factors Between Eating and Substance Use Disorders

Regarding psychological factors, impulsivity has been linked to both bulimia nervosa and substance abuse. Individuals with eating and alcohol use disorders are often characterized by both anxious, perfectionistic traits and impulsive, dramatic dispositions. Bulimic patients with traits of a "multi-impulsive" personality may engage in a variety of other impulsive behaviours, such as substance abuse [56]. Furthermore, abnormal eating behaviours and substance abuse may be efforts for self-medication developed by patients with other psychiatric symptomatology, like psychological distress, social anxiety or even depression. Patients with eating disorders and those with bulimia nervosa report also frequent feelings of guilt.

As regards possible biological factors, Krahn (1991) suggested that food deprivation, caused, for instance, by dieting behaviours might cause changes in the reward pathways of the central nervous system, which may increase the consumption of substances like alcohol [56]. At the same time, studies suggest that both disorders may be related to atypical activity of the endogenous opioid peptide (EOP) and brain neurotransmitter systems, including the serotonin, dopamine and gamma-aminobutyric acid (GABA) systems [57].

Finally, regarding family and genetic factors, many studies have demonstrated that patients with eating disorders are more likely to have family histories of substance use disorders [58]. However, other studies show that there is little evidence of common familial or genetic risk factors. A large epidemiological study of female twins showed that most of the genetic factors associated with alcoholism in women do not influence the risk for development of bulimia nervosa [59].

Assessment and Treatment Implications

A thorough and comprehensive assessment of patients is essential for a successful treatment. Assessment protocols should include special instruments sensitive enough to identify patients with possible co-morbid problems, who may need further evaluation. During the assessment, physicians should always take into account the high levels of co-morbidity of eating and substance use disorders. When an eating disorder is suspected, screening for substance use disorders should always be performed, by using, for instance, one of the many screening instruments that have been developed for alcohol problems. Respectively, patients with substance use disorders should also be screened for eating disorders. The reason for that is that a possible failure to identify the total number of problems that possibly co-occur may contribute to poor treatment outcomes even for the targeted problem. The influence of eating disorders on alcohol use disorder appears to be greater than the reverse. Many patients who initially present with an eating disorder develop alcohol problems over the course of time, suggesting that the risk is an ongoing one that should be monitored by clinicians [60]. Furthermore, for eating-disordered patients, who already have an elevated risk for morbidity and mortality, co-morbid alcoholism is expected to further increase this risk.

Both pharmacological and psychological treatments have been used to treat patients with co-morbid eating and substance use disorders. Among antidepressants, selective serotonin reuptake inhibitors (SSRIs) such as fluoxetine may be useful in treating those patients [61], while opioid antagonists such as naltrexone have also been used. Furthermore, heavy use of alcohol increases the requirement for B vitamins, and eating-disordered patients should use proper supplements [33]. Psychological treatments such as cognitive-behavioural therapy (CBT) seem to have the best treatment outcomes. Properly modified CBT-based treatments represent a good option when starting to treat co-morbid alcohol use and eating disorders.

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Chapter 31
Viral Infections and Cancer During Alcohol Use

Malgorzata Schlegel-Zawadzka

Key Points

- Alcohol consumption and human cancer
- Alcohol consumption and viral infection
- Influence of alcohol consumption on human cancer caused by viral infection
- World development of alcoholic beverage consumption

Keywords Viral infections • Epstein–Barr virus • Hepatitis viruses • Human papillomavirus • Human lymphotrophic virus type 1 • Human herpesvirus 8 • Human immunodeficiency virus (HIV) • Viral human carcinogens • Alcoholic beverage consumption

The relationship between viral infections, cancers, and alcoholic beverages or alcohol surrogate intake is not well recognized.

The World Health Organization (WHO) recently published two large monographs on alcohol consumption and human carcinogens (biological agents). However, those monographs do not contain clear information about how consumption of alcohol can influence an increase in cancer-dependent viral infectious, apart from hepatitis B and C viruses [1, 2].

One of the issues is that these three components – viral infections, cancer, and alcohol intake –fall under different disciplines: viruses and their epidemic spread are part of microbiology, genetic bases of cancer development, clinical experience in health care, alcohol intake is a subject of study in nutritional epidemiology, and human behavior in connection with alcohol drinking falls under social and cultural studies.

Carcinogenic agents belong to one of four categories (groups): the evidence is derived from human and experimental animal studies and other relevant data. *Group 1* includes agents that are carcinogenic to humans – there is *sufficient evidence of carcinogenicity*. *Group 2* has, from on the one hand, agents whose carcinogenicity for humans has *almost sufficient* evidence or, on the other hand, for which there are presently no human data. However, there is evidence from experimental animal studies. This category consists of two subgroups: Group 2A (*probably carcinogenic to humans*) and Group

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Table 31.1 United nations classification registry of alcoholic beverages – standard international trade classification, Rev. 4

1.	Beverages and tobacco
11.	Beverages
112.	Alcoholic beverages
112.1.	Wine of fresh grapes (including fortified wine); grape must in fermentation or with fermentation arrested
112.11	Grape must in fermentation or with fermentation arrested otherwise than by the addition of alcohol
112.13	Vermouth and other wines of fresh grapes flavored with plants or aromatic substances
112.15	Sparkling wine
112.17	Wine of fresh grapes (other than sparkling wine); grape must with fermentation prevented or arrested by the addition of alcohol
112.2.	Fermented beverages, n.e.s. (e.g., cider, perry, mead); mixtures of fermented beverages and mixtures of fermented beverages and non-alcoholic beverages, n.e.s
112.3.	Beer made from malt (including ale, stout, and porter)
112.4.	Undenatured ethyl alcohol of alcohol strength by volume of less than 80% vol; spirits, liqueurs, and other spirituous beverages
112.41	Whiskies
112.42	Spirits obtained by distilling grape wine or grape marc
112.44	Rum and other spirits obtained by distilling fermented sugar cane products
112.45	Gin and Geneva
112.49	Spirits and distilled alcoholic beverages, n.e.s

Based on data from Ref. [3]

2B (*possibly carcinogenic to humans*). *Probably* indicates a higher level of evidence than *possibly carcinogenic*. *Group 3* (the agent is not classifiable as to its carcinogenicity to humans) is used for agents for which the evidence of carcinogenicity is *inadequate* in humans and *inadequate* or *limited* in experimental animals. The last group, *Group 4*, possesses agents that are probably not carcinogenic to humans – the *evidence suggests a lack of carcinogenicity* in humans and experimental animals. More details about agent classifications are covered by monographs edited by the World Health Organization International Agency for Research on Cancer – *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans* [1, 2].

According to the Standard International Trade Classification (SITC) ver. 4, alcoholic beverages are divided into four categories: wine from fresh grapes, fermented beverages (e.g., cider, perry, mead), beer made from malt, undenaturated ethyl alcohol of alcohol strength by volume of less than 80% – spirits, liqueurs, and other spirituous beverages (Table 31.1) [3].

There are some difficulties in assessing the influence of alcohol consumption on human health due to a confounding factor – smoking [1]. Alcoholic beverage intake differs in terms of quantity consumed – different countries have different standard volumes (liter, ounce, pint) – and specific beverages typical regional cultures, which includes religious practices (Table 31.2) [4].

Epidemiological studies provide basic knowledge about the relationship between alcoholic beverage intake and various human cancers. The research covers large cohort studies, case controls, and meta-analyses. It is almost impossible to compare large pieces of information because of the different methods in data collection and their poor level of standardization [1, 5].

What is more important the amount of alcohol consumed or the amount of time over which the alcohol was consumed? The Patterns of Drinking Score (PDS) attempts to reflect *how* people drink instead *how much* they drink. Alcohol consumption behavior is reflected on a scale from 1 (least risky pattern of drinking) to 5 (most risky pattern of drinking). The following drinking attributes are taken

Table 31.2 Data of alcohol consumption using average recorded alcohol consumption 2003–2005, by WHO region and the world, 2005

		WHO Region					
	World	Africa	The Americas	Eastern Mediterranean	Europe	South-East Asia	Western Pacific
Total adult per capita consumption (15+ years; L pure alcohol; 2005)							
Total adult per capita consumption (APC)	6.13	6.15	8.67	0.65	12.18	2.20	6.23
Unrecorded APC (15+)	1.76	1.93	2.01	0.36	2.67	1.52	1.63
Proportion of unrecorded APC of total APC	28.7	31.4	23.1	56.2	21.9	69.0	26.2
Distribution of recorded adult per capita consumption of alcoholic beverages (%; 2005)							
Spirits	45.7	12.0	32.9	25.2	34.6	71.0	54.0
Beer	36.3	34.1	54.7	37.8	37.1	25.5	35.5
Wine	8.6	5.6	12.0	5.7	26.4	2.5	3.6
Other	10.5	48.2	0.6	31.3	2.5	1.0	6.9
Prevalence of alcohol consumption (% of the world's population; 2004)							
Lifetime abstainers							
Total	45.0	57.3	21.5	87.8	18.9	80.4	29.2
Men	34.9	49.1	15.2	82.4	12.6	68.4	14.3
Women	55.0	65.1	27.4	93.4	24.6	92.8	44.5
Former drinkers							
Total	13.1	13.5	20.2	8.7	12.3	8.9	14.5
Men	13.8	14.1	17.8	12.3	11.0	13.5	13.9
Women	12.5	12.9	22.4	4.8	13.5	4.2	15.1
Past-year abstainers							
Total	58.2	70.8	41.7	96.5	31.2	89.3	43.7
Men	48.7	63.1	33.0	94.7	23.5	81.9	28.2
Women	67.5	78.1	49.8	98.7	38.1	97.1	59.5
Former drinkers among past-year abstainers							
Total	22.6	19.1	48.4	9.0	39.4	10.0	33.1
Men	28.4	22.3	54.0	13.0	46.5	16.5	49.2
Women	18.5	16.5	45.0	4.9	35.5	4.4	25.3
Prevalence of weekly heavy episodic drinking among drinkers in the past 12 months by sex, 2005							
Total	11.5	25.1	12.0	24.7	11.0	21.7	8.0
Men	16.1	30.5	17.9	24.9	16.8	23.0	11.6
Women	11.5	16.2	4.5	17.9	4.6	12.9	1.3

Best estimate for abstention rates in 2004 based on surveys carried out within the time period 1993–2009
Reprinted from Global status report on alcohol and health. Geneva, WHO; 2011. With permission from WHO

into account: (a) the usual quantity of alcohol consumed per occasion; (b) holiday drinking; (c) proportion of drinking events when drinkers get drunk; (d) proportion of drinkers who drink daily or almost daily; (e) drinking with meals; (f) drinking in public places. Table 31.3 presents the patterns of drinking scores in different WHO regions. The lowest drinking scores are found in western European countries; however, these countries have high adult per-capita consumption rate. This information is necessary when it comes to examining alcohol-dependent cancers because even moderate consumption has an effect on cancer development. The epidemiological evidence of alcohol consumption should take into consideration the fact that in many regions of the world a large proportion of alcohol is produced locally and remains unrecorded (Table 31.2) [4, 6].

Alcohol consumption weakens the human immune system and encourages risky sexual behavior, leading to different infectious diseases [7–9], depending on drinking patterns and the diet-enhanced

Table 31.3 Patterns of Drinking Score (PDS) of alcohol consumption in WHO regions: 2005

Region (n = 100%)	Median (arithmetic mean)	Score	Country	n (%)
Africa (38)	3 (2.95)	2	Algeria, Benin, Mali, Mauritania	4 (10,5)
		3	Angola, Botswana, Burkina Faso, Burundi, Cameroon, Cape Verde, Central African Republic, Chad, Congo, Côte d'Ivoire, Democratic Republic of the Congo, Equatorial Guinea, Eritrea, Ethiopia, Gabon, Ghana, Guyana, Kenya, Lesotho, Liberia, Malawi, Mauritius, Namibia, Nigeria, Rwanda, Senegal, Seychelles, Sierra Leone, Swaziland, Uganda, United Republic of Tanzania, Zambia	32 (84,2)
		4	South Africa, Zimbabwe	2 (5,3)
The Americas (29)	3 (2.79)	2	Argentina, Bahamas, Barbados, Canada, Cuba, Dominica, Dominican Republic, Jamaica, Saint Lucia, Trinidad and Tobago, United States of America	11 (37.9)
		3	Bolivia (Plurinational State of), Brazil, Chile, Colombia, Costa Rica, El Salvador, Haiti, Honduras, Paraguay, Peru, Suriname, Uruguay, Venezuela (Bolivarian Republic of)	13 (44.8)
		4	Belize, Ecuador, Guatemala, Mexico, Nicaragua	5 (17.3)
Eastern Mediterranean (11)	2 (2.45)	2	Egypt, Jordan, Kuwait, Morocco, Saudi Arabia, Syrian Arab Republic	6 (54.5)
		3	Djibouti, Iran (Islamic Republic of), Lebanon, Pakistan, Sudan	5 (45.5)
Europe (50)	3 (2.48)	1	Andorra, Austria, Belgium, Cyprus, France, Germany, Italy, Luxembourg, Malta, Netherlands, Portugal, Spain, Switzerland	13 (26.0)
		2	Armenia, Bulgaria, Denmark, Georgia, Greece, Iceland, Israel	7 (14.0)
		3	Albania, Azerbaijan, Bosnia and Herzegovina, Croatia, Czech Republic, Estonia, Finland, Hungary, Ireland, Kyrgyzstan, Latvia, Lithuania, Norway, Poland, Romania, Serbia, Slovakia, Slovenia, Sweden, Tajikistan, former Yugoslav Republic of Macedonia, Turkey, Turkmenistan, United Kingdom, Uzbekistan	25 (50.0)
		4	Belarus, Kazakhstan, Republic of Moldova	3 (6.0)
		5	Russian Federation, Ukraine	2 (4.0)
Southeast Asia (7)	3 (2.86)	2	Myanmar (Burma)	1 (14.3)
		3	Bangladesh, Democratic People's Republic of Korea, India, Indonesia, Sri Lanka, Thailand	6 (85.7)
Western Pacific (15)	3 (2.67)	2	Australia, China, Japan, New Zealand, Singapore	5 (33.3)
		3	Cambodia, Fiji, Lao People's Democratic Republic, Malaysia, Mongolia, Papua New Guinea, Philippines, Republic of Korea, Samoa, Viet Nam	10 (66.7)

n number of countries
Based on data from Ref. [6]

effect of carcinogenesis-generating reactive oxygen species that cause damage to DNA or have an inhibitory effect [10]. The enzymes responsible for the majority of ethanol oxidation are alcohol dehydrogenases (ADHs). They are grouped into classes I–V and are encoded by appropriate genes. The ethanol metabolite acetaldehyde is metabolized by aldehyde dehydrogenases (ALDHs), which

are classified into three groups, I–III. The human genes that code for ALDHs have been classified into 18 major families. Ethanol can be metabolized by the microsomal oxidizing system mostly via CYP2E1. The polymorphism of gene CYP2E1 depends on its continental origin, just like the aforementioned genes. Recent genetic epidemiological data suggest several positive relations between genotype and risk of cancer.

Excessive alcohol consumption, apart from low folate intake with food products, causes folate deficiency, too. This folate depletion is caused by two main mechanisms: (1) decreasing intestinal absorption and hepatic update; (2) increasing renal excretion through a reduction in tubular reabsorption. Folate metabolism influences DNA methylation and synthesis associated with carcinogenesis. The polymorphism of methylenetetrahydrofolate reductase (MTHFR), 5-methyltetrahydrofolate-homocysteine *S*-methyltransferase (MTR), and thymidylate synthase (TS) have been investigated in relation to the risks for colorectal, breast, esophageal, gastric, and pancreatic cancers, as well as for hepatocellular carcinoma with alcoholic liver cirrhosis [1]. However, the results from a recent study in the Australian population raise the possibility that folic acid supplementation may increase the risk of Barrett's esophagus with dysplasia and esophageal adenocarcinoma [11].

Eight viruses were recognized as carcinogenic: HHV-4 Epstein-Barr virus (EBV), hepatitis B virus (HBV), hepatitis C virus (HCV), human papillomavirus (HPV), human lymphotrophic virus type 1 (HTLV-1) and type 2 (HTLV-2), Kaposi sarcoma herpesvirus (KSHV) – human herpesvirus 8 (HHV-8), and human immunodeficiency virus (HIV). According to the genetic material within virus particles, viruses EBS, HBV, HPV, and KSHV are of the DNA type; the rest, HCV, HTLV-1, and HIV-1, belong to the DNA type. The aforementioned viruses with types of cancer having sufficient evidence and limited evidence are presented in Table 31.4. Also, this table provides information about cancers caused by alcohol consumption with the same limitations.

There are three major mechanisms of viruses' carcinogenesis: (1) direct (several types of the human papillomavirus family, T-cell lymphotropic virus type 1, Epstein-Barr virus, Kaposi sarcoma herpesvirus) – the viral genome is usually detected in each cancer cell, and virus can immortalize target cells in vitro; (2) indirect carcinogens that act via chronic inflammation (hepatitis viruses B and C); (3) indirect carcinogens that act via immune suppression (human immunodeficiency virus).

The methods of transmission of viral infection are varied (Table 31.5). However, in almost all of them there exists a sexual component. Taking into account that alcohol is a recognized marker for risky sexual behavior, this is one of the components of the effect of alcohol on cancer development [1, 7, 8].

Aerodigestive Tract Cancers: Oral Cancer and Cancers of the Oropharynx, Hypopharynx, and Esophagus

In 2008, the highest percentage of deaths due to mouth and oropharynx cancers (0.9%) was in Southeast Asia, whereas the lowest was in Africa (0.1%) (Table 31.6) [12]. The results from different studies confirmed the influence of alcohol consumption and human papilloma viruses on mouth, pharynx, and larynx cancer development.

Independent studies on alcohol consumption (3 cohorts, 62 case controls; 1982–2004 year) have shown a relative risk of highest versus lowest exposure category of greater than 1.0 [relative risk (95% CI); 0.80 (0.52–1.22) – 60.40 (20.98–173.86)]. The same finding was made regarding relative risk, drink/week consumption [2 cohorts, 31 case controls; 1969–2005; 1.01 (1.01–1.04) – 1.26 (1.10–1.44)]. The relationship between dose and response was independent of the type of study (cohort or case control) and was significantly positive [5].

The prevalence of HPV-16 detected in various tumor specimens ranged from 16 (oral cavity) to 90% (tonsil) [2]. Ongoing studies did not give a simple answer regarding the relation between HPV, alcohol intake, and cancer due to many confounding factors. It is difficult to find nonsmokers and

Table 31.4 Human cancers caused by viral infections or alcoholic beverage consumption

Viral human carcinogens			Alchohol originated cancers		
Viral agent	Limited evidence	Sufficient evidence	Sufficient evidence	Limited evidence	Alcohol beverage consumption
HHV Epstein-Barr Virus (EBV)[a]	Gastric, lymphoepitheliomalike	Nasopharyngeal, lymphomas: Burkitt's, immune-suppression-related non-Hodgkin, extranodal NK/T-cell (nasal type), Hodgkin	Nasopharyngeal	Leukaemia, lymphoma (Hodgkin disease, non-Hodgkin l lymphoma)	The risk increases with the level of alcohol consumption. Drinkers rarely consume one type of alcoholic beverage. The types of alcoholic beverage that are the largest contributors to alcoholic beverage consumption are usually associated with the greatest increases in risk.
Hepatitis B Virus (HBV)[b]	Cholangiocarcinoma non-Hodgkin lymphoma	Hepatocellular carcinoma	Liver cancer		
Hepatitis C Virus (HCV)[b]	Cholangiocarcinoma	Hepatocellular carcinoma, non-Hodgkin lymphoma			
Human papillomavirus (HPV)[c] (118 HPV types)	Larynx	Cervix, vulva, vagina, penis, anus, oral cavity, oropharynx and tonsil	Oral cavity, pharynx, oropharynx, larynx, uterine cervix, testis	Vulva cancer, vagina cancer	
Human lymphotrophic virus type 1 (HTLV-1)[a] Human lymphotropic virus type 2 (HTLV-2)		Adult T-cell leukemia/ hairy-cell leukemia		Lack of strong evidence for cancers of lymphatic and hematopoietic systems – multiple myeloma, Leukemia, lymphoma (Hodgkin disease, non-Hodgkin)	

Human herpesvirus 8 (HHV-8 – KSHV – Kaposi sarcoma-associated herpesvirus)[1]	Multicentric Castleman's disease	Kaposi sarcoma, primary effusion lymphoma	
Human immunodeficiency virus (HIV)[1]	Vulva, vagina, penis, nonmelanoma skin, hepatocellular carcinoma	Kaposi sarcoma, non-Hodgkin lymphoma, Hodgkin lymphoma, cervix, anus, conjunctiva	Vulvar cancer, vaginal cancer
			Cancer of the oesophagus, breast cancer, cancer of the stomach, colon and/or rectum, pancreas, lung, urinary bladder, endometrium, ovary, prostatę, kidney, melanoma, thyroid

[a]Group 1 – agent is carcinogenic to humans

[b]Group 1 – chronic infection with hepatitis B virus or with hepatitis C virus is carcinogenic to humans

[c]Groups 1 to 4 – among 118 types of HPV viruses – Group 1 – HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59; Group 2A – HPV type 68; Group 2B – HPV types 26, 30, 34, 53, 66, 67, 69, 70, 73, 82, 85, 97; Group 3 – HPV types 6, 11; Group 4 – HPV types

Adapted from Refs. [1] and [2]

Table 31.5 Methods of transmission of viral human carcinogens and possibility of alcohol influence

No.	Viral agent	Method of transmission	Influence of alcohol consumption
1.	HHV Epstein-Barr virus (EBV)	Oral route: young age, low socioeconomic status, poor hygiene standards	–
		Transfusion	–
		Sexual intercourse	+
2.	Hepatitis B virus (HBV)	Percutaneous and permucosal exposure to infected blood and other bodily fluids	±
		Transmission includes mother-to-infant, child-to-child, unsafe injection practices	–
		Blood transfusions	–
		Sexual contact	+
3.	Hepatitis C virus (HCV)	Transfusion of blood and blood products (eliminated in several countries due to routine HCV testing)	–
		Transplantation of solid organs from infected donors	–
		Injection, drug abuse, and unsafe therapeutic injections	±
		Occupational exposure to blood perinatal HCV	–
		Perinatal HCV transmission possible when HCV RNA is detectable in maternal serum at delivery	–
		Sexual transmission depending on type of relationship	±
4.	Human Papillomavirus (HPV) (118 HPV types)	Direct skin-to-skin or skin-to-mucus contact	±
		Anogenital HPV: sexual transmission	+
		Perinatal transmission	–
5.	Human Lymphotrophic Virus type 1 (HTLV-1)	Vertical transmission: prolonged breastfeeding	–
		Sexual route: unprotected sex with infected partner, multiple partners	+
		Infection with sexually transmitted diseases	+
6.	Human Herpesvirus 8 (HHV-8 – KSHV – Kaposi sarcoma associated herpesvirus)	Transmission primarily via saliva; infection occurs during childhood and increases with age. Risk factor immunovirus HIV	±
		Sexual transmission; mostly homosexual	+
		Blood-borne transmission	±
		Transmission by organ donation possible	–
7.	Human Immunodeficiency Virus (HIV)	Blood contact: blood transfusion, occupationally through needle	–
		Sexual intercourse: unprotected vaginal or anal intercourse	+
		Mother-to-child transmission during pregnancy, labor,and delivery, and postpartum through breastfeeding	–
		Needle sharing by intravenous drug users	±

– lack of evidence; + evident influence; ± possible evidence

nondrinkers among oral and oropharyngeal subjects and control groups in published papers, as well as only drinkers or only smokers [2, 9, 13–18].

The analysis of HPV DNA crude prevalence among women with normal cytology by world region (meta-analysis including 157,879 women from 36 countries) confirmed a range of 6.6–22.9% [2]. Comparing this fact with the risks for sexually transmitted diseases due to early adolescent alcohol use and sexual experience we obtained an explanation of other results showing a decrease in the age of oropharyngeal cancer patients [2, 9, 16–18].

Table 31.6 Deaths (000s) by cause (viral disease, cancer, violence), countries grouped by WHO subregion (a), estimates for 2008

Cause	World (b)		Africa		The Americas		Eastern Mediterranean		Europe		Southeast Asia		Western Pacific	
	6,737,480		804,865		915,430		580,208		889,70		1,760,486		1,787,321	
Population (000)	(000)	% total	(000)	% total	(000)	% total	(000)	% total	(000)	% total	(000)	% total	(000)	% total
Total deaths	**56,888**	**100.0**	**10,125**	**100.0**	**6,170**	**100.0**	**4,198**	**100.0**	**9,223**	**100.0**	**14,498**	**100.0**	**12,674**	**100.0**
I. Communicable diseases, maternal and perinatal conditions, and nutritional deficiencies	**15,637**	**27.5**	**6,577**	**65.0**	**723**	**11.7**	**1,524**	**36.3**	**532**	**5.8**	**5,033**	**34.7**	**1,248**	**9.9**
Infectious and parasitic diseases	**8,721**	**15.3**	**4,190**	**41.4**	**289**	**4.7**	**660**	**15.7**	**237**	**2.6**	**2,806**	**19.4**	**540**	**4.3**
HIV/AIDS	1,776	3.1	1,303	**12.9**	69	**1.1**	25	**0.6**	77	**0.8**	244	**1.7**	58	**0.5**
Hepatitis B (d)	128	0.2	12	**0.1**	4	**0.1**	15	**0.4**	5	**0.1**	53	**0.4**	39	**0.3**
Hepatitis C (d)	69	0.1	5	**0.1**	11	**0.2**	7	**0.2**	6	**0.1**	20	**0.1**	20	**0.2**
II. Noncommunicable conditions	**36,122**	**63.5**	**2,861**	**28.3**	**4,853**	**78.7**	**2,229**	**53.1**	**8,027**	**87.0**	**7,914**	**54.6**	**10,238**	**80.8**
Malignant neoplasms	**7,583**	**13.3**	**407**	**4.0**	**1,193**	**19.3**	**315**	**7.5**	**1,872**	**20.3**	**1,135**	**7.8**	**2,660**	**21.0**
Mouth and oropharynx cancers	281	0.5	13	**0.1**	24	**0.4**	16	**0.4**	50	**0.5**	125	**0.9**	54	**0.4**
Esophageal cancer	414	0.7	24	**0.2**	33	**0.5**	15	**0.4**	50	**0.5**	61	**0.4**	231	**1.8**
Stomach cancer	758	1.3	18	**0.2**	70	**1.1**	22	**0.5**	147	**1.6**	63	**0.4**	439	**3.5**
Colon/rectal cancer	647	1.1	21	**0.2**	111	**1.8**	18	**0.4**	245	**2.7**	67	**0.5**	186	**1.5**
Liver cancer	695	1.2	43	**0.4**	48	**0.8**	13	**0.3**	66	**0.7**	62	**0.4**	462	**3.6**
Pancreatic cancer	270	0.5	7	**0.1**	62	**1.0**	5	**0.1**	102	**1.1**	16	**0.1**	77	**0.6**
Trachea/bronchus/lung cancers	1,387	2.4	16	**0.2**	248	**4.0**	25	**0.6**	376	**4.1**	136	**0.9**	588	**4.6**
Melanoma and other skin cancers	77	0.1	5	**0.1**	22	**0.4**	2	**0.1**	30	**0.3**	4	**0.0**	14	**0.1**
Breast cancer	482	0.8	37	**0.4**	91	**1.5**	32	**0.8**	155	**1.7**	94	**0.6**	74	**0.6**
Cervix uteri cancer	277	0.5	51	**0.5**	36	**0.6**	11	**0.3**	30	**0.3**	103	**0.7**	47	**0.4**
Corpus uteri cancer	78	0.1	2	**0.0**	15	**0.2**	1	**0.0**	26	**0.3**	8	**0.1**	25	**0.2**
Ovary cancer	140	0.2	8	**0.1**	26	**0.4**	7	**0.2**	45	**0.5**	33	**0.2**	21	**0.2**
Prostate cancer	272	0.5	24	**0.2**	83	**1.3**	10	**0.2**	101	**1.1**	20	**0.1**	34	**0.3**
Bladder cancer	160	0.3	8	**0.1**	27	**0.4**	14	**0.3**	62	**0.7**	16	**0.1**	32	**0.3**
Lymphomas, multiple myeloma	305	0.5	31	**0.3**	66	**1.1**	28	**0.7**	76	**0.8**	53	**0.4**	51	**0.4**
Leukaemia	267	0.5	13	**0.1**	49	**0.8**	19	**0.5**	65	**0.7**	45	**0.3**	76	**0.6**
Other neoplasms	**188**	**0.3**	**18**	**0.2**	**35**	**0.6**	**18**	**0.4**	**44**	**0.5**	**22**	**0.2**	**51**	**0.4**
Neuropsychiatric disorders	**1,310**	**2.3**	**132**	**1.3**	**352**	**5.7**	**85**	**2.0**	**329**	**3.6**	**232**	**1.6**	**181**	**1.4**
Alcohol use disorders	79	0.1	4	**0.0**	23	**0.4**	2	**0.0**	25	**0.3**	14	**0.1**	12	**0.1**

(continued)

Table 31.5 (continued)

Cause	World (b)		Africa		The Americas		Eastern Mediterranean		Europe		Southeast Asia		Western Pacific	
	6,737,480		804,865		915,430		580,208		889,70		1,760,486		1,787,321	
Population (000)	(000)	% total	(000)	% total	(000)	% total	(000)	% total	(000)	% total	(000)	% total	(000)	% total
Digestive diseases	**2,206**	**3.9**	**226**	**2.2**	**312**	**5.1**	**184**	**4.4**	**424**	**4.6**	**662**	**4.6**	**398**	**3.1**
Peptic ulcer disease	298	0.5	27	**0.3**	17	**0.3**	13	**0.3**	33	**0.4**	144	**1.0**	64	**0.5**
Cirrhosis of the liver	849	1.5	31	**0.3**	114	**1.8**	78	**1.9**	185	**2.0**	284	**2.0**	157	**1.2**
III. Injuries	**5,129**	**9.0**	**687**	**6.8**	**594**	**9.6**	**445**	**10.6**	**664**	**7.2**	**1,552**	**10.7**	**1,187**	**9.4**
Intentional injuries	**1,510**	**2.7**	**242**	**2.4**	**239**	**3.9**	**152**	**3.6**	**177**	**1.9**	**420**	**2.9**	**280**	**2.2**
Violence	535	0.9	162	**1.6**	157	**2.5**	22	**0.5**	46	**0.5**	102	**0.7**	47	**0.4**

(a) WHO Regions

African Region: African Region D (AFR D): Algeria, Angola, Benin, Burkina Faso, Cameroon, Cape Verde, Chad, Comoros, Equatorial Guinea, Gabon, Gambia, Ghana, Guinea, Guinea-Bissau, Liberia, Madagascar, Mali, Mauritania, Mauritius, Niger, Nigeria, Sao Tome and Principe, Senegal, Seychelles, Sierra Leone, Togo; *African Region E* (AFR E): Botswana, Burundi, Central African Republic, Congo, Côte d'Ivoire (Ivory Coast), Democratic Republic of the Congo, Eritrea, Ethiopia, Kenya, Lesotho, Malawi, Mozambique, Namibia, Rwanda, South Africa, Swaziland, Uganda, United Republic of Tanzania, Zambia, Zimbabwe

Region of the Americas: Region of the Americas A (AMR A): Canada, Cuba, United States of America; *Region of the Americas B* (AMR B): Antigua and Barbuda, Argentina, Bahamas, Barbados, Belize, Brazil, Chile, Colombia, Costa Rica, Dominica, Dominican Republic, El Salvador, Grenada, Guyana, Honduras, Jamaica, Mexico, Panama, Paraguay, Saint Kitts and Nevis, Saint Lucia, Saint Vincent and the Grenadines, Suriname, Trinidad and Tobago, Uruguay, Venezuela (Bolivarian Republic of); *Region of the Americas D* (AMR D): Bolivia, Ecuador, Guatemala, Haiti, Nicaragua, Peru

Eastern Mediterranean Region: Eastern Mediterranean Region B (EMR B): Bahrain, Iran (Islamic Republic of), Jordan, Kuwait, Lebanon, Libyan Arab Jamahiriya, Oman, Qatar, Saudi Arabia, Syrian Arab Republic, Tunisia, United Arab Emirates; *Eastern Mediterranean Region D* (EMR D): Afghanistan, Djibouti, Egypt, Iraq, Morocco, Pakistan, Somalia, Sudan, Yemen

European Region: European Region A (EUR A): Andorra, Austria, Belgium, Croatia, Cyprus, Czech Republic, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Israel, Italy, Luxembourg, Malta, Monaco, Netherlands, Norway, Portugal, San Marino, Slovenia, Spain, Sweden, Switzerland, United Kingdom; *European Region B* (EUR B): Albania, Armenia, Azerbaijan, Bosnia and Herzegovina, Bulgaria, Georgia, Kyrgyzstan, Montenegro, Poland, Romania, Serbia, Slovakia, Tajikistan, Former Yugoslav Republic of Macedonia, Turkey, Turkmenistan, Uzbekistan; *European Region C* (EUR C): Belarus, Estonia, Hungary, Kazakhstan, Latvia, Lithuania, Moldova, Russian Federation, Ukraine

Southeast Asia Region: Southeast Asia Region B (SEAR B): Indonesia, Sri Lanka, Thailand; *Southeast Asia Region D* (SEAR D): Bangladesh, Bhutan, Democratic People's Republic of Korea, India, Maldives, Myanmar, Nepal, Timor-Leste

Western Pacific Region: Western Pacific Region A (WPR A): Australia, Brunei Darussalam, Japan, New Zealand, Singapore; *Western Pacific Region B* (WPR B): Cambodia, China, Cook Islands, Fiji, Kiribati, Lao People's Democratic Republic, Malaysia, Marshall Islands, Micronesia (Federated States of), Mongolia, Nauru, Niue, Palau, Papua New Guinea, Philippines, Republic of Korea, Samoa, Solomon Islands, Tonga, Tuvalu, Vanuatu, Viet Nam

(b) World totals for males and females; do not include populations living outside WHO member states

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Chapter 32
Ethanol and Hepatocarcinogenesis

Helmut K. Seitz and Felix Stickel

Key Points

The present chapter addresses specifically:

- The epidemiology of alcohol-associated hepatocellular carcinoma and the link to coexisting non-alcoholic liver diseases
- Molecular mechanisms of alcohol-associated liver cancer development as evidenced by animal experimentation
- Key events of alcohol-mediated hepatocarcinogenesis including cirrhosis as a precancerous condition, inflammation and cytokine abnormalities facilitating HCC evolution, co-infection with hepatitis B and C viruses, iron storage and non-alcoholic fatty liver disease (NAFLD)
- Molecular interactions of alcohol with transmethylation processes and retinoic acid metabolism
- The central role of acetaldehyde and reactive oxygen species in liver cancer initiation

Keywords Acetaldehyde • Alcohol dehydrogenase • Cytochrome P450 2E1 • Gene methylation • Liver cirrhosis • Non-alcoholic fatty liver disease • Retinoic acid • Viral hepatitis

Introduction

The incidence of hepatocellular cancer (HCC) is rising worldwide. HCC is the most frequent complication of hepatic cirrhosis, and its increase may also be explained by the fact that therapy of liver cirrhosis has improved and cirrhotic patients live longer as compared to decades ago and may, therefore, develop HCC more frequently [1]. In addition, hepatitis B and C infections leading to cirrhosis and HCC are still not under control in certain geographic areas of the world [2]. Furthermore, in the

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Western world, non-alcoholic fatty liver disease (NAFLD) is almost endemic, and data available show an increased burden of HCC in patients with this disease [3].

Subsequently, chronic alcohol consumption is a major health problem worldwide associated with addiction and organ damage. The Global Burden of Disease Project of the WHO concludes that alcohol accounts for approximately 1.8 million deaths per year and one of the most significant diseases caused by chronic alcohol consumption is cancer [4]. In February 2007, an international group of specialist met at the International Agency for Research on Cancer (IARC) in Lyon, France, to evaluate the role of alcohol and its first metabolite acetaldehyde, as potential carcinogens. This working group concluded finally that the occurrence of malignant tumours of the oral cavity, pharynx, larynx, oesophagus, liver, colorectum and female breast is causally related to the consumption of alcoholic beverages [5]. Worldwide, a total of approximately 389,000 cases of cancer representing 3.6% of all cancers derive from chronic alcohol consumption [6].

In this review, a brief analysis of epidemiology and experimental data of HCC will be given. Major emphasis, however, will be put on molecular mechanisms of alcohol-derived HCC.

Epidemiology

HCC is among those cancers that present with a rising incidence worldwide, particularly in Western industrialized countries. For example, in the USA, HCC is the fastest growing cause of cancer-related death in men with incidence rates increasing more than twofold between 1985 and 2002 [7]. Overall, HCC is the fifth most common cancer and the third most frequent cause of cancer mortality, only surpassed by cancers of the lungs and the stomach [8]. Incidence of HCC closely corresponds with mortality from HCC with some 626,000 cases diagnosed each year and 598,000 deaths due to HCC. However, the burden of HCC is not evenly distributed throughout the world, and important differences between countries and regions have been recorded. For example, HCC is as high as 99/100,000 in the Mongolian Republic, around 30–35/100,000 in China and Japan and similar figures in sub-Saharan Western Africa. Countries with a moderate incidence of HCC (~10–15/100,000) include Italy, Spain and Greece, while typical low-incidence countries (1–5/100,000) comprise France, Great Britain, Germany, Canada, Northern America and Scandinavia [9]. What has been observed over the last decade is a gradually decreasing incidence of HCC in many high-prevalence areas of the world, whereas the incidence of HCC in low-prevalence regions such as the United States and Europe has nearly doubled [2, 10]. While the former decline is likely the result of large-scale vaccination against hepatitis B virus infection and decreased exposure to dietary aflatoxins, the latter increase has been ascribed to the rising incidence of progressively fibrosing viral hepatitis C and persistently high alcohol consumption. In an analysis of 1,605 patients diagnosed with HCC between 1993 and 1998, rates of HCC due to chronic hepatitis C infection increased threefold, while age-adjusted rates for HCC following chronic hepatitis B infection and alcohol abuse remained stable [11]. Noteworthy, recent compelling scientific evidence suggests that non-alcoholic fatty liver disease (NAFLD) likely accounts for a substantial proportion of “cryptogenic” cirrhosis and HCCs that develop in this context [12].

Animal Experiments

For a long time, alcohol has not been considered a carcinogen rather than a co-carcinogen and/or a tumour promoter, since its administration alone did not induce tumours. However, in an important study by Beland and co-workers in B6C3F1, mice of female and male sex were exposed to alcohol 2.5% and 5.0% in the drinking water for 104 weeks without any additional carcinogen. As a result, more male animals developed hepatocellular adenoma and hepatocellular carcinoma with a significant

dose-related trend with $p<0.05$ [13]. This was for the first time that chronic alcohol consumption shows a carcinogenic effect in the liver without administration of an additional carcinogen.

More than 50 studies were performed to determine whether ethanol can modify chemically induced carcinogenesis, using various mouse and rat strains and various carcinogens to induce tumours. In most of the studies, the co-administration of ethanol increased chemically induced carcinogenesis (for review, see IARC Monograph Vol 96, 2010).

With respect to hepatocarcinogenesis, most of the studies have been performed with nitrosamines as inducing agents. Almost all these studies showed an inhibition of carcinogenesis with alcohol but on the other hand an enhancement in the incidence of extrahepatic tumours such as those in the nasal cavity, trachea and oesophagus (IARC Monograph Vol 96, 2010). Only if additional manipulations were added, such as administration of methyl-deficient or low-carbohydrate diet [14, 15] or partial hepatectomy [16], was hepatic carcinogenesis stimulated by alcohol. A striking enhancement of hepatic carcinogenesis was also observed when alcohol and the procarcinogen were given strictly alternatively to avoid an interaction between alcohol and carcinogen metabolism.

In most recent animal experiments in which rats were fed with alcohol-containing liquid diets for 4 weeks with and without a small single dose of diethylnitrosamine given prior to the alcohol administration, exiting results were found. These animals also received chlormethiazole, a strong cytochrome P450 2E1 (CYP2E1) inhibitor. Ethanol feeding resulted in a significant increase in p-GST-positive altered hepatic foci, a procarcinogenic lesion. This was associated with a significant increase in hepatic CYP2E1 and nuclear accumulation of NFκ(kappa)B protein. Simultaneous chlormethiazole treatment inhibited hepatocellular regeneration, NFκ(kappa)B protein and the occurrence of hepatic p-GST foci [17]. Furthermore, even more puzzling, 10 months feeding of the alcohol-containing diet resulted in hepatic adenoma formation in almost all animals which was completely blocked by chlormethiazole (Wang and Seitz, unpublished observation). These animal experiments contribute to the understanding of the underlying mechanisms of the co-carcinogenic effect of ethanol. Both, the induction of CYP2E1 by chronic ethanol administration resulting in oxidative stress as well as in the depletion of retinoic acid may be responsible for the findings observed since inhibition of CYP2E1 prevents carcinogenesis.

Pathophysiology

Hepatic Cirrhosis as a Major Prerequisite for HCC

The vast majority of alcohol-associated HCCs develop in patients who have alcoholic cirrhosis. Alcohol-related HCC without pre-existing cirrhosis is rare; however, case series have shown that this may occasionally occur [18–20]. Fattovich et al. summarized and analysed available data on the annual incidence of HCC in different aetiologies of liver cirrhosis and calculated the 5-year incidence of HCC in alcoholic cirrhosis at 8% (Fig. 32.1) [21]. These findings correspond to former data investigating the independent and joint effects of alcohol drinking, its cessation and chronic hepatitis C on the risk of HCC [22]. Interestingly, authors of the same study showed that former drinkers who had been abstinent for less than 10 years carry a higher risk of developing HCC than those who continue to drink. Explanations could be that cessation of drinking rather reflects advanced liver cirrhosis which is per se associated with HCC occurrence, or stimulated liver cell regeneration following alcohol abstinence enhancing cell turnover, expansion of dysplastic cell clones and the likelihood of tumour initiation.

Certain histological features typically seen in established HCC are already present, albeit less pronounced, in alcoholic cirrhosis indicating that pathogenic events leading to cirrhosis precede those causing HCC [23]. To these premalignant lesions belong enzyme-altered foci and preneoplastic nodules which can also be induced in certain rodent HCC models [24]. Interestingly, Mallory body (MB)

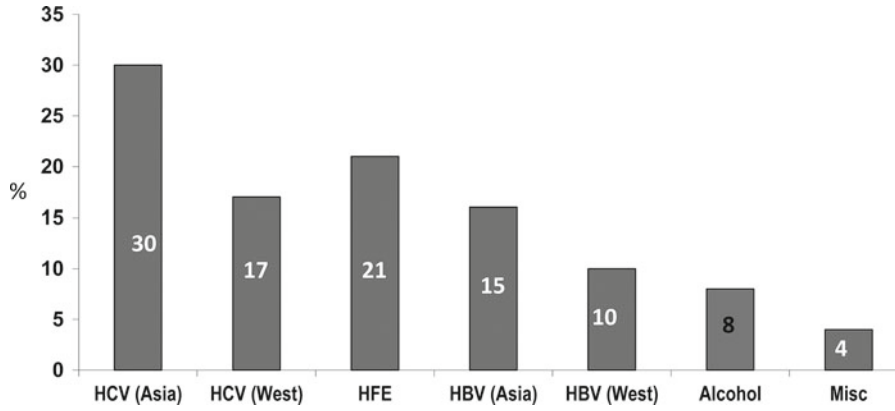

Fig. 32.1 Five-year cumulative incidence of HCC in different aetiologies of liver cirrhosis (Based on data from Ref. [21])

formation is high in HCC, and the incidence of HCC is significantly higher in cirrhosis with MBs than without leading to the hypothesis that MBs may represent an initial phenotypical alteration in the carcinogenic transformation of hepatocytes [25]. In addition, oval cells – pluripotent liver progenitor cells – are present in premalignant liver tissues HCC and adjacent tissues, and evolve in response to long-term alcohol exposure [26].

In summary, hallmarks of cirrhotic transformation including alterations of matrix composition, growth factor and cytokine milieu, disturbed vascularization and reduced capacity of cirrhotic tissue to handle oxidative and/or toxic insults create an environment that favours dedifferentiation and malignant growth.

Hepatic Inflammation, Intracellular Signal Transduction and HCC

HCC evolution is closely linked to chronic liver injury from various causes including alcohol, but rarely develops in healthy liver during physiological ageing. One possible explanation for this tight correlation is that HCC development requires cell division, leading to the stepwise accumulation of genetic hits necessary for dysplastic changes. The most common and unifying condition associated with hepatocarcinogenesis is cirrhosis which takes long to develop (20–40 years). As mentioned above, cirrhosis induces alterations of the microenvironment including altered cytokine secretion from activated hepatic stellate cells and portal fibroblasts, as well as inflammatory signalling from infiltrating immune cells. In association with the latter, molecular signals derived from pro-inflammatory tumour necrosis factor-α (TNF-α) are considered pivotal in ALD [10]. Excessive alcohol consumption can lead to an increased portosystemic uptake of endotoxins from gut bacteria which contribute to necroinflammation and fibrosis progression via various molecular mechanisms including tumour necrosis factor-α (TNF-α) and the CD14/toll-like receptor 4 complex to produce ROS via NADPH oxidase [27–29]. In fact, elevated TNF-α levels and corresponding cytokines are a prominent feature of ALD compared with other liver diseases, finally resulting in hepatocyte proliferation or apoptotic/necrotic death, recruitment of inflammatory cells and tissue remodelling. Molecular responses are triggered upon binding of TNF-α to its cellular receptors on hepatocytes and other liver cells leading to activation of adaptor protein 1 (AP-1; c-jun/c-fos), crosstalk with epidermal growth factor signalling and subsequently enhanced cell proliferation and potentially to apoptosis via caspase activation [30]. Beyond that, TNF-α activates sphingomyelinase to increase intracellular ceramide which inhibits the mitochondrial electron transport chain. Consequently, increased production of ROS promotes lipid peroxidation and apoptosis independently of caspases. However, increased oxidative stress also contributes to activation of transcription factor nuclear factor κB which is

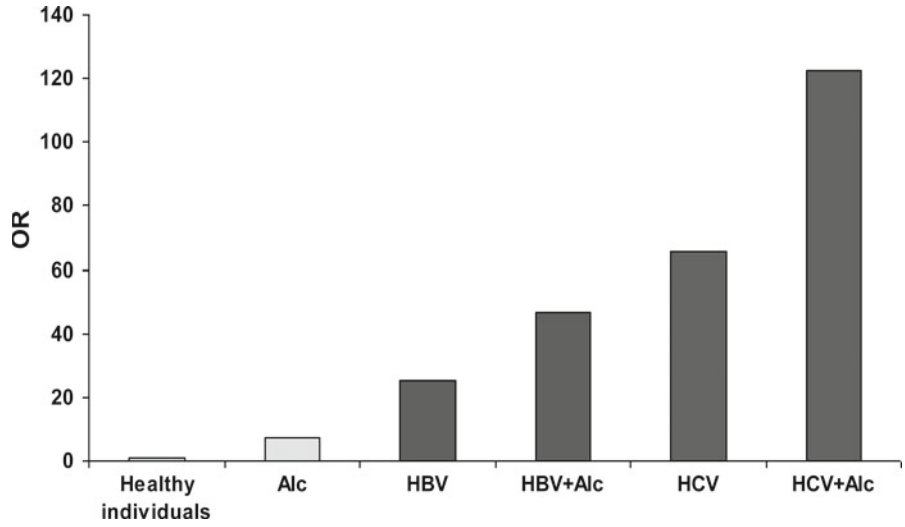

Fig. 32.2 Odds ratios (*OR*) for HCC in drinkers with/without chronic viral hepatitis B or C. Coexisting alcohol drinking doubles the risk of HCC in patients infected with hepatitis B or C virus (Based on data from Ref. [22])

instrumental for the initiation of cell survival mechanisms involving the upregulation of antiapoptotic proteins such as Bcl-2, manganese superoxide dismutase and nitric oxide synthase that can all protect mitochondrial integrity and function. Indeed, upregulation of nuclear factor κB expression has been convincingly demonstrated both in human and experimental ALD [31, 32]. Hence, TNF-α may dose dependently activate cellular survival mechanisms, or elicit apoptosis and/or necrosis. This may provide an explanation why hepatocytes challenged by inflammatory insults below the threshold to cause cell death may become more susceptible to proliferative stimuli and to dedifferentiation triggered by carcinogens such as (alcohol-derived) acetaldehyde.

Alcohol as a Risk Modifier for HCC in Other Liver Diseases

Chronic alcohol consumption may enhance the risk of HCC development in other liver diseases including viral hepatitis [33], hereditary hemochromatosis (HH) [34] and non-alcoholic fatty liver disease (NAFLD) [3]. Hepatitis B and C infections account for the magnitude of chronic liver diseases potentially leading to HCC in the developing world, whereas NAFLD along with the obesity epidemic is a rising aetiology of HCC in Western countries. In these diseases, which render the liver susceptible to additional oncogenic insults, chronic alcohol consumption even at moderate levels could have a striking influence on the risk of HCC in millions of people.

Viral Hepatitis

Epidemiological data from the study by Donato and co-workers mentioned above [22] show that both infections with hepatitis B and C viruses cause an approximately twofold increase in the risk of HCC in subjects drinking >60 g/day of alcohol in both instances (Fig. 32.2).

Not surprisingly, the coexistence of two liver diseases (alcohol + chronic infection with hepatitis viruses) synergistically enhances the risk of liver disease progression, and regarding hepatitis B and C, that of HCC. However, the mechanisms leading to hepatoma evolution are imprecisely defined and may be distinct between the two types of viral hepatitis.

Hepatitis B

At present, there are few human studies on the interaction between HBV infection and alcohol intake; most were conducted in Mediterranean Europe. A large multicenter study from France in 2001 analysed causes of death and covariates in 999 patients extracted from 65,000 death certificates listing HBV, HCV, hepatitis, liver disease, possible complication of cirrhosis, bacterial infection, HIV or transplantation and found that death related to HBV or HCV infection occurred at an earlier age in patients with a history of excessive alcohol consumption, however, without providing alcohol quantities that defined "excessive drinking" [35]. Data from East Asia are similar such as from a Japanese prospective cohort study which demonstrated that heavy alcohol intake with a cumulative lifetime consumption of >500 kg of alcohol can increase the risk of progression to cirrhosis sixfold relative to alcohol abstinence among patients chronically infected with HBV [36]. Similarly, another study by the same authors among patients with compensated HBV-related cirrhosis showed that heavy alcohol intake was associated with a threefold increased risk for HCC [37]. A population-based cohort study from Korea found that in the subgroup of chronic HBV carriers, the HCC risk rose dose dependently with an alcohol intake of 50–99 g/day with a relative risk of 1.2 (95% CI 1.0–1.5) and of 1.5 (95% CI 1.2–2.0) for >100 g/day [38]. Whether this synergistic effect on the risk of HCC from alcohol and coexistent HBV infection is additive or exponential is not known.

Putative mechanisms are yet unknown, but may relate to distinct pattern of methylation of certain HCC-associated genes as evidenced by Lambert and co-workers [39] who showed a high frequency of aberrant hypermethylation of specific genes (RASSF1A, GSTP1, CHRNA3 and DOK1) in HCCs as compared to control cirrhotic or normal liver tissues. An association between alcohol intake and hypomethylation of the methylguanine methyltransferase gene promoter was demonstrated, whereas HBV infection was linked to promoter hypermethylation of glutathione S-transferase, indicating that hypermethylation of the genes analysed in HCC tumours exhibits remarkably distinct patterns depending on associated risk factors.

Hepatitis C

Abundant evidence exists testifying a clear synergistic effect of coexisting alcohol abuse and chronic infection with hepatitis C virus. This circumstance is important since the prevalence of HCV infection is significantly higher among alcoholics than in the general population; for example, while HCV antibody positivity in the general population in the USA is approximately 1%, this figure raises to 16% among alcoholics and even 30% in individuals with ALD [40–42].

A large observational study from Northern Italy analysed risk factors of progression of chronic hepatitis C and development of HCC in anti-HCV-positive subjects extracted from known the Dionysos cohort and found alcohol above 90 g/day to be a significant risk factor for HCC [43]. Hassan and co-workers conducted a hospital-based, case–control study among 115 HCC patients and 230 non-liver cancer controls matched by 5-year age groups, sex and year of diagnosis [44]. Factors independently associated with HCC were chronic hepatitis B and C, alcohol consumption (>80 g/day) and type II diabetes. Significant synergistic interactions were observed between heavy alcohol consumption and chronic hepatitis C virus infection (OR 53.9; 95% CI 7.0–415.7) and diabetes mellitus (OR 9.9; 95% CI, 2.5–39.3). The study emphasized that heavy alcohol consumption contributes to the majority of HCC cases (32%), whereas 22%, 16%, and 20% were explained by HCV, HBV and diabetes mellitus, respectively. Similar data have been gathered for Europe and Asia as well in which concomitant alcohol consumption in HCV-infected individuals increases the risk of HCC additively, if not exponentially [45–47].

The underlying pathophysiology of this synergistic impact on HCC evolution is still not completely understood but may relate to joint effects of both alcohol and HCV on certain effects conveyed by

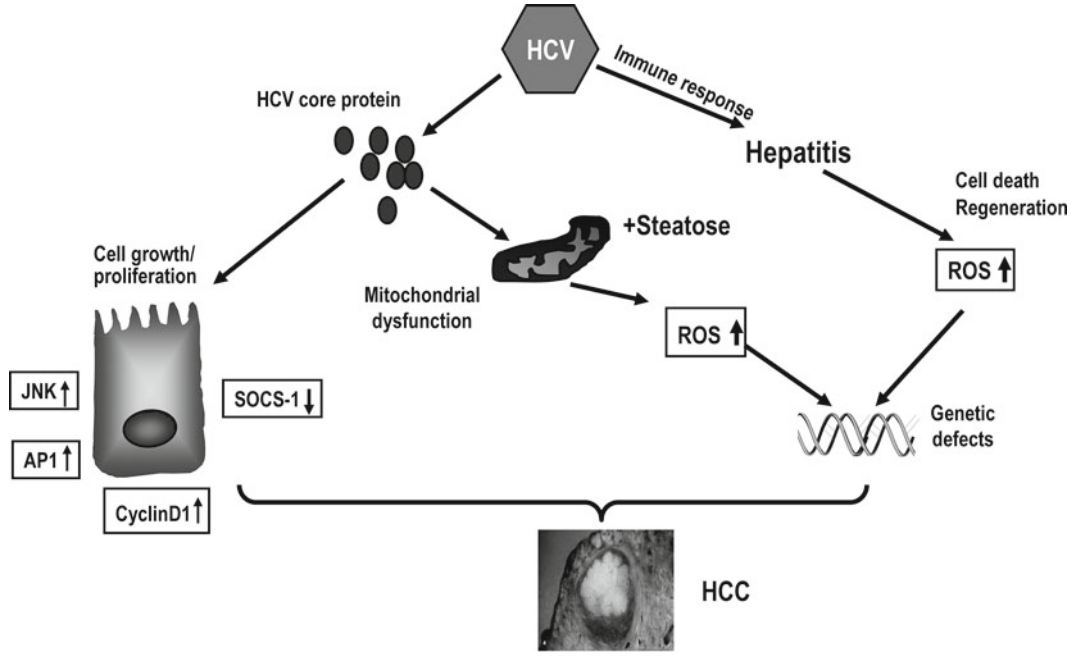

Fig. 32.3 HCV core protein promotes hepatocarcinogenesis indirectly via causing chronic inflammation and mitochondrial dysfunction. However, evidence exists that HCV may also directly cause malignant transformation through stimulating hyperproliferation, reduction of cytokine release and upregulation of cell survival mechanisms

HCV epitopes on key molecular events instrumental in hepatocarcinogenesis. In keeping, experimental evidence generated by Moriya and associates is highly suggestive of a direct oncogenic effect of HCV core protein in mice [48]. In their study, the development of HCC in two independent lines of mice transgenic for the HCV core gene, but not of envelope or non-structural (NS) proteins, was reported. The same mice spontaneously develop steatosis early in life as a feature of chronic hepatitis C infection and of alcohol. The latter similarity allows for speculations with regard to coexisting alcohol abuse [49]: the downstream events of the core protein are segregated into two components. One is the augmented production of oxidative stress along with the activation of scavenging system, including catalase and glutathione, in the putative pre-neoplastic stage with steatosis in the liver. Thus, oxidative stress production in the absence of inflammation by the core protein would partly contribute to the development of HCC. The generation of oxidative stress is estimated to originate from mitochondrial dysfunction in hepatocytes by HCV infection. Obviously, oxidative stress from concomitant alcohol consumption would further intensify these effects from HCV. The other component is the alteration of intracellular signalling cascade of mitogen-activated protein kinase (MAPK) and activating factor (AP)-1, leading to the activation of cell growth and proliferation. Notably, AP-1 upregulation is a key observation in alcohol-mediated liver cell regeneration via retinoic acid receptors, and MAP kinase cascades and their regulation by the phosphoinositide-3-kinase/Akt signalling cascade appear to be crucial in the onset of alcohol-mediated cell injury [50].

The combination of these pathways, collective with HCV-associated alterations in lipid and glucose metabolism, and inflammation-associated initiation of tumorigenesis would lead to the frequent development of HCC in persistent HCV infection. Since all of these mechanisms are also hallmarks of alcohol-associated hepatocarcinogenesis, concurrent attacks on these molecular targets from both alcohol and HCV represent an attractive explanation for the incidence of HCC in HCV-infected subjects with harmful drinking (Fig. 32.3).

Hereditary Hemochromatosis

HH is classified into four subtypes of which type 1 is of clinical importance in Europe and the USA. An autosomal recessive inborn error of metabolism (homozygous C282Y mutation of the HFE gene) on chromosome 6 results in general iron overload of various organs, especially of the liver, since intestinal iron absorption is dysregulated resulting in an enhanced uptake of iron [51]. The other subtypes affect the hemojuvelin, the hepcidin, the transferring receptor 2 and the ferroportin-1 gene.

The increased iron content of the liver may catalyze the generation of reactive oxygen species (ROS) with consecutive risks for the development of HCC [52]. Chronic alcohol consumption by itself results in a decrease of hepcidin and thus in an iron increased absorption of iron from the duodenum into the liver which further enhances iron accumulation [53]. In addition, as pointed out under 4.5.2., chronic alcohol consumption results in oxidative stress and ROS production. Both factors contribute to an enhanced risk for HCC in HH.

Non-alcoholic Fatty Liver Disease

Since hepatic histological changes in non-alcoholic steatohepatitis (NASH) as well as in alcoholic steatohepatitis (ASH) are indistinguishable, similar pathogenetic mechanisms may occur. In a recent animal study in rats fed with a high-fat diet for 6 weeks to induce NASH, it was clearly shown that the additional administration of alcohol (16% of total calories) resulted in an increased number of hepatic inflammatory foci and apoptotic hepatocytes. The aggravated inflammatory response and cellular apoptosis mediated by the high-fat alcohol diet were associated with elevated mRNA expression of Fas/FasL and cleaved caspase-3 protein [54]. Data of this animal experiment suggested that even moderate alcohol consumption can cause more hepatic inflammation and cellular apoptosis in pre-existing NASH condition.

Furthermore, using insulin-resistant, leptin-deficient Zucker rats, an animal model for NASH, chronic alcohol consumption resulted in a significant increase in highly carcinogenic exocyclic etheno-DNA adducts in the liver. In this study, both obesity and alcohol enhanced the generation of these DNA lesions [55].

It has also been shown in humans that hepatic fat accumulation [56] as well as hepatic fibrosis [57, 58] observed in obese individuals is found to be enhanced if alcohol is consumed. The Dionysos study from Northern Italy showed very clearly that fatty liver diagnosed per ultrasound is found more frequently in obese people (BMI <30) as compared to individuals with a BMI <25. This is even more pronounced if alcohol is consumed additionally with more than 60 g/day. Obesity and chronic alcohol consumption lead in almost 100% of cases to fatty liver [56].

With respect to fibrosis, it has been shown that alcohol is an important factor that increases fibrosis significantly in obese individuals. In patients with an alcohol consumption of more than 120 g/day, the prevalence of hepatic cirrhosis was found approximately double as high in individuals with a BMI of 29 as compared to 21 [57]. Finally, in a recent epidemiological study, it has been reported that even moderate alcohol consumption increases significantly the risk for HCC in patients with NASH [3]. In this study, it was shown that an even very small amount of alcohol has a similar risk for HCC in NASH patients as in patients with HCV infection.

Although it has been emphasized that small amounts of alcohol may improve peripheral insulin resistance, taking all these data together, especially the data with respect to HCC development, it is strongly recommended to avoid chronic alcohol consumption in patients with NAFLD.

Alcohol Enhances HCC Risk Due to Activation of Environmental Carcinogens

Alcoholics may be exposed to carcinogens or procarcinogens ingested along with alcoholic beverages which may contain nitrosamines, polycyclic hydrocarbons, asbestos fibres and fusel oils [59]. In addition, many alcoholics are smokers, and epidemiological surveys have shown a hyperadditive effect of alcohol and smoking in increasing the risk of developing HCC [60]. Similarly, dietary carcinogens and exposure to carcinogens at the working place have to be taken into account.

Some of these procarcinogens are activated by cytochrome P450 2E1 (CYP2E1), which is induced by chronic ethanol consumption (see "the role of oxidative stress"). Thus, nitrosamines, aflatoxins as well as vinyl chloride are all hepatocarcinogens and need cytochrome P450 activation to exert their carcinogenic potency [61].

Aflatoxin B1 can induce mutation in codon 249 of the p53 tumour suppressor gene which is frequently found in human HCC [62]. Although animal experiments have been controversial as to whether ethanol enhances AFB_1-induced hepatocarcinogenesis, an epidemiological study on AFB_1 exposure demonstrated that even a moderate daily consumption of 24 g ethanol increases the risk of developing HCC induced by 4 μg of dietary AFB_1 by 35-fold [63].

Vinyl chloride is also metabolized by CYP2E1, and its exposure is associated with the development of HCC which is again increased several fold by additional alcohol consumption [64].

Ethanol Metabolism and HCC

More than 90% of ethanol metabolism takes place in the liver catalyzed either by alcohol dehydrogenase (ADH), the microsomal ethanol oxidizing system (MEOS) which includes CYP2E1 or catalase. While catalase is of minor importance in hepatic ethanol metabolism, ADH and MEOS produce acetaldehyde (AA), the first and most toxic metabolite of ethanol as well as reactive oxygen species (ROS), and both of them may contribute to hepatocarcinogenesis (Fig. 32.4).

The Role of Acetaldehyde (AA)

AA is highly toxic, mutagenic and carcinogenic [65]. It interferes with DNA synthesis and DNA repair. In vivo and in vitro experiments in prokaryotic and eukaryotic cell cultures as well as in animal models have shown that AA has direct mutagenic and carcinogenic effects. AA causes point mutations in the hypoxanthine-guanine-phosphoribosyltransferase locus in human lymphocytes and induces sister chromatid exchanges and gross chromosomal aberrations [66–71]. AA induces inflammation and metaplasia of tracheal epithelium, delays cell cycle progression and enhances cell injury associated with cellular hyperregeneration in the mucosa of the oesophagus and colon [72, 73]. AA also binds to protein and DNA [74, 75]. Thereby, structure and function of various proteins are altered including the antioxidative defence system with glutathione, DNA repair enzymes and cell organelles such as mitochondria and microtubules [76]. Decreased mitochondrial function results in inhibition of fatty acid oxidation and ATP formation. Decreased microtubular function leads to inhibition of the secretion of macromolecules such as very-low-density lipoproteins from the liver. Both factors favour the generation of fatty liver. In addition, apoptosis as well as survival factors such as NFκ(Kappa)B are induced [77]. AA directly inhibits O6-methylguanosyl transferase, an enzyme that repairs DNA adducts [78].

Most importantly, however, AA binds to DNA and forms stable adducts [71, 79–85]. Binding to DNA represents one mechanism by which AA could trigger replication errors and/or mutations in oncogenes and tumour suppressor genes. It has been shown that the major stable DNA adduct

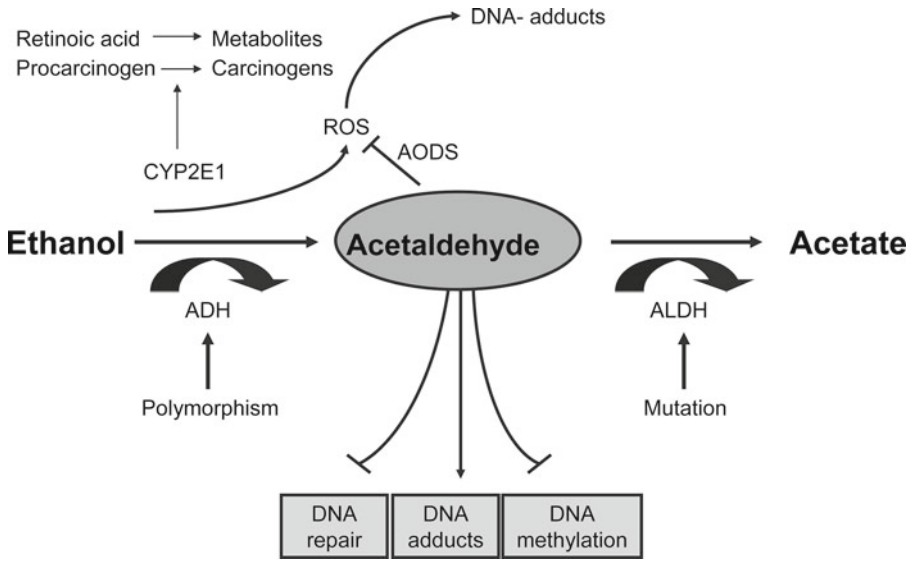

Fig. 32.4 Ethanol is metabolized to acetaldehyde via alcohol dehydrogenase (ADH) and further metabolized to acetate via acetaldehyde dehydrogenase (ALDH). Acetaldehyde has toxic, mutagenic and carcinogenic properties; inhibits DNA repair; decreases DNA methylation; and forms DNA adducts. Thus, acetaldehyde accumulation is associated with increased carcinogenesis as with ADH isoenzymes that reveal increased alcohol-metabolizing activity (ADH 1B*2 and ADH 1C*1). Acetaldehyde may also accumulate with ALDH isoenzymes with low acetaldehyde-degrading capacity as observed in 50% of Asians. Ethanol is also metabolized via cytochrome P2E1 (CYP2E1) to reactive oxygen species (ROS) which leads to lipid peroxidation, and lipid peroxidation products bind to DNA resulting in highly carcinogenic DNA adducts. There is an interaction with acetaldehyde since acetaldehyde inhibits the antioxidant defence system (AODS) resulting in a further increase of ROS. Finally, alcohol-induced CYP2E1 may also activate procarcinogens to carcinogens such as nitrosamines and can transform retinoic acid to inactive metabolites and/or to reactive polar metabolites which cause liver cell apoptosis. Retinoic acid depletion can promote cell proliferation and, thus, carcinogenesis. For more details, see text

N2-ethyl-desoxyguanosine (N2-Et-dG) serves as a substrate of eukaryotic DNA polymerase. However, N2-Et-dG seems rather a marker for chronic ethanol consumption than a major risk lesion for cancer. In addition, another DNA adduct of AA, 1,N2-propano-desoxyguanosine (PdG), has been identified, especially in the presence of basic amino acids, histones and polyamines. While N2-Et-dG is non-mutagenic and may represent a marker of chronic alcohol ingestion, PdG has mutagenic properties.

Most striking evidence of the causal role of AA in ethanol-mediated carcinogenesis is due to genetic candidate gene case–control studies in alcoholics. Individuals who accumulate AA due to polymorphisms and/or mutations in the genes coding for enzymes responsible for AA generation and degradation have been shown to have an increased cancer risk. Thus, individuals who drink alcohol and have a deficient AA dehydrogenase such as 40% of the Asian population with increased AA levels after drinking also have a high risk for various cancers such as those of the upper aerodigestive tract and the colon [86]. Similarly, individuals who produce more AA due to a rapid alcohol dehydrogenase (ADH1C1*1) also have an increased risk for these cancers including HCC [87].

In the liver, the situation seems to be more complex. On one hand, hepatic ethanol metabolism results in relatively high AA concentration, but on the other hand, the liver has also a high capacity to remove AA. AA removal depends primarily on the activity of mitochondrial ALDH 2. This enzyme activity, however, decreases with mitochondrial ethanol-mediated damage.

Besides the removal of AA, its generation is also of importance. Due to ADH polymorphisms, the gene product of ADH1B and ADH1C varies with respect to the enzyme activity and thus AA generation. While ADH1B does not play an important role in Caucasians, ADH1C polymorphisms may be

of relevance with respect to AA generation and thus cancer development [88]. In this context, it is interesting to note that Caucasians with ADH1C1.1 homozygosity associated with approximately 2.5 times higher production of AA compared to the ADH1C2.2 homozygosity seem to have an increased risk for HCC when they consume alcohol regularly [87].

According to the International Agency for Research on Cancer (IARC), there is sufficient evidence to classify AA as a carcinogen in experimental animals and humans.

The Role of Oxidative Stress

The formation of ROS such as superoxide anion and hydrogen peroxide causes oxidative injury. Several enzyme systems are capable to produce ROS, including the cytochrome P450 2E1 (CYP2E1)-dependent microsomal mono-oxygenase system, the mitochondrial respiratory chain and the cytosolic enzymes xanthine oxidase and aldehyde oxidase [89]. Ethanol-mediated ROS formation may be due to an increased electron leakage from the mitochondrial reparatory chain associated with the stimulation of reduced nicotinamide adenine dinucleotide (NADH) shuttling into mitochondria and to the interaction between N-acetylsphingosine (from tumour necrosis factor-alpha) and mitochondria. The induction of sphingomyelinase by TNF-α(alpha) increases the levels of ceramide, an inhibitor of the activity of the mitochondrial electron transport chain, leading to increased mitochondrial production of ROS [90]. ROS can also be generated in alcoholic hepatitis with activated hepatic phagocytes [91]. Hepatic iron accumulation as observed in alcoholic liver disease increases ROS and finally nitric oxide production due to ethanol-mediated stimulation of inducible nitric oxide synthase results in the formation of peroxynitrite which is highly reactive [92].

Most important, however, is the production of ROS via CYP2E1. It has been shown that alcohol induces CYP2E1 in the liver. This induction is an adaptive process and is associated with an increased metabolism of ethanol to acetaldehyde and also to ROS. The induction differs individually and is most likely due to the fact that the degradation of CYP2E1 by the ubiquitin proteasome pathway is inadequate since alcohol has an effect on this pathway. A significant increase in hepatic CYP2E1 activity occurs already following the ingestion of 40 g of ethanol daily for 1 week which is further enhanced after 4 weeks [93]. However, this occurs not in all individuals.

In animal experiments, the induction of CYP2E1 correlates with NAD phosphate (NADPH) oxidase activity, the generation of hydroxyethyl radicals, lipid peroxidation and the severity of hepatic damage, all of which could be prevented by the CYP2E1 inhibitor chlormethiazole [94]. In addition, DNA lesions have been found to be lower in CYP2E1 knock-out mice as compared to wild-type mice [95], and hepatic injury was significantly increased in transgenic mice that overexpressed CYP2E1 [96].

In an animal model using Lieber-DeCarli alcohol-containing and control diet hepatocarcinogenesis was induced by a single small dose of diethylnitrosamine given prior to the alcohol administration. One month of ethanol feeding resulted in a significant increase of preneoplastic lesions in the liver associated with an increase in NFκB protein and cellular regeneration which was not observed in control animals. Furthermore, chlormethiazole almost completely inhibited these changes induced by ethanol (Wang and Seitz, unpublished data). In the same experimental model, hepatic adenoma was observed following 10 months of ethanol feeding [63] which was completely inhibited by chlormethiazole (Wang and Seitz, unpublished data). Two explanations may exist for these observations: (1) ROS is responsible for enhanced hepatocarcinogenesis, (2) decreased retinoic concentrations are responsible for enhanced hepatocarcinogenesis (see under “alcohol retinoid interaction”) or (3) both.

ROS produced by CYP2E1 results in lipid peroxidation. Various lipid peroxidation products including 4-hydroxynonenal may bind to various purine and pyrimidine bases forming exocyclic DNA adducts. It has been shown that these adducts are highly mutagenic and carcinogenic [97, 98]. We have investigated biopsies from patients with various degrees and severities of alcoholic liver disease and found that in these biopsies, exocyclic DNA adducts are significantly increased.

This takes already place at the stage of alcoholic fatty liver [99]. More recently, we found a highly significant correlation between these adducts, CYP2E1 expression and 4 HNE, in liver biopsies from patients with ALD [55]. By using CYP2E1 overexpressing cells, we also found that the generation of etheno-DNA adducts can be correlated with the degree of CYP2E1 expression and can be inhibited by the CYP2E1 inhibitor chlormethiazole. In addition, etheno-adduct formation also correlates with CYP2E1 as well as with lipid peroxidation products such as 4-hydroxynonenal in human liver biopsies [55].

However, another factor which may be of major importance is the presence of the antioxidative defence system. Most exocyclic etheno-DNA adducts have been observed in cells with a high expression of CYP2E1 and a low concentration of mitochondrial glutathione. Thus, both factors may play an important role in the production of this important mutagenic DNA adduct. In addition, this adduct can also be detected in the urine of patients. Using HPLC for determination of these adducts, we found increased concentrations not only in patients with viral hepatitis such as hepatitis B and C but also in patients with alcoholic liver disease [100, 101]. Thus, measurement of exocyclic etheno-DNA adducts in the urine of patients with alcoholic liver disease could be a predictive marker for risk assessment of HCC in the alcoholic.

Alcohol and Altered DNA Methylation

Apart from genetic changes along with chronic alcoholism, i.e. mutations, DNA cross links or impaired DNA repair, chronic and acute alcohol intake may affect epigenetic mechanisms of gene expression such as methylation of DNA. DNA methylation is an important determinant in controlling gene expression whereby hypermethylation has a silencing effect on genes and hypomethylation may lead to increased gene expression. And indeed, alcohol intercepts with these epigenetic mechanisms [102].

Alcohol interacts with absorption, storage, biologic transformation and excretion of compounds which are essential for methyl group transfer including folate, vitamin B6 and certain lipotropes. Especially, the production of S-adenosyl-L-methionine (SAMe), the universal methyl group donor in methylation reactions, is impaired. Alcohol interacts with SAMe synthesis through inhibition of crucial enzymes involved in SAMe generation. This can lead to compromised formation of endogenous antioxidants such as glutathione and also lead to impaired cellular membrane stability [103].

In addition, alcohol interacts with methylation of certain genes and thereby contributes to liver damage and tumour development. Accordingly, alcohol-induced depletion of lipotropes may cause hypomethylation of oncogenes leading to their activation. The decrease in methylation capacity caused by chronic alcohol consumption can therefore contribute to epigenetic alterations of genes involved in hepatocarcinogenesis.

Alcohol Retinoid Interaction

It has been shown for decades that chronic alcohol consumption lowers hepatic vitamin E levels, especially in advanced alcoholic liver disease [104]. Retinoic acid plays an important role in controlling cell growth differentiation and apoptosis and is of potential clinical interest in cancer prevention and treatment. Therefore, the interaction with the retinoic acid metabolism by ethanol has important impacts on the aetiology, prevention and treatment of alcohol-related diseases.

The mechanism of alcohol-associated decrease in retinol and retinoic acid has multiple causes. Since ADH and ALDH share the common substrates ethanol and retinol as well as AA and retinal to

form retinoic acid, an interaction at these enzyme sites is not surprising. It has been demonstrated that ethanol acts as a competitive inhibitor of retinol oxidation [105]. Besides the fact that ethanol competes with retinol for the binding side of ADH, there are other mechanisms explaining the decrease in retinoic acid. Since chronic ethanol consumption increases CYP2E1 activity, an enhanced catabolism of vitamin A and retinoic acid into polar metabolites due to an induction of cytochrome P450 2E1 occurs [105]. Although a variety of cytochrome isoenzymes such as CYP1A1, CYP2B4, CYP2C3, CYP2C7, CYP2E1 and CYP26 are involved in retinoic metabolism, CYP2E1 seems of major importance [105]. The involvement of CYP2E1 in the metabolism of retinoic acid was proven by the fact that the CYP2E1 inhibitor chlormethiazole can completely inhibit this degradation [106]. The inhibitory effect of chlormethiazole on CYP2E1 may be related to its regulatory effect on CYP2E1 transcription in vivo in CYP2E1 catalytic activity in vitro mediated by binding to the heme iron of the enzyme. The prevention of reduced retinoic acid status in the liver of ethanol-fed rats by chlormethiazole treatment indicates that the breakdown of retinoids by microsomal CYP2E1 is a key mechanism for the ethanol-enhanced catabolism of retinoids in hepatic tissue after treatment with alcohol. Chronic ethanol consumption with low hepatic retinoic acid concentrations results in a downregulation of retinoic acid receptors and an up to eightfold expression of the AP-1 (c-jun and c-fos) transcriptional complex [105]. This explains parenchymal hyperproliferation as AP-1 is a central complex downstream of various growth factors, oncogenes and tumour promoters. Supplementation of retinoic acid to animals not only results in a decrease of AP-1 gene expression but also in reduced hepatic proliferation.

In addition to an increased degradation of retinol and retinoic acid by CYP2E1, this catabolism leads to polar retinoid metabolites which are identified as 4-oxo- and 18-hydroxy retinoic acid as well as some still unidentified metabolites [107]. However, these metabolites have been shown to have apoptotic properties leading to a change in the mitochondria, membrane potential, the liberation of mitochondrial cytochrome C, activation of caspases and finally apoptosis. This may explain why chronic alcohol consumption together with the administration of retinol or retinoic acid may lead to hepatic damage [108].

Summary and Conclusion

The incidence of HCC is rising worldwide. Chronic hepatitis B and C, alcohol abuse and a rising incidence of non-alcoholic fatty liver disease in many affluent countries are among the major causes. The pathogenic role of alcohol in the development of liver cirrhosis has been studied extensively, whereas our understanding of its importance as a modulating factor in hepatocarcinogenesis is only beginning to emerge. To date, a number of possible cofactors and mechanisms are well-investigated by which alcohol may enhance the development of HCC. These include dietary or environmental carcinogens ingested along with alcoholic beverages, alcoholic cirrhosis as a precancerous condition and the effects of alcohol metabolism such as the toxicity of its metabolite acetaldehyde, increased lipid peroxidation due to reactive oxygen species, activation of procarcinogens via induction of cytochrome P450 2E1 and DNA lesions derived from oxidative stress by-products. Alterations of DNA methylation through interactions with one carbon metabolism can lead to aberrant methylation of tumour suppressor genes and oncogenes, and alcohol metabolism reduces hepatic retinoic acid levels and alleviates retinoic acid-mediated silencing on hyperproliferation. Important environmental cofactors are alcohol and hepatitis B and especially C viruses, synergistically promoting HCC development. Formerly considered a tumour promoter, mounting evidence from human and experimental studies indicate that alcohol may also contribute to tumour initiation.

These insights underscore the importance of alcohol as an important aetiologic factor in hepatocarcinogenesis and potentially pave the way for preventive and therapeutic measures.

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Chapter 33
Alcohol, Diet, and Their Interaction in Colorectal and Urinary Tract Tumors

María Marta Andreatta, Aldo R. Eynard, and Alicia Navarro

Key Points

- Based on available evidence, we show data indicating that diet and alcohol strongly influences the risk for the development of colorectal and urinary tract tumors.
- For colorectal cancer, diet has shown to be one of the most significant factors, and alcoholic consumption is considered a toxic habit related to this cancer. Furthermore, a possible co-synergistic effect between high intake of alcoholic beverages and red meat – preferably eaten with heavy burn surface – becomes relevant in populations with a Western dietary pattern.
- The occurrence of urinary tract tumors may be related to a Western dietary pattern which includes high and frequent intake of red meat, potatoes, sugars, and alcoholic drinks. However, a moderate consumption of red wine, together with a healthy diet, would be protective.
- Dietary practices are a complex field of study, even more in relation to cancer, whose etiology is recognized as multicausal. The habit of consuming alcoholic beverages is one of those practices. Consequently, epidemiological studies should consider the type of alcoholic drink, the amount consumed, and also the frequency of consumption in order to achieve valid and reliable results.

Keywords Alcohol • Diet • Colorectal tumors • Urinary tract tumors • Epidemiology

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Introduction

Since the intoxicating effects of the fermented products of plant foods were discovered – probably in the Paleolithic or even at earlier times – alcoholic drinks have been largely consumed by humans [1]. Ancient records and art from Babylon, Mesopotamia and Egypt, and other early civilizations indicate that the use of alcohol as a beverage, medicine, and ceremonial drink was common [2]. Alcoholic beverages have been some of the few socially and legally permissible drugs in most societies – except for Muslims – and its use has even been recommended as a food or "tonic" for the sick and the children [3]. However, it is important to take into account that nowadays, about 21% all deaths worldwide from disease or trauma are attributable to alcoholic drink abuse [4].

After an alcoholic drink is consumed, blood alcohol levels reach their peak around 30–60 min and alter the functions of the central nervous system [1]. Hence, the alcohol blood concentration and the speed of saturation of the central nervous system with ethanol will determine its acute effect [3]. Thus, alcohol gradually depresses brain function and impacts on emotions, rational thought, and judgment. If alcoholic drink intake continues, motor control becomes impaired, causing slurred speech, slower reactions, and loss of balance (MedlinePlus 2011). It also could cause decreased alertness, impaired reflexes, vision changes, tremors, and hallucinations by altering the action of neurotransmitters. The above-combinated effects are linked to multiple traffic and occupational accidents, which claim for the lives of millions of people around the world every year [5].

By altering the action of neurotransmitters, results in decreased alertness, impaired reflexes, vision changes, tremors and hallucinations. It also decreases self-control and affects memory, concentration, and motor function. The combination of the above effects is because of multiple accidents and traffic, which each year claim the lives of millions of people around the world (Gisbert Calabuig and Villanueva Cañadas 2005). The blood alcohol levels reach their peak around 30–60 min after consumption and alter the functions of the central nervous system.

Worldwide, alcoholic drinks supply an average of 2–3% of total dietary energy. This ranges from around 10% in some northern European countries to – as said – practically zero in Islamic countries, where alcohol is illegal. Average consumption is nearly four times higher in high-income compared with low-income countries. However, consumption widely varies within countries: many people do not consume alcoholic drinks, some drink occasionally, and others consume even 15–25% or more of their dietary energy as alcohol [1].

Most of the research regarding food, nutrition and the prevention of chronic diseases has often excluded alcohol. This misleading exclusion is perhaps due to the fact that alcohol is considered a drug and its impact is mainly behavioral and social, as well as biological. Only recently, alcoholic beverages have been included in such research because it has been observed that low to moderate consumption protects against coronary heart disease together with the fact that may increase the risk of cancer, since ethanol is a human carcinogen [1].

Globally, there is a wide variation in alcoholic drink consumption among people older than 15 years old. The highest levels are found mainly in the developed world, especially in the northern hemisphere, but also in Australia, New Zealand [4], and Argentina [6–8]. South Africa, together with the North and the remaining South American countries, shows medium consumption levels. The lowest levels appear in Islamic populations, such as North Africa, sub-Saharan Africa, the Eastern Mediterranean region, South Asia, and oceanic India [4].

American countries have experienced significant changes in the consumption pattern of alcoholic beverages. Thus, it has been gradually turned from the use of traditional drinks – usually fermented and with low alcohol level – circumscribed to certain social occasions, traditional holidays or festivals, to the consumption of spirits on multiple situations without a specific social purpose. Even worst, these drinks are marketed and promoted as a feature of the so-called cosmopolitan urban lifestyle [9].

Since the mid-1990s, the epidemiological evidence linking alcohol with certain types of cancer has been increasing [1]. In the Americas, 14% of all malignant tumors are considered alcohol-related [9, 10].

Evidences point out that a certain degree of immunosuppression caused by nutritional deficiencies and/or direct effects of alcohol and its derivatives on immunocompetent cells may be a complex factor for cancer risk in alcohol abusers [11]. One of the explanations is that ethanol may favor tumorigenesis via free radical products released during its metabolism [12].

Our research group has studied the link between cancer and diet for several decades with an integrated experimental-epidemiological approach. Therefore, we proposed to analyze the role of alcoholic beverages, diet, and their interaction in colorectal and urinary tract tumors.

Some Methodological Aspects on Epidemiological Studies on Alcohol and Cancer

A Matter of Words

On scientific literature, the terms alcohol and alcoholic beverages are often confused with each other, although they refer to different things.

Alcohol is a misleading noun for ethanol or ethyl alcohol, a compound belonging to the family of alcohols. Certainly, it is the type of alcohol widely found in alcoholic beverages. However, it is also a universal vehicle for substances for human topics or consumption, including scents, flavorings, colorings, and many medicines. In laboratory practices, it is used as an essential solvent and a feedstock for the synthesis of other products as well. Its use as a fuel for heat and light has a long history, more recently, as a fuel in the mixtures for internal combustion engines [2]. Alcohol is easily produced in the nature when sugar molecules are split to release energy by several varieties of yeasts. Since it releases 7 kcal/g, it is also a strong source of energy unfortunately seldom considered in nutritional evaluation of the patients [1].

In many epidemiological studies, the terms "alcohol" and "alcoholic beverages" are used as synonyms, which can complicate further interpretation of the results. Indeed, sometimes it is difficult to understand if the authors of one research are referring to ethanol containing in a beverage or to alcoholic beverages.

Obviously, alcoholic beverages are drinks that contain ethanol. Those with lower alcohol content, such as beer and wine, are produced by fermentation of sugar or starch-containing plant material. Beverages of higher alcohol content, such as spirits, are produced by fermentation followed by distillation [2]. Other alcoholic drinks that may be locally important for certain populations include fermented milks, fermented honey-water, and fermented apples [1].

The main sources of ethanol for human consumption are:

- Beer: It contains between 3% and 7% of ethanol and several compounds with antioxidant properties [1]. It is the most consumed beverage worldwide, especially in Europe, North America, Oceania, and several African countries [4].
- Wine: Its content of ethanol varies from 9% to 15% [1]. Wine is consumed mainly in Europe and the Americas – especially in Argentina, Uruguay, and Chile [4]. Red wine has significant amounts of resveratrol, an antioxidant which is derived from the skin of grapes and seems to have anticancer properties [13].
- Spirits: These drinks contain between 35% and 50% of ethanol, although some reach even higher values, since they are obtained by distillation. Spirits include whiskey, vodka, grappa, gin, and tequila, among others [1]. They are consumed primarily in Asia and Eastern Europe [4].

The Matter of Epidemiological Studies on Alcohol and Cancer

Epidemiological studies consider different manners of the exposure to alcoholic beverages, such as:

- Drinkers versus abstainers
- Number of alcoholic drinks per time period
- Alcoholic drink consumption in grams or milliliters per time period
- Ethanol intake in grams or milliliters per time period
- Type/s of alcoholic drink/s consumed are commonly identified

Just comparing drinkers with nondrinkers is an oversimplification since this way of assessing does not reveal how much, how often, or what kind of drinks is consumed. Other measures are needed to estimate with certain precision the risk of disease in relation to alcohol intake.

Measurement of number of drinks per time period is likely to be less precise because the size of each drink usually remains unknown [1]. For instance, the standard measure for a unit of alcohol varies by country, as well as the standard measure used: a glass of wine can contain from 114 to 432 ml, and beer is sold in cans or bottles of different sizes [9].

Even if this issue can be resolved by locally designing and validating quali-quantitative food questionnaires [14], comparing alcohol intake within different studies still remains a complex issue on epidemiological research.

Otherwise, self-reporting of alcoholic beverage consumption is usually underestimated, since these drinks are known to be unhealthy and undesirable [1]. This could be even more notorious in cancer patients who suspect that their disease could be related to the consumption of alcohol. This particular aspect requires taking into consideration the manner that such issues should be addressed at the time of interview or survey.

Colorectal Cancer

Colorectal cancer is the third most common cancer worldwide, with around one million new cases recorded in 2002, having a mortality of approximately half of its incidence, which makes it the fourth most common cause of cancer death. This disease is slightly more common in men than in women, by 7–5. Additionally, risk increases with age [1].

In Latin America and the Caribbean, it is one of the most frequent types of cancer, both for women and men [15]. In Argentina, it is the third most common tumor type for both sexes, presenting also a high mortality rate [16]. Recently, the first study on geolocation of this and other tumors in Córdoba, Argentina, was published showing striking differences among counties [17–21]. Colorectal cancer is the third in incidence among men and the second for women from this Argentinean province [17].

Both environmental – lifestyle, especially diet – and genetic factors play key roles in colorectal cancer etiology. Strong genetic proneness has been observed in 5–10% of colorectal cancers [1].

Genetic proneness varies from strong defined inherited syndromes, such as familial adenomatous polyposis, to ill-defined familial clustering. Genetic and molecular mechanisms underlying are different. Some recent research indicates two main chains of sequence: a mutational pathway, involving microsatellite instability which appears mainly in hereditary nonpolyposis colon cancer and in a low proportion of sporadic carcinomas. The starting lesion is the adenoma, which is frequently detected and treated by routine endoscopic techniques. Nonneoplastic polyps are not considered precancerous unless they occur in polyposis syndromes. Inflammatory bowel diseases, such as chronic ulcerative colitis, require control by endoscopic surveillance due to the risk for colorectal cancer. Full recovery after surgery is linked to early diagnosis and anatomic compromise, which makes precise staging by histopathology very important. Other varieties of tumors are seldom diagnosed in the colon and rectum [22].

Unknown carcinogens ingested unwittingly with food and drinks can interact directly with the cells of the colon and rectum mucosa if they are not previously inactivated, absorbed, or metabolized in the stomach and small intestine. Increasing epidemiological evidence indicates that certain dietary patterns, alcohol consumption, overweight, and a sedentary lifestyle are consistent risk factors for colorectal cancer [1, 22].

Recently, a panel of world experts has concluded that red meat, processed meat, and substantial consumption of alcoholic drinks have a strong influence on the development of colorectal cancer, based on the epidemiological evidences in convincing meta-analysis. Food containing dietary fiber, as well as garlic, milk, and calcium, probably protects against this disease [1].

Our previous results showed a significant association of colorectal cancer risk with high consumption of fatty red meat, heavily browned surfaces when meats were barbecued or iron-pan cooked, and alcoholic beverages [6, 23, 24]. In one of the scarce case–control studies devoted to identify specifically promotion/antipromotion activity of dietary fatty acids, we show that high intake of saturated fatty acids and cholesterol increases the risk for colorectal cancer [25]. Additionally, insoluble fibers and lean red meat were associated with a decreased risk [23, 25].

Fatty red meat products, such as cold cuts, sausages, and bovine viscera would increase risk probably due to their high saturated fat content. High-fat diets, rich mainly in cholesterol and saturated lipids, may favor colon cancer because of their high caloric content. Alternatively, they may lead to increased levels of biliary acids in the colonic lumen or unbalanced ratio of conjugated linoleic acid – CLA [26–28]. Further, consumption of protein, iron, and heterocyclic amines produced by cooking and N-nitroso compounds has also been involved. Heterocyclic amines formed during cooking of red meat are powerful mutagens and carcinogens. The type of beef meat preferred by South American population for barbecuing or iron-pan cooking is usually fatty rich (30–33% of total lipids). Thus, undesirable quality of cuts increases when other risky cooking procedures are added, such as high cooking temperatures with close and prolonged contact to charcoal smoke. These combinations probably enhance the production of heterocyclic amines [23, 24, 29].

Since different kinds of meat have similar levels of protein, it is possible to assume that the major difference lays in the amount and quality of lipid components. The fat content of meat ranges from 4.5% to at least 37% for fatty meat [29]. Fats from bovine milk and meat contain variable amounts of CLA, a strong anticarcinogen. Interestingly, CLA is located within interstitial nonvisible fat, evenly distributed along muscle fibers. As a consequence, beneficial effects of conjugated linoleic acid may be relatively enhanced in lean meat in comparison to fatty meats and fatty meat subproducts [23, 28].

Several epidemiological studies have established a causal association between alcohol consumption and colorectal cancer [30]. They also suggest some sexual dimorphism, with a possibly greater effect in men than in women. This could be linked with a generally higher consumption of alcohol among men and also with different preferences of alcoholic drinks, hormone-related differences in alcohol metabolism, or gender susceptibility [1].

Previous results of our group showed a strong association between colorectal cancer and alcoholic drinks in Córdoba, Argentina. The association was observed for red wine, the most commonly consumed beverage, but also with beer and spirits, and the risk was similar for men and women. With regard to the frequency of consumption, regular intake of two or more glasses of wine per day (about 400 cc per day) increases the risk of colorectal cancer. Furthermore, a dose–response relationship was found since increasing consumption caused a rise in the risk of developing the disease [6].

It has been demonstrated that ethanol per se increases the levels of saturated fatty acids and decreases ω(omega)-6 and ω(omega)-3 essential fatty acids in rodents and in human normal and tumor cells, a condition that has been postulated as protumorigenic condition [27, 31]. Taken as a whole, high consumption of alcohol, together with high intake of fatty red meat, would play a cosynergistic role on colorectal tumorigenesis [6].

On the other hand, some dietary features such as low-folate intake are believed to favor the risk for colorectal cancer by 2–5 times, and alcohol induces perturbations in folate metabolism. Hence, alcohol

consumption and low-folate intake might interact synergistically, or alcohol could act through folate metabolism to increase risk of colorectal cancer [30].

Summing up, diet plays an important role in colorectal cancer development. Among its components, alcoholic drinks have been established as a convincing cause to this type of cancer in men and probably also in women.

The possible co-synergistic action found between high intake of alcoholic beverages and beef meat is particularly relevant in populations with a similar dietary pattern of these features, such as Argentineans, Uruguayans, and Chileans.

Urinary Tract Tumors

We refer to urinary tract tumors, including in this category the transition cell carcinoma varieties of the bladder including also cancers of the upper part of the urinary tract. These tumors are ranked in the tenth place within the most common malignancies worldwide. Their mortality is estimated around 2% of all cancer deaths [1]. Our earlier studies on geolocation showed, for the first time, that urinary tract tumors are the fourth in incidence among men in Córdoba, Argentina, with different patterns in several counties of this region [17–19]. Even if their mortality is not remarkably high, the morbidity and recurrence of these tumors provides a serious challenge for oncological treatment and follow-up [32].

Regarding to sex, urinary tract tumors are 2–5 times more common in men than in women, and risk increases with age [1]. Interestingly, in South America, the highest rates of incidence and mortality were recorded in Uruguay and Argentina, particularly among men [16, 33–35].

The etiology of urinary tract tumors is poorly understood; however, it is suspected to be multifactorial. Since genetic background seems to play an unimportant role in their proneness, environmental factors become the main cause of suspicion [32].

Indeed, it is known that tobacco smoking is the main risk factor for bladder cancer, and it is estimated that 30–50% of all cases around the world are caused by this habit. Occupational exposure also accounts for a small fraction of cases [1]. Other risk factors include medicinal drugs, chronic infections, and pollutants, such as arsenic [36]. Accidental intoxication with melamine – as happened recently in China [37] – has been proven to have procarcinogenic capabilities in urinary mucosa when administered per os in rodents [38, 39], and it should be considered also in humans.

However, there are a large unexplained number of cases, which may be linked with dietary habits. In fact, differences in diet could be responsible for the great variation in urinary tract tumor incidence and mortality rates in diverse areas of the world and across different social classes [40]. There is growing evidence that a considerable number of substances in the diet have an influence on urinary tract tumors [41]. Moreover, the urinary tract surfaces are in close contact with many potentially carcinogenic compounds present in foods and their metabolites, which are excreted through urine [42].

Available epidemiological data are yet not sufficient in order to reach univocal conclusions about the association between urinary tract tumors and diet. Nevertheless, several studies around the world have found that usual intake of vegetable and fruit [42], milk [1], and lean white meat [43] could protect against this type of cancer. On the contrary, barbecued meat [44], cold cuts and sausages [45], fried foods [46], infusions and alcohol [47], and artificial sweeteners [48] may increase risk for urinary tract tumors.

Experimental data indicates that chronic essential fatty acid (EFA) deficiency seems to induce both urolithiasis and transitional hyperplasias, followed by a tendency for tumorigenesis of the urinary passages. High intakes of saturated fats or non-EFAs are conditions that may induce EFA deficiency and increase the risk of bladder cancer. Thus, it is reasonable to assume that abnormal metabolism and/or nutritional deprivation of EFA, by inducing a chronic or a subclinical EFA deficiency, may enhance the risk of urothelial tumorigenesis [31].

Based on our previous research [48], we have also suggested that patients with cystitis or chronic inflammation caused by lithiasis, men with partially obstructive prostatism, or even people with chronic irritation caused by long-term use of artificial sweeteners, or those with low intake of EFA and/or trans-fatty acid high-consumption diets, may have a higher susceptibility to the action of artificial sweeteners promoting tumor growth. Moreover, one could further speculate that subjects on "healthy" diets – low fat and low calorie – that usually are enthusiastic artificial sweetener consumers may be under a particular risk group for urinary tract tumors [32].

Interestingly, recent epidemiological studies carried out in Argentina and Uruguay suggest that certain cultural dietary patterns shared by both South American populations – such as a very frequent consumption of red meat, potatoes, alcohol (mainly red wine), and sweet infusions as maté – play a role in the development of urinary tract cancer [40, 49].

However, our previous results on alcohol and urinary tract tumors showed that modest drinking of red wine (no more than 100 cc per day) as a part of a healthy diet seems to be related to a protective role [40]. Resveratrol and other parent flavonoids present in red wine have shown anticarcinogenic activity [13]. Actually, polyphenols isolated from red wine were able to inhibit the proliferation of tumor cells in vitro [41]. On the contrary, a high and frequent consumption of alcoholic beverages, mostly red wine, as a part of a Western dietary pattern – high consumption of red meat, potatoes, and sugars – has shown to be a promoting dietary habit for urinary tract tumors in both Argentinean and Uruguayan population [40, 49]. As said, ethanol contained in these drinks is a carcinogen by itself [1].

Summarizing, when wine is drunk with moderation and as part of a healthy diet, the protective influence perhaps linked to resveratrol would dominate. However, when taken too often and as part of an unhealthy diet, the harmful effect of ethanol would prevail.

Conclusions

Alcohol consumption is one of the most important known causes of human cancer after tobacco smoking, chronic infections, and possibly obesity [30].

Based on available and analyzed evidence, we assure that diet and alcohol strongly influences the risk for the development of colorectal and urinary tract tumors.

For colorectal cancer, diet has shown to be one of the most significant factors, and alcoholic drinks are considered a related habit for this disease. Furthermore, a possible co-synergistic effect between high intake of alcoholic beverages and red meat – preferably eaten with heavy burn surface – becomes relevant in populations with a Western dietary pattern.

Similarly, the occurrence of urinary tract tumors would be related to this type of dietary pattern which includes high and frequent intake of alcoholic drinks. However, a moderate consumption of red wine, together with a healthy diet, would be protective.

In general, the scientific community agrees that alcoholic drink consumption may provide some health benefits, but they are exceeding by the possible negative effects on people and their environment [9].

Nevertheless, total avoidance of alcohol, although optimum for cancer control, cannot be recommended in terms of a broad perspective of public health, in particular in countries with high incidence of cardiovascular disease [30]. Actually, the so-called Mediterranean diet, which among other food products includes red wine, has been strongly related as a protective dietary habit against several types of cancer [50].

As previously mentioned, alcoholic beverage consumption becomes a part of the dietary practices of most populations since ancient ages. Since they are cultural human behaviors, they may be oriented towards healthier ways, through culturally appropriate and scientifically substantiated educational strategies.

In this regard, it is interesting to note that most policies to prevent and reduce alcohol-related problems are based on external control of the behavior of consumers by using, for instance, regulations on advertising of these products, restrictions on the sale – for hours of sale, or minimum age for sale, among others – price increase, taxes, monitoring of alcohol in motor vehicle drivers, and punishments. So far, these strategies have not had a major impact on public health [9]. Thus, we can reasonably wonder whether this is the best approach to change an individual and social practice that goes back several millennia ago in human history.

Dietary practices are a complex field of study, even more in relation to cancer, an etiology recognized as multicausal. The habit of consuming alcoholic beverages is one of those practices. Consequently, epidemiological studies should consider the type of alcoholic drink, the amount consumed, and also the frequency of consumption in order to achieve valid and reliable results.

Furthermore, research on alcohol intake and cancer should be analyzed from multiple theoretical and methodological approaches, involving communities in both research and educational strategies on this issue.

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Chapter 34
Alcohol, Acetaldehyde, and Digestive Tract Cancer

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Key Points

- Alcohol and tobacco are the most important risk factors for upper digestive tract cancers.
- Acetaldehyde, derived from the alcoholic beverage itself and formed endogenously from ethanol, is a group 1 carcinogen to humans.
- Acetaldehyde is also the most abundant carcinogen of tobacco smoke.
- Microbes are responsible for most of the acetaldehyde production in the digestive tract.
- Some ALDH2 and ADH gene polymorphisms associate with markedly increased risk for upper digestive tract cancer and with enhanced local acetaldehyde exposure via saliva.
- At individual level, acetaldehyde exposure can be markedly reduced.

Keywords Acetaldehyde • ADH • Alcohol • ALDH2 • Cancer • Digestive tract • Tobacco • Microbes • Gene polymorphism

Introduction

The worldwide incidence of upper digestive tract cancer is characterized by large geographical variations and periodical changes [1]. In 2008, the yearly worldwide incidence of new digestive tract cancers was over three million representing 25.1% of all cancers [2]. Stomach cancer alone is still the leading cause of cancer deaths in the world [2]. With such a poor prognosis, it is essential to explore all possible means of prevention by identifying specific etiological factors, possible risk groups, and mechanisms of carcinogenesis and by intervening where possible. In industrialized countries, alcohol and tobacco are the main risk factors for oral, pharyngeal, and esophageal cancers [3–5]. Furthermore, tobacco is an independent risk factor for stomach cancer [6–8] and alcohol is a significant risk factor for colorectal cancer [9].

Acetaldehyde is the key intermediate both in alcoholic fermentation and ethanol oxidation. Furthermore, it is the most abundant carcinogenic compound of tobacco smoke. In October 2009, the International Agency for Research on Cancer (IARC), World Health Organization (WHO), concluded

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that acetaldehyde associated with alcohol consumption is a group 1 carcinogen to humans [10]. This includes acetaldehyde present in alcoholic beverages and acetaldehyde formed from ethanol endogenously [10]. The new IARC classification is based on the uniform epidemiological, genetic, biochemical, and microbiological evidence derived from alcohol-consuming individuals carrying aldehyde (ALDH2) and alcohol (ADH) dehydrogenase gene mutations. In the presence of ethanol, these mutations lead via saliva to increased acetaldehyde exposure of the upper digestive tract [11, 12]. The scientific evidence strongly supports the concept that acetaldehyde acts as a cumulative and local carcinogen especially in the oral cavity and the esophagus but also in the stomach and large intestine [11–13]. By and large, all known environmental and genetic risk factors of upper digestive tract cancers appear to be associated with enhanced exposure to carcinogenic acetaldehyde. These mechanisms and possible preventive actions will be discussed in detail in the following sections.

Environmental Risk Factors for Digestive Tract Cancers

Tobacco and Alcohol

Tobacco and alcohol are independent and multiplicative risk factors for oral, pharyngeal, laryngeal, and esophageal cancers, especially in industrialized countries [3]. In smokers, the risk for oral cancer is 7–10 times higher than that in never smokers [14]. With increasing tobacco consumption, the risk increases linearly. The relative risk of oral, pharyngeal, and laryngeal cancers is 3.9 for those smoking 10–19 g and 15.4 for those smoking over 30 g daily [15]. The multiplicative and dose-dependent effect of alcohol and tobacco on the risk of esophageal cancer in France is demonstrated in Table 34.1 [16].

Smoking and alcohol drinking have been estimated to account for up to 77% of oral cancers in Spain [17]. Six glasses of wine (16.7 g pure alcohol/glass) consumed daily over many years is associated with 6.1- and 4.2-fold risk of oropharyngeal and esophageal cancers, respectively [4]. A significantly increased risk is found even for an ethanol intake of 25 g (about two drinks) per day. The multiplicative effect of alcohol and tobacco on upper digestive tract cancer risk has been documented in many studies and confirmed in a meta-regression analysis including 14 studies and 4,585 cases [5, 18]. The relative risks for individuals consuming over 30 cigarettes and 4 or more drinks daily were 21.2 for oropharynx, 35.6 for pharynx, 34.6 for larynx, and 12.7 for esophagus [5].

Tobacco smoking is an independent risk factor for stomach cancer. In the USA, 28% of stomach cancer deaths in men and 14% among women have been estimated to be attributable to tobacco use [6]. In a prospective European study including 521,468 individuals and 10 European countries, the hazard ratio for ever smokers was 1.45 and for current smokers 1.73 in males and 1.87 in females [7]. The risk of stomach cancer increased with intensity and duration of cigarettes smoked. In a more

Table 34.1 Relative risk of esophageal cancer by level of smoking and drinking

	Smoking (g/day)		
Alcohol consumption (g/day)	0–10	10–30	Over 30
0–40	1.0	3.9	7.8
40–80	7.3	8.6	33.6
80–120	11.7	13.1	87.0
> 120	49.7	78.7	149.1

Source: Tuyns et al. [16]

detailed study, cigarette smoking has been shown to be associated positively with an increased risk for both esophageal squamous cell and adenocarcinomas, as well as for gastric cardia and non-cardia cancers [8]. In a prospective follow-up study from Japan, the risk for stomach cancer was 11.4 among *Helicobacter pylori*-positive smokers, 5.8 among *H. pylori*-negative smokers, 6.9 among *H. pylori*-positive nonsmokers, and 1.0 among *H. pylori*-negative nonsmokers [19].

The epidemiological evidence for the possible association between alcohol consumption and stomach cancer is controversial. However, in a meta-analysis of alcohol-related cancers including 235 studies and over 117,000 cases, the relative risk for gastric cancer was 1.32/100 g alcohol daily [4]. This number may, however, be biased by the unrecorded alcohol and acetaldehyde present in foodstuffs and in so-called nonalcoholic beverages, as will be discussed in the following sections.

Pooled results from eight cohort studies and data from meta-analyses provide evidence for an increased risk of about 1.4 for colorectal cancer with regular consumption of about 50 g alcohol per day [9].

Diet and Type of Alcoholic Beverage

In Linzhou, China, the incidence of esophageal squamous cell carcinoma (OSCC) has been particularly high – over 100 cases/100,000 per year for both sexes [20]. Poor oral hygiene, heavy use of pickled vegetables, heating stoves without chimneys, and some nutritional deficiencies have been shown to be associated with the increased risk for esophageal cancer in that area [21–25]. In Yanting, another high OSCC incidence area in China, alcohol consumption and tobacco smoking were associated with a 3.16 and 3.76 odds ratio (OR) for esophageal cancer, respectively [26].

So far, it has been believed that congeners do not play any significant role in the pathogenesis of alcohol-related cancers. However, consumption of hot Calvados has been reported to explain about two-thirds of the interregional and urban/rural differences in the incidence of esophageal cancer in Northwest France [27]. Even after adjustment for all other alcoholic beverages, consumption of hot Calvados explained almost half of the peak incidence of esophageal cancer and half of the urban/rural differences in incidence [27].

Helicobacter pylori and Atrophic Gastritis

The most important risk factor for stomach cancer is atrophic gastritis caused by either *H. pylori* infection or autoimmune disorder [28–31]. Consequently, *H. pylori* infection has been classified as a group 1 carcinogen to humans [32]. *H. pylori* infection associates with 4.2-fold risk of stomach cancer [33]. The risk is 11.2-fold among those with both *H. pylori* infection and atrophic gastritis [33]. Highest risk (up to 90-fold) is seen among those with severe panatrophy occupying the whole stomach [28]. The successful eradication of *H. pylori* reduces significantly the incidence of gastric cancer in patients without precancerous lesions such as atrophy, intestinal metaplasia, and dysplasia [34]. *H. pylori* eradication prevents the development of stomach cancer also in patients with mild gastric atrophy identified by low serum pepsinogen levels [35]. It has been calculated that in China, the screening and treatment of *H. pylori* infection might prevent one in every four to six cases of gastric cancer and even to be cost-effective [36].

The evidence from Sweden and Linzhou, China, suggests that atrophic gastritis is an additional significant and independent risk factor also for esophageal cancer and for esophageal squamous dysplasia [37–39].

Acetaldehyde-Related Genetic Risk Factors for Digestive Tract Cancers

ALDH2 Polymorphism

More than a decade ago, it was demonstrated that the risk for upper digestive tract cancer is markedly increased in alcoholics who have a deficient ability to eliminate acetaldehyde due to a gene mutation (Table 34.2) [40, 41]. A single-point mutation in ALDH2 gene results in an enzyme with a deficient ability to remove the first metabolite of ethanol oxidation, acetaldehyde [42, 43]. In affected individuals, drinking of alcohol leads to flushing of the face and body, tachycardia, drop in blood pressure, and nausea [44]. As a consequence, ALDH2-deficient homozygotes (<5% of Asians) rarely use alcohol because of the severity of the flushing reaction, while ALDH2-deficient heterozygotes (30–50% of Asians) with a limited capacity to metabolize acetaldehyde adapt and may become heavy drinkers and alcoholics [45].

The increased upper digestive tract cancer risk associating with ALDH2 deficiency and alcohol consumption has been confirmed in several studies from Japan, China, and Taiwan [26, 46–57]. In some of the latest studies, an increased risk has been found also among occasional and moderate drinkers and even among nondrinkers [26, 50, 53] In a study from Taiwan including 406 cases with OSCC and 656 matched controls, ALDH2 deficiency and the risk of OSCC correlated not only with the drinking behavior but also with the quantity of alcohol and tobacco consumption [53]. The risk for ALDH2-deficient heterozygotes drinking at a low-to-moderate rate (0.1–30 g/day) was 14.5 and that of homozygotes 17.3, whereas the risk of those with the active ALDH2 genotype was 7.2. The risk of those drinking over 30 g/day was as high as 102.5 (Table 34.3). Furthermore, a significant risk for OSCC was observed among low-to-moderate drinking and smoking ALDH2-deficient individuals but not in nonsmokers [53].

Only a few studies have examined the association between ALDH2 deficiency and gastric cancer. A relative risk of 3.5 among ALDH2-deficient alcoholics was found by Yokoyama et al. (Table 34.2) [41]. In a more recent Japanese study including 45 alcoholic cases with gastric cancer and 281 controls, OR for those with severe atrophic gastritis in combination with ALDH2 deficiency was 39.2 as compared with 17.6 for those with atrophic gastritis alone and 9.7 for those with ALDH2 deficiency alone [58].

A 3.4-fold risk for colorectal cancer has been found among ALDH2-deficient alcoholics (Table 34.2) [41]. This has been confirmed in two other studies but only among heavy drinkers [59, 60]. However, in two later studies, the association was not found, but these studies may not have included enough heavy drinkers [61, 62].

Asian-type ALDH2 mutation is rare in Europe. However, in Poland, another ALDH2 variant with deficient ability to metabolize acetaldehyde has been shown to be associated with a 2.3-fold risk of stomach cancer among daily drinkers [63]. A threefold risk was found among those with 40 or more

Table 34.2 Relative risk (odds ratios) of digestive tract cancers among Japanese alcoholics after adjustment for confounders among ALDH2-deficient subjects compared with those with the normal ALDH2 enzyme. ALDH2 = low K_m mitochondrial aldehyde dehydrogenase

Type of cancer	Odds ratios
Oropharyngolaryngeal	11.1
Esophageal	12.5
Stomach	3.5
Colon	3.4
Esophageal cancer concomitant with oropharyngolaryngeal and/or stomach cancer	54.2

Adapted from Yokoyama et al. [41]. With permission from Oxford University Press

Table 34.3 Carcinogenetic impact of slow ADH1B- and ALDH2-deficiency genes on the risk for esophageal cancer with regard to the consumption of alcohol or tobacco. Nondrinkers as a reference group

Interaction between slow ADH1B and ALDH2 deficiency		
Genes/polymorphisms	Alcohol 0.1–30 g/day	Alcohol >30 g/day
Combined odds ratios		
ALDH2 deficiency	14.5	102.5
Slow ADH1B	10.6	71.9
Slow ADH1B + ALDH2 deficiency	37.5	382.3
Combined odds ratios associated with smoking status		
Nonsmokers		
Slow ADH1B	6.7	19.2
ALDH2 deficiency	3.6	82.3
Smokers		
Slow ADH1B	25.9	199.6
ALDH2 deficiency	16.5	79.3

Adapted from Lee et al. [53]. With permission from John Wiley & Sons, Inc.

drink-years [63]. In an earlier European multicenter case–control study including 811 cases and 1,083 controls, the same ALDH2 variant was found to be associated with a 1.76-fold risk of upper aerodigestive tract cancers among moderate drinkers [64]. The OR was 5.79 among heavy drinkers [64].

ADH Polymorphism

ADH has several isoenzymes. The two enzymes responsible for the most of alcohol elimination are ADH1B and ADH1C. ADH1B*2 is a mutant allele with a particularly high prevalence in East Asia, e.g., 93–95% of Japanese carry it [45]. The less active isoenzyme ADH1B*1/*1 (activity 1/40 of the normal) is a strong risk factor for esophageal and oropharyngolaryngeal cancers among the Japanese, Chinese, Thai, and Central European alcohol-drinking populations [26, 49, 51, 53, 54, 56, 57, 65].

Among Caucasians, the main enzyme for alcohol metabolism is ADH1C, which has two isoenzymes. The ADH 1 C*1 allele with a 2.5-fold enzyme activity as compared to the ADH1C*2 allele has been shown to be associated with a significantly increased risk for squamous cell carcinoma of the head and neck among smoking heavy drinkers [66–69]. In a German study including 110 cancer cases and 508 controls with other alcohol-related diseases, the ORs for the development of esophageal, hepatocellular, and head and neck cancers were 2.93, 3.56, and 2.2, respectively [67]. However, discrepant results have been obtained in some other studies [70, 71]. The differences in the findings have been explained to be due to variations in the geographic distribution of ADH1C genotypes in Europe [67]. Furthermore, the negative studies have generally included controls and patients with minor or moderate alcohol consumption [67].

Alcohol-drinking individuals homozygous for ADH1C*1 have been shown to have an increased risk also for both esophageal and gastric adenocarcinomas [72]. Moreover, in a recent German study including 173 cases and 788 controls, subjects homozygous for high-activity ADH1C*1/1 were found to have a 1.7-fold risk for the development of high-risk adenomas and colorectal cancer [73].

*ALDH2 Deficiency Combined with Low-Activity ADH1B*1/*1*

There is confirming evidence indicating that the risk for upper digestive tract cancer is highest among ALDH2-deficient drinkers who simultaneously have the low-activity ADH1B*1/*1 genotype

(Table 34.3) [26, 49, 53, 56, 57, 74, 75]. In one study, the average OR for OSCC was 37.5 for those drinking from 0.1 to 30 g alcohol daily, and for those drinking over 30 g/day, the OR was as high as 382.3 (Table 34.3) [53]. There is also evidence that smoking may have an independent and interactive effect on esophageal cancer risk among slow ADH1B- and ALDH2-deficiency gene carriers (Table 34.3) [53].

In conclusion, the epidemiological and genetic studies provide strong evidence suggesting that an increased risk for upper digestive tract cancer is associated both with a deficient ability to detoxify acetaldehyde and with an enhanced or prolonged ability to produce it. All of these findings can be explained by the enhanced exposure of the upper digestive tract mucosa to locally formed acetaldehyde through saliva as will be described later.

Oral and Esophageal Cancer Among APECED Patients

Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) is a rare recessive disease caused by mutations of the AIRE (autoimmune regulator) gene. The disease is characterized by chronic mucocutaneous candidiasis, hypoparathyroidism, and adrenal insufficiency. Most patients have chronic oral candidiasis since early childhood. Of all the APECED patients in Finland that are over the age of 25 years, up to 10% have developed oral or esophageal cancer at the site of mucositis [76]. The age at cancer diagnosis has been markedly low (29–44 years) that is significantly lower than in general [76]. Locally in the oral cavity, formed acetaldehyde may provide a plausible explanation also for this association.

Acetaldehyde as a Common Denominator and Cumulative Carcinogen

Acetaldehyde as a Carcinogen

IARC/WHO concluded in October 2009 that acetaldehyde associated with alcohol beverage drinking and formed endogenously from ethanol is carcinogenic to humans (group 1) [10]. Acetaldehyde interferes with DNA synthesis and repair. It can cause point mutations and form covalent bonds with DNA [77]. Inhalation and oral administration studies in rats have proven that acetaldehyde is carcinogenic to animals [78–80]. Acetaldehyde can form mutagenic DNA adducts in concentrations of 100 μM and above [81]. This is in line with in vivo findings in humans showing that after a moderate dose of alcohol acetaldehyde concentrations of saliva range between 18 and 143 μM [82].

Strongest evidence for the local carcinogenic potential of acetaldehyde in the upper digestive tract can be derived from the studies focusing on the regulation of acetaldehyde concentration in the saliva. Indisputably, most of the known risk factors for upper digestive tract cancers appear to be associated with an enhanced exposure to acetaldehyde (Table 34.4). Acetaldehyde is so efficiently detoxified in the liver by mitochondrial ALDH2 enzyme that measurable levels of acetaldehyde are not seen in blood of normal individuals after an alcohol challenge [83]. Thus, there is no evidence of the systemic carcinogenic effects of acetaldehyde. On the other hand, the mucosal cells of gingiva and tongue have been shown to lack low K_m aldehyde dehydrogenase enzymes and thus suggested to be more vulnerable to the toxic effects of acetaldehyde [84].

Table 34.4 Acetaldehyde exposure from environmental and genetic sources is cumulative and includes by and large all known risk factors for upper digestive tract cancer [13]. Acetaldehyde has been shown to produce mutagenic DNA adducts at 100-μM concentrations [81]

Cancer risk factor	Acetaldehyde (Ach) exposure via saliva
Alcohol intake Instant effect	Up to 200 μM acetaldehyde concentrations in saliva instantly after a small sip of a strong alcoholic beverage. The exposure continues for at least 10 min [85]
Prolonged effect	After about three doses (0.5 g/kg) of alcohol, peak salivary acetaldehyde concentrations range from 19 to 144 μM and decrease slowly with decreasing salivary ethanol concentrations during the subsequent 4 h [82]. Mouth rinsing with chlorhexidine results in decreased acetaldehyde levels in saliva [82]
Acetaldehyde as a congener	A small sip of an alcoholic beverage containing acetaldehyde as a congener has a short term (1–2 min) peaking effect on salivary acetaldehyde [85]
Smoking	Mean acetaldehyde concentration in saliva during active smoking is 260 μM and lasts for about 5 min [96]. Thus, daily acetaldehyde exposure depends on the number of cigarettes smoked
Heavy drinking, chronic smoking, and poor oral hygiene	Modify oral flora to produce more acetaldehyde from ethanol. The increase in acetaldehyde exposure through saliva after a dose of alcohol is 60–75% in vitro and 100% in vivo [94–96]
Smoking + drinking	Have a synergistic (sevenfold) effect on acetaldehyde exposure through saliva [96]
ALDH2 deficiency	Two- to threefold increase in salivary acetaldehyde after a dose of alcohol [101–103]
Low active ADH1B	Decreased elimination rate of ethanol associates with prolonged presence of ethanol in blood and saliva and consequently also with prolonged exposure to microbially derived acetaldehyde [106]
High active ADH1C	Increased acetaldehyde exposure via saliva after a dose of alcohol [66]
Atrophic gastritis, gastric acid secretor inhibitors, and *H. pylori*	Achlorhydric stomach is colonized by oral microbes, which produce acetaldehyde both from ethanol and glucose [109–112]. Many *H. pylori* strains possess also ADH and are able to produce acetaldehyde [114]
"Nonalcoholic" beverages and foodstuffs	Official alcoholic beverages contain 2.8% or more ethyl alcohol. However, many so-called nonalcoholic beverages and foodstuffs produced by fermentation may contain 0.05–2.7% ethanol. 0.05% (10 mM) ethanol concentration is more than enough for local microbial acetaldehyde production in the mouth. Furthermore, many nonalcoholic beverages and foodstuffs contain mutagenic concentrations of acetaldehyde, which exceed significantly the safe limits [131]

Exposure to Microbially Produced Acetaldehyde Via Saliva

Measurable levels of acetaldehyde are not found in saliva without ethanol administration. Oral microflora appears to be the main determinant of acetaldehyde concentration in the saliva [82]. The major source for local acetaldehyde production in saliva is ethanol that is distributed to the saliva either immediately after a sip of an alcoholic beverage or later on after the distribution of ethanol to the whole water phase of human body including blood and saliva [82, 83, 85]. Another source for salivary acetaldehyde provides some alcoholic beverages containing high concentrations of acetaldehyde and ethanol [86–88].

Many microbes representing normal oral flora possess ADH activity and are able to oxidize ethanol to acetaldehyde [83, 89]. However, the capacity of the microbes and oral mucosal cells to remove acetaldehyde is limited, and therefore, acetaldehyde accumulates in the saliva [83, 90, 91]. Mutagenic amounts of acetaldehyde can be detected in the saliva of healthy volunteers even after a moderate dose of ethanol [82]. Rinsing the mouth with chlorhexidine before drinking decreases salivary microbial counts and acetaldehyde production about 50% [82]. In vitro salivary acetaldehyde production from ethanol can be totally prevented if microbes are destroyed or removed from the saliva samples [82]. With increasing alcohol doses, the salivary acetaldehyde concentration increases linearly because

microbial ADHs are not saturated with ethanol [82, 83]. This is concordant with well-established epidemiological findings of an increased cancer risk associated with heavier and more intoxicating drinking. ADH activity and the capacity to produce acetaldehyde vary between different oral microbial strains [92, 93]. In vitro acetaldehyde production from ethanol is strongly dependent on alcohol concentration and pH [82, 83]. The marked acetaldehyde production capacity of the clinical strain of *Streptococcus salivarius* may be particularly important, since this bacterium colonizes the mucosal surfaces of the oral cavity, which is rarely colonized by other normal flora bacteria [93].

Chronic smoking, heavy drinking, and poor oral hygiene are established risk factors for oral and esophageal cancers. All these factors are also known to increase microbial acetaldehyde production in saliva (Table 34.4). Smoking and heavy drinking independently increase in vitro acetaldehyde production from ethanol by 60–75% and their combined effect is about 100% [94]. Poor dental status increases in vitro acetaldehyde production by 100% [95]. Chronic smoking increases also in vivo acetaldehyde production by about 100% after a moderate dose of alcohol [96]. Some *Candida albicans* strains and some Gram-positive aerobes have been found more often and in higher amounts in high acetaldehyde-producing saliva samples [92, 97]. Moreover, *Candida albicans* strains isolated from APECED patients, that have high risk of developing oral cancer due to chronic oral mucositis, have been shown to produce significantly higher amounts of acetaldehyde from glucose than control isolates or isolates from cancer patients [98]. In addition, non-albicans yeasts can also produce carcinogenic amounts of acetaldehyde from ethanol and glucose in vitro [99].

Synergistic Effect of Smoking and Alcohol on Acetaldehyde Exposure Via Saliva

In tobacco smoke, there are 11 known and 7 probable human carcinogens. However, the concentration of acetaldehyde in tobacco smoke is more than 1,000 times greater than that of some other well-known carcinogens, e.g., polycyclic aromatic hydrocarbons or tobacco-specific nitrosamines [100]. Most importantly, acetaldehyde of tobacco smoke – as a water-soluble agent – dissolves readily in saliva during smoking [96].

In the presence of ethanol, smoking results in 300–500-μM concentrations of acetaldehyde in saliva lasting for as long as the active smoking continues (Fig. 34.1) [96]. Because chronic smoking modifies the oral flora to produce more acetaldehyde from ethanol, the concomitant smoking and drinking have a synergistic, i.e., sevenfold, effect on the upper digestive tract's exposure to acetaldehyde [96].

Effect of Gene Polymorphisms on Acetaldehyde Exposure

ALDH2 Deficiency

ALDH2-deficient alcohol consumers form an exceptional human model for long-term acetaldehyde exposure. Their risk for alcohol-related upper digestive tract cancers is particularly high, and they have markedly elevated concentrations of acetaldehyde in their saliva after drinking of alcohol [101–103]. After ingestion of a moderate dose (0.5 g/kg) of alcohol, ALDH2-deficient individuals have 2–3 times higher acetaldehyde levels in their saliva than those with the normal genotype during the whole observation period of 240 min (Fig. 34.2) [101]. The most probable source for additional salivary acetaldehyde in ALDH2-deficient individuals is the deficient capacity of the oral mucosa and parotid glands to remove acetaldehyde produced by their own ADH [84]. During an alcohol challenge, sterile saliva, obtained from the main duct of the parotid gland, contained acetaldehyde only in

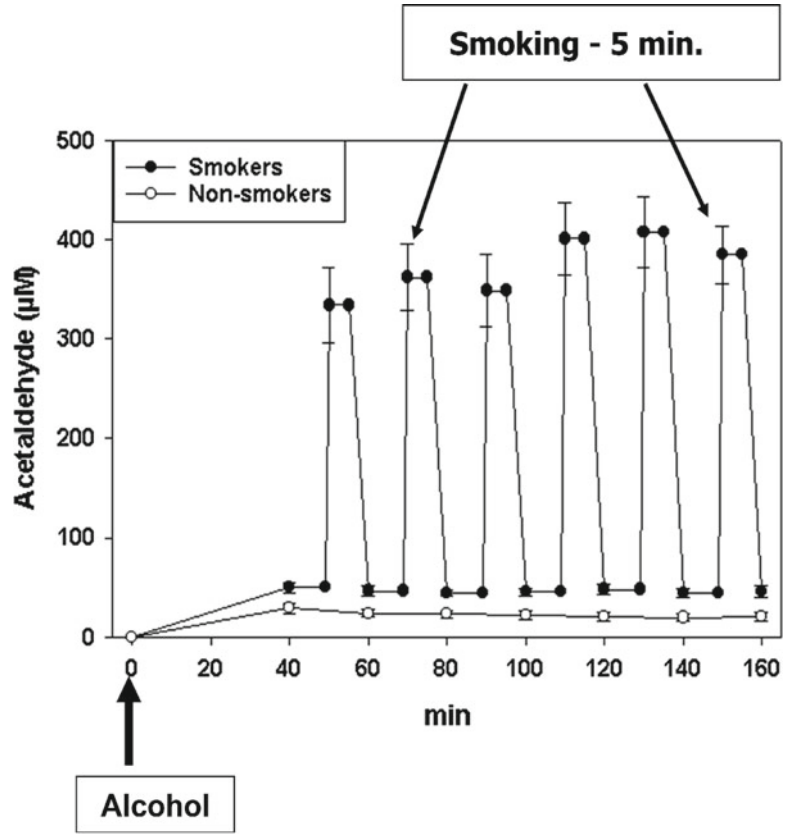

Fig. 34.1 The effect of smoking on acetaldehyde concentration in saliva. During active smoking, acetaldehyde of tobacco smoke becomes dissolved in saliva. In this study, the smokers and nonsmokers at first ingested a moderate dose of alcohol and smoked thereafter six cigarettes at 20-min intervals. The area under the curve is sevenfold in smokers as compared to nonsmokers. Accordingly, smoking and alcohol drinking have a synergistic effect on salivary acetaldehyde. (Adapted from Salaspuro et al. [96]. With permission from John Wiley & Sons, Inc.)

ALDH2-deficient subjects [101]. In addition, 4-methylpyrazole, which is an effective inhibitor of somatic ADH enzymes but a weak inhibitor of microbial ADH enzymes [82], totally prevented the increase in salivary acetaldehyde only in ALDH2-deficient subjects [102]. Furthermore, salivary acetaldehyde cannot not be derived from blood, since in ALDH2-deficient subjects, acetaldehyde concentrations in blood are much lower than those in the saliva (Fig. 34.2) [101–103].

Low-Activity ADH1B*1/*1 Genotype

The Vmax of the low-activity ADH1B is only 1/40 of that of the superactive genotype in vitro [104], and this has been shown to be associated with a significantly decreased rate of ethanol elimination during intravenous alcohol infusion [105]. Consequently, after consumption of alcoholic beverages, ethanol remains elevated in the blood and saliva for hours longer in those with the low-activity enzyme than in those with the normal enzyme, resulting in a longer exposure time to microbially formed acetaldehyde [106]. Moreover, chronic heavy drinking has been shown to lead to quantitative and qualitative changes in oral microflora, with a consequent increase in their capacity to produce acetaldehyde from ethanol both in vitro and in vivo [94–96].

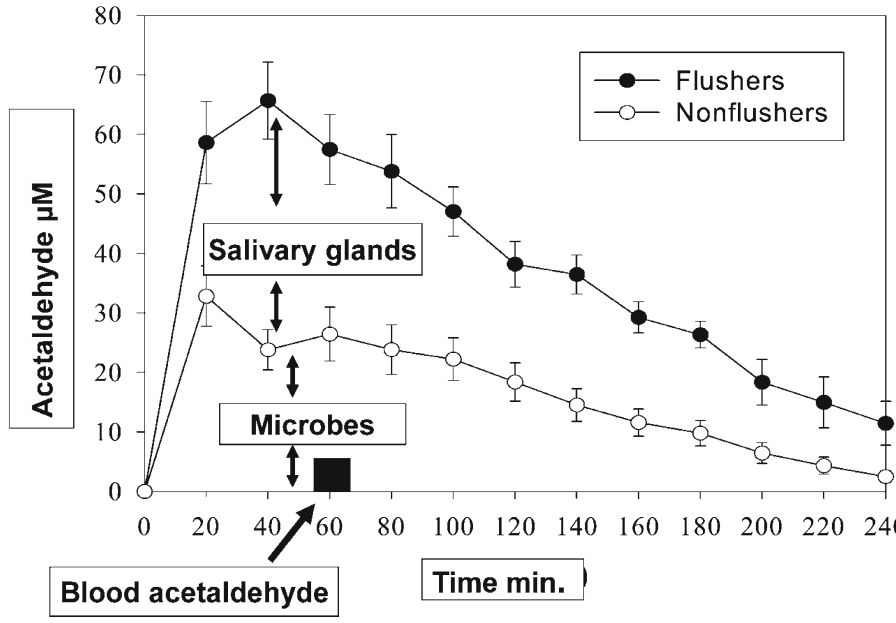

Fig. 34.2 "A human knockout model for long-term acetaldehyde exposure" ALDH2-deficients (flushers) have two- to threefold higher salivary acetaldehyde concentrations than those with the normal enzyme (nonflushers). Cannulation of the duct of the parotid gland proved that additional acetaldehyde is derived from parotid glands. The lower part of acetaldehyde curve is produced from ethanol by microbes. Blood acetaldehyde levels were considerably lower than those in saliva. Chinese students volunteered and participated in the study. (Adapted from Väkeväinen et al. [101]. With permission from John Wiley & Sons, Inc.)

High-Activity ADH 1 C*1 Genotype

The ADH 1 C*1 allele with a 2.5-fold activity as compared to ADH1C*2 allele has been shown to be associated with a significantly increased risk for squamous cell carcinoma of the head and neck among heavy drinkers [66, 67]. In our study, 11 subjects homozygous for the high-activity ADH1C*1 allele were found to have significantly higher acetaldehyde concentrations in their saliva after alcohol ingestion than 22 volunteers heterozygous for ADH1C or homozygous for the normal genotype ADH1C*2 [66].

Acetaldehyde and Stomach Cancer

The pathogenetic mechanism behind the increased risk for gastric cancer in patients with achlorhydric atrophic gastritis is still without a final explanation. Correa's hypothesis, which proposes that hypochlorhydria permits gastric bacterial colonization, the reduction of nitrates to nitrites, and the formation of potentially carcinogenic N-nitroso compounds, remains controversial [107]. Acetaldehyde derived either from tobacco smoke or microbial metabolism could provide another explanatory mechanism for the increased gastric cancer risk among smokers and/or patients with achlorhydria [13].

Because of its low pH, the normal human stomach is free of microbes. However, microbes representing normal oral flora can survive and even proliferate in increasing intragastric pH [108]. In some atrophic gastritis patients, bacterial overgrowth of the gastric juice resulted in the formation of minor concentrations of endogenous ethanol and acetaldehyde from glucose [109, 110]. Furthermore, after

administration of a small dose of alcohol, intragastric acetaldehyde production increased 6.5-fold in achlorhydric subjects as compared to healthy controls [110]. Hypochlorhydria induced by cimetidine has been shown to result in intragastric formation of endogenous ethanol by microbial fermentation from glucose [111]. One week of treatment with proton-pump inhibitors resulted in significantly increased intragastric production of acetaldehyde from ethanol, associating with a marked overgrowth of aerobic bacteria representing normal oral flora [112].

In addition to atrophic gastritis, *H. pylori* infection is also an established risk factor for gastric cancer [30, 31, 113]. Many *H. pylori* strains possess significant ADH activity and are able to produce acetaldehyde from ethanol under microaerobic conditions [114]. Many so-called nonalcoholic beverages and foodstuffs may contain low but significant amounts of alcohol and may, thus, function as relevant source for microbial acetaldehyde production in achlorhydric or *H. pylori*-infected stomach. Consequently, low concentrations of ethanol present in nonalcoholic beverages and foodstuffs produced by fermentation may be a potential confounder that has not been included in earlier epidemiological calculations with regard to the risk factors of gastric cancer [13].

As already described in earlier chapters, tobacco has been shown to be an independent risk factor for stomach cancer and the highest risk has been found among *H. pylori*-positive and ALDH2-deficient heavy drinkers and smokers [58]. The possible effect of tobacco smoke on acetaldehyde concentration of the gastric juice is so far not known. However, during active smoking, considerable amounts of salivary acetaldehyde can be expected to reach the stomach via swallowing.

Acetaldehyde and Colon Cancer

Chronic alcohol consumption is an established risk factor for colorectal cancer [9]. Some genetic linkage studies suggest that acetaldehyde could also be a causal factor in the pathogenesis of the cancer of the large intestine [41, 73, 115]. In experiments with animals, microbially mediated ethanol oxidation results in high acetaldehyde concentrations in the colon after alcohol administration [83, 116–118]. This has been shown to be associated with the depletion of folate in the large intestine as well as with enhanced colorectal proliferative status [119, 120]. In animal experiments, chronic alcohol feeding leads to DNA hypomethylation, and one factor explaining this is probably low folate concentration [121].

Acetaldehyde Exposure Via the Type of Alcoholic Beverage and Diet

Combined epidemiological and biochemical findings suggest that a high concentration of acetaldehyde present as a congener in Calvados might explain the particularly high incidence of esophageal cancer in the Northwest France [27, 86]. Even a single sip of a strong alcoholic beverage without ingestion leads to carcinogenic salivary acetaldehyde concentration, and the exposure to acetaldehyde continues at least for 10 min [85]. Exposure to acetaldehyde is shortly (1–2 min) but significantly higher with calvados or other alcoholic beverages containing high levels of acetaldehyde than with ethanol containing no acetaldehyde [85, 88, 103].

In a large chemical survey including over 1,500 samples of different alcoholic beverages, very high acetaldehyde concentrations have been found especially in many fruit spirits and fortified wines [87]. A subsequent risk assessment analysis showed that the lifetime risks for acetaldehyde from alcoholic beverages greatly exceed the usual limits for cancer risks from the environment [122]. The cumulative cancer risk that includes all possible sources of acetaldehyde is still much higher and supports strong regulatory measures for acetaldehyde in alcoholic beverages and foodstuffs [11, 87, 122].

The burden of upper digestive tract cancers is especially high in some Central European countries, and this appears to be associated with a high consumption of fruit-based spirits containing particularly high concentrations of acetaldehyde as a congener [123]. Based on these findings, it has been suggested that the acetaldehyde levels of alcoholic beverages should be monitored and high-level exposure should be avoided [122, 123].

In addition to official alcoholic beverages containing over 2.8% of ethanol, also many so-called non-alcoholic beverages and foodstuffs produced or preserved by fermentation may in fact contain small amounts (0.05–2.7%) of ethanol. Already 0.05% (10 mM) ethanol concentration is more than enough for microbial acetaldehyde production in saliva [82]. Furthermore, many widely used nonalcoholic beverages and food may contain high concentrations of acetaldehyde as a congener or aroma agent. These include products such as yogurts, kefir, apple juices, soy products, tofu products, fermented vegetables, e.g., Chinese pickles and kimchi, vinegar, and home-brewed beers and meads [124–131]. Furthermore, many fruits, e.g., some apples, oranges, and bananas, may have their own metabolic pathways for acetaldehyde production [131, 132]. Acetaldehyde is also widely used as a food additive and aroma agent [124]. This is possible, since it is still considered to be a GRAS (generally regarded as safe) product [133]. The discrepancy between the views of cancer researches (IARC/WHO) and authorities responsible for the food safety (JEFCA) is obvious, which warrants for further combined actions.

Fermented products have been used for centuries worldwide, but so far, neither their acetaldehyde nor ethanol contents have been measured. Furthermore, there is no systematic data about their worldwide consumption in different geographical areas to be used in epidemiological studies focusing on the risk factors of upper digestive tract cancers. Recently, it has been shown that the average acetaldehyde exposure from food (without alcoholic beverages) is around 40 μg/kg bw/day for the German population [131]. By using this data, the authors concluded that the margin of exposure (MOE) would be 1,175, which is in similar region to the MOEs of other food carcinogens such as acrylamide, furan, aflatoxins, or nitrosamines [131]. MOEs above 10,000 are normally judged as of low relevance for health by the European Food Safety Authority (EFSA) [134]. Consequently, at population level in Germany, the daily mean acetaldehyde exposure derived from food (without alcoholic beverages) exceeds the officially accepted safety limits by over fivefold.

Minimization of Acetaldehyde Exposure: Cancer Prevention

There are several means toward minimizing acetaldehyde exposure (Table 34.5), and these measures could have an enormous impact on cancer prevention worldwide. The cumulative cancer risk of acetaldehyde strongly suggests worldwide screening of ethanol and acetaldehyde levels in thousands of beverages and foodstuffs as well as giving high priority to regulatory measures and consumer guidance. Toward that aim, semiautomatic gas chromatographic methods are available, and as in the case of other potentially dangerous food additives, manufacturers should be responsible for these analyses as well as for consumer guidance.

The ALARA (As Low As Reasonably Achievable) principle should be applied to acetaldehyde levels of alcoholic beverages, tobacco smoke, and to other beverages and foods produced by fermentation, as has been suggested [11, 122, 131]. To that aim, the standards of Codex Alimentarius for dealing with contaminants and toxins and the corresponding EU legislation could be used [134–136]. Oral hygiene can be improved. Risk groups with the ADH and ALDH2 gene polymorphisms and/or hypo- or achlorhydric atrophic gastritis can be screened and informed about the possible risks that are associated with enhanced acetaldehyde exposure. Serum biomarkers, which provide an accurate method for diagnosis of atrophic gastritis in the general population, are available [137]. Commercially available DNA tests for screening of high-risk ALDH2 and ADH gene polymorphisms can be developed.

Table 34.5 Reduction of acetaldehyde exposure at individual and population level

Risk group	Recommended measures
Tobacco smoking	Reduction or quitting from tobacco smoking
Excessive alcohol consumption	Moderation to light drinking
Drinking habits	Avoid drinking to intoxication
	Higher ethanol in saliva ➤ higher acetaldehyde in saliva
	Prefer light drinks
	Local microbial acetaldehyde production increases with increasing ethanol concentrations
	Take a gulp of water after each drink
	Water dilutes acetaldehyde in the oral cavity
	Take care of good oral hygiene
	Decreased number of oral bacteria associates with decreased local production of acetaldehyde
	Use alcoholic beverages with nil or low acetaldehyde concentration
	Free acetaldehyde of the beverage dissolves in saliva
	Manufacturers should inform the consumers about the acetaldehyde concentration of alcoholic beverages
	Avoid drinking of alcoholic beverages known to contain high levels of acetaldehyde, e.g., sherries, madeiras, Calvados, strong fruit spirits, some sakes. Avoid especially homemade products
Foodstuffs	Avoid use of foodstuffs and so-called nonalcoholic beverages without knowing their ethanol and acetaldehyde concentrations
	The GRAS status of acetaldehyde should be reevaluated
	ALARA principle (As Low As Reasonably Achievable) according to the standards of *Codex Alimentarius* should be extended to the ethanol and acetaldehyde present in foodstuffs and "nonalcoholic" beverages
Risk groups	All above-mentioned measures should at first be applied to those with highest upper digestive tract cancer risk related to acetaldehyde exposure
Gene polymorphisms	Screening of individuals with ALDH2 deficiency and low active ADH among East Asians and those with high active ADH among Caucasians
Achlorhydric atrophic gastritis and *H. pylori* infection	Use of biochemical markers (*H. pylori* antibodies, pepsinogens I and II) and gastroscopy for the screening of individuals with atrophic gastritis and/or *H. pylori* infection especially among alcohol-consuming ALDH2-deficient subjects
L-Cysteine releasing medical devices	Decreases markedly acetaldehyde exposure through saliva and gastric juice during an alcohol challenge

Alternatively, specific questionnaires for the detection of alcohol-flushing reactions can be used. Further studies for the evaluation of the cost-effectiveness of these methods in different populations are warranted.

Microbial ADH-mediated acetaldehyde production is pH dependent. By decreasing intracolonic pH, lactulose also decreases intraluminal acetaldehyde concentrations after alcohol administration in the large intestine of rats [138]. An earlier report has already demonstrated that lactulose significantly decreases the recurrence rate of colorectal adenomas [139].

Acetaldehyde exposure can be decreased or even totally abolished by using special medical devices that slowly release L-cysteine. L-Cysteine is a semi-essential, natural, and safe sulfur-containing amino acid. L-Cysteine binds effectively to acetaldehyde forming inactive methyltiazolidinecarboxylic acid. A slow-release buccal tablet of L-cysteine is able to remove about two-thirds of acetaldehyde after consumption of a moderate dose of alcohol [140]. During smoking, already 5 mg of L-cysteine releasing slowly from a lozenge or chewing gum is enough to remove all the acetaldehyde from saliva

[141, 142]. L-Cysteine has also been shown to decrease acetaldehyde concentration in the achlorhydric stomach of atrophic gastritis patients after a dose of ethanol [143]. During 40-min follow-up period, the area under the curve for acetaldehyde decreased by a mean 63% with L-cysteine capsules as compared to a placebo [143]. Thus, medical devices slowly releasing L-cysteine provide a safe means for the reduction of acetaldehyde exposure in the gastrointestinal tract. Intervention studies involving L-cysteine and other measures aimed at the minimization of acetaldehyde exposure are warranted both at the population level and especially among high-risk groups such as heavy drinkers, smokers, and those with ALDH2 deficiency or achlorhydric atrophic gastritis.

Conclusions

The key issue in cancer prevention is the identification of specific etiologic factors. Acetaldehyde is the most important intermediate of alcoholic fermentation and ethanol oxidation. Thus, it is present in most alcoholic beverages and in many foodstuffs produced by fermentation. Microbial formation of acetaldehyde from ethanol is one of the key mechanisms in acetaldehyde exposure of the digestive tract mucosa. During and after drinking of alcoholic beverages, acetaldehyde derived from microbial oxidation of ethanol accumulates in the oral cavity and is transported via saliva further to the pharynx, esophagus, and stomach. Furthermore, acetaldehyde is the most abundant carcinogenic compound of tobacco smoke, which is readily dissolved in saliva during active smoking. Epidemiological, genetic, biochemical, and microbiological evidence derived from alcohol-consuming individuals carrying ALDH2-deficiency gene resulted in the reclassification of acetaldehyde as a group 1 carcinogen to humans. The evidence strongly suggests worldwide screening of acetaldehyde levels in thousands of beverages and foodstuffs as well as giving high priority to regulatory measures and consumer guidance. The screening and provision of information to hundreds of millions of people with gene polymorphisms and hypochlorhydric atrophic gastritis associating with enhanced acetaldehyde exposure should be seriously considered. New methods for the elimination of acetaldehyde, such as medical devices that slowly release L-cysteine, should be developed. Most importantly, the GRAS status of acetaldehyde, which allows it to be used as a food additive, should be reevaluated according to its classification as a group one carcinogen.

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Chapter 35
Alcohol Intake and Esophageal Cancer: Epidemiologic Evidence

Jill Layton and Jianjun Zhang

Key Points

- Most epidemiologic studies have demonstrated that alcohol drinking is associated with an increased risk of esophageal cancer. This association is more consistent and pronounced for squamous cell carcinoma than adenocarcinoma.
- Substantial epidemiologic evidence supports the synergistic effect of alcohol drinking and cigarette consumption on the occurrence of esophageal cancer.
- Molecular epidemiological studies revealed that genetic variants in alcohol metabolic pathway modulate individual susceptibility to the carcinogenic effect of alcohol consumption.
- Abstinence from alcohol or avoidance of heavy drinking could lead to a considerable reduction in the incidence and mortality of esophageal cancer especially among cigarette smokers.

Keywords Esophageal squamous cell carcinoma • Esophageal adenocarcinoma • Epidemiology • Acetaldehyde • Carcinogen • Alcohol drinking • Genetic susceptibility • Cancer prevention

History of Alcohol and Cancer

Alcohol has long been established as a risk factor for cancers of the oral cavity, pharynx, esophagus, and liver [1, 2]. According to Kamangar et al. [3], two papers published in 1932 and 1939 reported an association between excessive use of alcohol (among other risk factors) and esophageal cancer on the basis of clinical observations alone. Subsequent studies conducted in the 1950s noted a similar relation between alcohol use and head and neck cancers but with an observation of a linear trend with both duration and amount of alcohol consumption [3]. Since tobacco smoking also has been consistently associated with esophageal and other head and neck cancers, studies performed among nonsmokers in the 1960s were important in establishing alcohol as a risk factor independent of smoking [3]. Furthermore, studies carried out in the 1970s helped demonstrate the role of the synergism between smoking and alcohol in human carcinogenesis [1, 4]. Finally, in 1988, the International Agency for Research on Cancer (IARC) published a report that summarized the epidemiologic data concerning

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alcohol use and cancer and offered convincing evidence on the causal effect of alcohol intake on some cancers, including esophageal cancer [4]. With respect to esophageal cancer, they evaluated data from eight cohort studies in which seven showed a significantly increased risk for esophageal cancer among heavy alcohol drinkers. They also evaluated 13 case-control studies in which 11 showed a statistically significant association between alcohol intake and esophageal cancer risk [2, 4]. For this chapter, studies published after the 1988 IARC report also were reviewed to reflect the current and comprehensive epidemiologic evidence on this topic.

Descriptive Epidemiology of Esophageal Cancer

Worldwide, esophageal cancer is currently the eighth most common cancer with 481,000 incident cases estimated to have occurred in 2008 and is ranked sixth with respect to mortality with 406,000 deaths attributed to esophageal cancer in the same year [5, 6]. The estimated mortality rate from esophageal cancer worldwide in 2008 was 128.6 per 100,000 in men and 87.6 per 100,000 in women. Bosetti et al. [7] analyzed trends in esophageal cancer mortality in Europe and found that for men in the European Union (EU), the age-standardized mortality rates were 6 per 100,000 during the 1980s and 1990s and decreased slightly to 5.4 per 100,000 in the early 2000s, giving an annual percent change of −1.1%. Mortality rates among EU women were stable with 1.1–1.2 per 100,000 over the past 20 years [7].

Incidence of esophageal cancer varies considerably throughout the world. According to the IARC [6], the estimated incidence of esophageal cancer worldwide in 2008 was 203.8 per 100,000 per year in men and 165.1 per 100,000 per year in women. However, the incidence rates of esophageal cancer vary by more than 15-fold internationally among men (22.3 per 100,000 for Southern Africa vs. 1.4 per 100,000 for Western Africa) and by almost 20-fold among women (11.7 per 100,000 for Southern Africa vs. 0.6 per 100,000 for Polynesia). Furthermore, countries in Asia and Eastern and Southern Africa have incidence rates that are 3–10 times higher than that of most Western populations. For example, the male age-standardized incidence rate of esophageal cancer in 2008 was 22.9 per 100,000 in China as compared with only 2.1 per 100,000 in Mexico [6]. Data in Table 35.1 provide further evidence that remarkable differences in esophageal cancer incidence exist among world populations.

In the United States, data from the Surveillance, Epidemiology, and End Results (SEER) Program of the National Cancer Institute show an age-adjusted incidence rate of 4.5 per 100,000 for the period 2003–2007 with an almost fourfold difference between men (7.8 per 100,000) and women (1.9 per 100,000) [8]. This gender difference holds across all ethnic groups. For example, the incidence rate of esophageal cancer among US white men and women was 8.0 per 100,000 and 1.9 per 100,000, respectively, which was similar in gender difference to the reported rates of black men (8.9 per 100,000) and women (2.9 per 100,000) [8]. In the United States, African Americans had the highest incidence rates (8.9 per 100,000 for men and 2.9 per 100,000 for women) followed by Caucasians (8.0 per 100,000 for men and 1.9 per 100,000 for women), American Indians (5.2 per 100,000 for men and 2.9 per 100,000 for women), Hispanics (5.1 per 100,000 for men and 1.1 per 100,000 for women), and finally Asian Americans (4.1 per 100,000 for men and 1.0 per 100,000 for women) [8]. Thus, the incidence rates of esophageal cancer not only vary considerably by geographic region but also by gender and ethnicity.

There are two main histological subtypes of esophageal cancer, esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC) [9]. The demographic profile of ESCC mirrors that of overall esophageal cancer, with higher rates in men (vs. women) and in African Americans (vs. Caucasians) [7–9]. The demographic profile of EAC differs from that of ESCC in that the former has a much higher male-to-female ratio (approximately 7:1–4:1) than the latter and incidence rates are higher in Caucasians than in blacks [7–10]. Overall, ESCC is prevalent in three main regions: Asia (extending from Turkey through Iran and Iraq and to China), Africa (Southern and Eastern regions), and northwestern France [7, 9]. Conversely, EAC is more prevalent in Western countries [7, 8].

Table 35.1 Comparison of age-standardized incidence rates of esophageal cancer (/100,000) in 2008 among 24 countries, World Health Organization, GLOBOCAN, World Cancer Statistics[a]

Country	Men	Women
South African Republic	23.5	12.6
China	22.9	10.5
Mongolia	21.8	16.1
Kenya	17.5	9.9
Zimbabwe	15.1	6.3
Kazakhstan	14.7	7.7
Japan	10.6	1.5
Myanmar	9.6	5.6
United Kingdom	9.5	3.6
Brazil	8.2	2.5
Iran	7.4	6.3
Argentina	6.9	2.4
South Korea	6.6	0.4
India	6.5	4.2
France	6.5	1.5
Germany	6.4	1.4
United State of America	5.8	1.2
Spain	5.2	0.7
Canada	4.4	1.3
Columbia	3.3	1.3
Italy	3.2	0.8
Mexico	2.1	0.7
Peru	1.5	0.7
Greece	1.4	0.2

[a]Countries are ranked by the descending order of the incidence rates for men (Based on data from reference [6])

The epidemiology of esophageal cancer has changed dramatically over the past few decades with respect to histological subtype [7, 9]. Previously, the most common histological subtype of esophageal cancer was squamous cell carcinoma, with adenocarcinoma reported to account for only 0.8–3.7% of esophageal cancers [9]. However, in Western countries such as the USA, Denmark, Sweden, Scotland, and Switzerland, the incidence rates of ESCC have either stabilized or declined, whereas the incidence rates of EAC have increased [7, 11]. Furthermore, in some areas of the world (e.g., USA, Northern Europe), EAC is now the most common subtype of esophageal cancer [7, 9, 11]. For example, in a study performed by Devesa et al. [12] using SEER data, they found an increase of over 350% in the annual rate of adenocarcinoma from 1974–1976 to 1992–1994. According to Pandeya et al. [13], the recent changes in the incidence and distribution of esophageal cancers are suggestive of a change in the prevalence of exposure to causal risk factors.

Carcinogenic Effect of Alcohol

The carcinogenic mechanisms of alcohol are not yet fully understood. Until recently, it was believed that pure ethanol was not a carcinogen itself based on animal studies [1]. However, new research has shown that when rats were given ethanol in their drinking water, they developed malignancies [14]. Ethanol itself can prevent DNA methylation by inhibiting S-adenosyl-L-methionine (SAM), a universal methyl group donor, which is important in the regulation of gene transcription [5]. By inhibiting

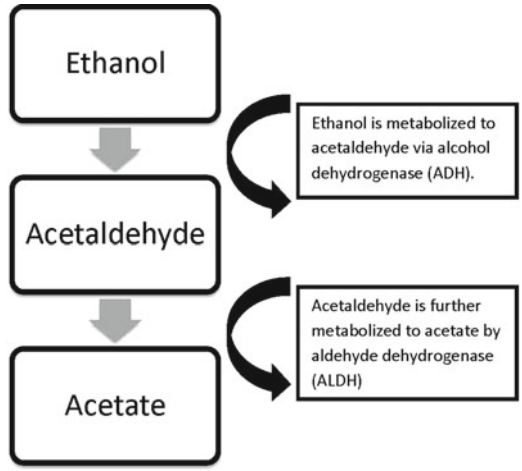

Fig. 35.1 A basic schematic diagram of alcohol metabolism and the key enzymes involved in the pathway

SAM synthesis, oncogenes are upregulated and tumor-suppressor genes are downregulated [5]. Ethanol is also considered to be a cocarcinogen by acting as a solvent for other carcinogens to penetrate the mucosa of upper aerodigestive organs, which could help explain the excessive risk of esophageal cancer associated with alcohol drinking among cigarette smokers [15].

There is ample evidence from both animal studies and in vitro studies of human cells indicating that the carcinogenicity of ethanol is related to its metabolism [1, 5, 14–17] (see Fig. 35.1). For example, inhalation of acetaldehyde, the primary metabolite of ethanol, in rats and hamsters resulted in increased rates of carcinomas [14]. Acetaldehyde (AD) has been shown to be carcinogenic by interfering with DNA synthesis and repair and inducing gene mutations by interacting with DNA to form mutagenic DNA adducts [5, 14]. These adducts can eventually lead to miscoding and permanent gene mutation if they are not removed by cellular repair mechanisms [5]. Chronic alcohol consumption has also been shown to induce the hepatic cytochrome P450 2E1 (CYP2E1)-dependent microsomal monooxygenase enzyme at concentrations 10–20 times higher than those without chronic alcohol consumption [5, 14]. The CYP2E1 enzyme generates reactive oxygen species (ROS) that result in oxidative stress, a critical pathophysiological mechanism in cancer [5]. Finally, heavy alcohol use can lead to nutritional deficiencies caused by changes in metabolic pathways such as the ones listed above (i.e., DNA methylation) [1, 5]. Boffetta and Hashibe [1] postulated that alcohol consumption may influence the intake, absorption, and metabolism of vitamins B6 and B12. Disruption of vitamin A metabolism in heavy drinkers may also promote carcinogenesis because retinoic acid (a metabolite of the vitamin) regulates genes involved in cellular growth and differentiation [1, 5]. In fact, a recent study that evaluated micronutrient intake and esophageal cancer risk found a protective effect for folate, vitamin B6, and vitamin A [18].

Alcohol Exposure Assessment in Epidemiologic Studies

Studies of alcohol consumption and esophageal cancer typically use alcohol frequency questionnaires to assess usual intake of alcohol in study participants during the past year [19]. Total alcohol intake is calculated by multiplying the amount of alcohol in each type of alcoholic beverage by the frequency and/or duration of alcohol use [19]. Diet records and 24-hour recalls are usually not used to assess alcohol exposure in case-control and cohort studies because of their methodological limitations (i.e., assessment of only short-term intake of alcohol). However, these instruments are widely used in

questionnaire validation studies, with correlation coefficients of 0.7–0.9 between alcohol intakes assessed from questionnaires and from diaries and/or recalls [20, 21]. Excessive alcohol consumption also can be indirectly assessed using biomarkers such as serum levels of gamma (γ)-glutamyl transferase, estrogens, and lipids (e.g., high-density lipoprotein cholesterol); however, none of these biomarkers alone is sufficient to accurately evaluate the amounts of excessive alcohol use [22].

Epidemiologic Evidence on Alcohol Intake and Esophageal Cancer

As mentioned above, the relation between ESCC and alcohol has been noted since the early 1900s [3]. To date, most epidemiologic studies (including migrant, ecologic, case-control, and cohort studies) have found a statistically significant association between alcohol intake and ESCC risk.

Ecologic and Migrant Studies

Some ecologic studies have investigated the influence of alcohol intake on esophageal cancer risk. A study performed by Audigier et al. [23] found a positive correlation between the mortality rates from esophageal cancer and mortality rates from alcoholism and cirrhosis in France. A recent study compared lifestyle and other environmental factors between high-risk immigrants and low-risk host residents in China and demonstrated that lifestyle factors play a potentially significant role in esophageal cancer etiology [24]. Immigrants from a region in China that had a very high prevalence of ESCC resettled in an area that had a low prevalence of ESCC. It was found that the immigrants had maintained their high mortality rate of ESCC despite having relocated to an area of low ESCC prevalence, suggesting importance of early exposure to environmental risk factors and/or genetic susceptibility [24].

Case-Control Studies

Eleven case-control studies (including six hospital-based and five population-based) have been conducted in the USA, the UK, Sweden, Central and Eastern Europe, and South America [13, 25–35]. Overall, these studies demonstrated a consistently significant relation between alcohol use and esophageal cancer.

All of the hospital-based case-control studies generally revealed a significant association between alcohol intake and ESCC. Kabat et al. [25] found an adjusted odds ratio (OR) of 10.9 [95% Confidence Interval (CI): 4.9, 24.4] for men in the highest alcohol consumption group compared with male nondrinkers. The corresponding OR (95% CI) was 13.2 (95% CI: 6.1, 28.8) for women. When potential interaction between smoking and drinking was examined, they observed an OR of 4.3 (95% CI: 1.4, 12.5) among nonsmokers in contrast to an OR of 7.6 (95% CI: 3.1, 18.6) in smokers, suggesting a multiplicative effect of smoking and alcohol consumption on the risk of esophageal cancer [25]. Other hospital-based case-control studies reported a similar synergistic effect of cigarette smoking and alcohol intake on ESCC risk, with the highest OR reported being 50.1 among subjects who were in the group with heaviest alcohol and tobacco consumption [31, 32, 34]. Launoy et al. [28] analyzed data obtained from various measurements of alcohol intake (including total lifetime intake, mean weekly intake, duration of consumption, and former and current consumption) to address weaknesses inherent in studies that used current alcohol consumption alone to assess exposure to this risk factor. Of note, only weekly consumption was included in the final model with an adjusted OR of 15.7 (95% CI: 7.4, 33.0) in the highest consumption group compared with the lowest group. The ORs increased with

increasing weekly consumption, exhibiting a statistically significant dose-response relationship between alcohol intake and ESCC [28]. The remaining hospital-based case-control studies, including a pooled analysis of five hospital-based case-control studies, reported ORs ranging from 2.86 to 5.34 in ever drinkers compared with never drinkers [31, 33, 34].

As in the hospital-based case-control studies, all of the population-based case-control studies and one nested case-control study [35] overall found a significant association between alcohol intake and ESCC risk. The ORs ranged from 3.1 to 9.5 when the highest consumption groups were compared with the lowest consumption groups [13, 26, 27, 29, 35]. The nested case-control study [35] was conducted using the UK General Practice Research Database (GPRD) that contained electronic medical records from general practitioners. The strengths of this study lied in the prospective nature of the exposure data collection and thus avoidance of misclassification of exposure data due to changes in drinking habits after diagnosis and treatment of esophageal cancer, a methodological issue inherent in case-control studies. As observed in hospital-based case-control studies, apparent interaction between alcohol drinking and cigarette smoking was also detected in some population-based case-control studies [13, 29].

Studies of EAC and alcohol consumption have yielded mixed results. Eight case-control studies have evaluated this potential association [13, 25–27, 29–31, 35]. Whereas the association between alcohol consumption and ESCC risk has been consistently observed in the aforementioned case-control studies, results for alcohol intake in relation to EAC risk are inconsistent. Significant association between alcohol use and EAC was observed in only two studies. In a hospital-based case-control study, Kabat et al. [25] reported an OR of 2.3 (95%CI: 1.3, 4.3) among male drinkers. Similarly, an OR of 1.8 (95%CI: 1.1, 3.1) was found in a study conducted by Vaughan et al. [26]. However, the remaining case-control studies did not find any statistically significant associations between EAC and alcohol consumption [13, 27, 29–31, 35]. The discrepancies between these studies could be due to differences in sample size (small studies may be underpowered to detect any true associations), populations studied (hospital-based studies versus community/population-based studies), and methods used to capture exposure to alcohol and other factors.

With regard to both histological subtypes of esophageal cancer and types of alcoholic beverages consumed, most case-control studies compared the effects of beer, wine, and liquor on esophageal cancer. Some studies revealed a statistically significant association of particular beverages with ESCC [27, 29, 32, 34], whereas others did not [26, 31]. None of the studies that examined alcohol and EAC found statistically significant associations by beverage type [26, 27, 29–31]. It should be pointed out that evaluation of esophageal cancer risk associated with types of alcoholic beverages is difficult because drinkers rarely consume only one type of alcoholic beverage. As a result, it is challenging to isolate the independent effect of each type of alcoholic beverage [13]. Collectively, it appears that the types of alcoholic beverages that are consumed in the greatest quantities are those that are associated with the greatest risk of ESCC. The lack of association between specific alcoholic beverages and EAC risk is in agreement with the overall inconsistent association between alcohol consumption and EAC risk [34].

Case-control studies also differed in the way alcohol intake was assessed in dietary surveys and treated in data analysis. Some studies defined exposure categorically whereas at least one study by Pandeya (2009) used both continuous and categorical analyses of alcohol consumption [13]. While the utilization of categories of alcohol consumption allowed for easily interpretable measures of risk, it also potentially obscured important differences across categories of alcohol intake [13]. Conversely, using continuous measures of alcohol intake could have masked potentially important differences in risk associated with different categories of intake [13]. However, the study performed by Pandeya et al. (2009) did show an increased risk of ESCC associated with alcohol consumption analyzed as both a continuous and a categorical variable, which lends further support to the association [13]. Some studies calculated alcohol consumption as daily, weekly, and/or lifetime intakes. In addition, several studies asked participants to recall alcohol drinking in a typical week, whereas other studies specified

a particular reference period of alcohol exposure (e.g., 20 years prior to diagnosis or typical alcohol consumption at particular ages). All these inconsistencies made it difficult to compare results between the studies reviewed in this chapter.

Finally, studies differed in the reference group used for calculating ORs. Some used never drinkers as referent, while others defined those in the lowest consumption category as referent. This difference in the definition of reference group precludes the calculation of overall summary ORs for risk of ESCC in relation to alcohol consumption. Nevertheless, compelling evidence that alcohol consumption has consistently been associated with ESCC in a wide variety of case-control studies serves to strengthen the notion that alcohol is an important risk factor for ESCC.

Cohort Studies

Three prospective cohort studies have addressed the association between alcohol intake and esophageal cancer. Freedman et al. [36] evaluated association of alcohol intake with the two histological subtypes of esophageal cancer in the National Institutes of Health-AARP Diet and Health Study. In that study, a total of 474,606 participants filled out a questionnaire and were followed up annually with linkage to the Social Security Administration Death Master File and cancer registry and by mailings to respondents. Alcohol intake per day was calculated in terms of responses to questions of frequency and portion size for usual consumption of wine, beer, and liquor over the past 12 months. The adjusted hazard ratios (HR) for ESCC in relation to total alcohol intake were 2.06 (95% CI: 1.16, 3.68) for 0 drinks/day, 2.33 (95% CI: 1.28, 4.24) for >1–3 drinks/day, and 4.93 (95%CI: 2.69, 9.03) for >3 drinks/day as compared with >0–1 drinks/day (p-trend: <0.0001). When alcoholic beverages were examined individually, only beer and liquor had a statistically significant influence on risk of ESCC. However, EAC was not significantly associated with either total alcohol intake or types of alcoholic beverages, which is in conformity with the results of most of the aforementioned case-control studies [36].

The Shanghai Cohort Study was conducted in a high-risk population of esophageal cancer in China [37]. A total of 18,244 men in Shanghai were enrolled during 1986–1989 and were prospectively followed up through 2006. Follow-up was implemented through in-person interviews of all surviving cohort members and review of reports from the population-based Shanghai Cancer Registry and the Shanghai Vital Statistics Office. Alcohol exposure was assessed by asking subjects about their weekly alcoholic beverage consumption over the past 6 months. After adjustment for education, BMI, years of smoking, and intakes of preserved food, fruits, and vegetables, the adjusted HR for esophageal cancer among regular drinkers compared with nondrinkers was 2.02 (95% CI: 1.31, 3.12). Moreover, a statistically significant trend (all p-trend values <0.0001) was observed of increasing risk for esophageal cancer with increasing years of regular drinking [adjusted HR: 3.22 (95% CI: 1.77, 5.86) for 40+ years of regular drinking], increasing number of alcoholic beverages per day [adjusted HR: 3.74 (95% CI: 2.12, 6.59) for 4+ drinks/day], increasing daily ethanol intake [adjusted HR: 4.65 (95%CI, 2.31, 9.36) for 80+ grams/day], and lifetime ethanol intake [adjusted HR: 4.26 (95% CI: 2.26, 8.01) for 800+ kg] when compared with nondrinkers. When specific alcoholic beverages were examined, only rice wine (1 to <2 drinks/day) and spirits (2 to <4 and 4+ drinks/day) were statistically significantly associated with risk of esophageal cancer. As expected, an apparent interaction between alcohol drinking and cigarette smoking was detected; the adjusted HR was 8.0 (95% CI: 3.4, 19.1) for subjects who smoked over 40 years and consumed over four alcoholic drinks per day compared with those who were nonsmokers and nondrinkers [37].

Finally, the Japan Public Health Center-based Prospective Study on Cancer and Cardiovascular Disease (JPHC) has evaluated the effect of alcohol consumption, smoking, and flushing response on the risk of ESCC [38]. The study started in 1990 and recruited 60,876 men. The participants filled out

a questionnaire to solicit data on alcohol consumption and other risk factors for ESCC. Subjects were followed up through 2004, and their cancer status was obtained through linkage to population-based cancer registries. Occasional drinkers did not exhibit an increased risk of ESCC when compared with nondrinkers. However, a statistically significant association was found for regular drinkers [compared with nondrinkers, HR: 2.59, 95% CI (1.57, 4.29) for subjects consuming 150–299 g of ethanol per week and HR: 4.64, 95% CI (2.88, 7.48) for subjects consuming 300 + grams of ethanol per week]. A clear interaction between alcohol drinking and cigarette smoking on ESCC risk also was demonstrated in this Japanese study.

Overall, the findings from cohort studies were consistent with those of case-control studies; alcohol consumption is a significant risk factor for ESCC but not for EAC [36–38]. Furthermore, a significant interaction exists between alcohol intake and cigarette smoking on risk of ESCC. Analysis by beverage type yielded mixed results. Current epidemiologic evidence suggests that it is the amount of alcoholic beverage consumption, rather than any particular type of alcoholic beverage, that is associated with increased risk of ESCC [36–38].

A major strength of the cohort studies is their prospective design. Alcohol intake was assessed before esophageal cancer was diagnosed, which ruled out the possibility of reverse causality. However, these cohort studies were also subject to some limitations. For example, the AARP Diet and Health Study [36] did not gather any information on alcohol use in different periods of life and smoking duration, which could have resulted in exposure misclassification. Additionally, the response rate in this study [36] was very low (only 17.6%), and the respondents were less likely to smoke, were more educated, and were more likely to be non-Hispanic white than the general US population, which limits the generalizability of the study results. A limitation of the cohort studies in Shanghai [37] and Japan [38] was that alcohol intake data from only men were analyzed in relation to esophageal cancer risk. Finally, the Japanese study [38] relied on a single baseline assessment of alcohol consumption and thus did not capture any potential changes in the drinking habits of the study subjects, which could lead to misclassification of exposure to alcohol.

Gene-Environment Interaction in Esophageal Cancer

A growing body of experimental and epidemiologic evidence demonstrates that alcohol drinking interacts with genetic variants in the alcohol metabolic pathway to modulate risk of esophageal cancer. As shown in Fig. 35.1, ethanol is metabolized to acetaldehyde and then to acetate by alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH), respectively [5]. Single nucleotide polymorphisms (SNPs) of *ADH1B* and *ALDH2* genes can affect the amount of acetaldehyde produced (*ADH1B*) or oxidized (*ALDH2*), resulting in differential acetaldehyde exposure among drinkers [14, 39]. For example, a polymorphism in the *ADH1B* gene influences ADH1B activity; *ADH1B*2* allele encodes for an enzyme that is 40 times more active than the enzyme encoded by the less active *ADH1B*1* allele. As a consequence, subjects with *ADH1B*2* allele have a much larger production of acetaldehyde [14, 39]. Conversely, the *ALDH2*2* allele of a polymorphism in the *ALDH2* gene encodes an enzyme that is unable to convert acetaldehyde to acetate due to an inactive protein subunit [40, 41]. The individuals who are homozygous for the *ADH1B*2* allele and/or the *ALDH2*2* allele experience a severe reaction involving facial flushing, nausea, and vomiting when exposed to alcohol. Actually, these genetic polymorphisms confer some form of protection against ESCC risk for those subjects because they cannot tolerate even small amounts of alcohol and therefore tend to avoid exposure to carcinogenic acetaldehyde [14, 40, 41]. Clearly, it is biologically plausible that genetic variability in alcohol metabolism modifies the association between alcohol intake and esophageal cancer risk.

Epidemiologic studies have shown that alcohol drinkers with the slower form of ADH1B enzyme (encoded by the *ADH1B* *1/*1 genotype) are at an excessive risk for ESCC [42, 43]. For example, Lee et al. (2008) found that *ADH1B* genotypes had no effect on ESCC risk among nondrinkers. However, among drinkers, elevated ESCC risk associated with the *ADH1B* *1/*1 genotype increased with increasing alcohol intake. Compared with nondrinkers with the *ADH1B* *2/*2 genotype, subjects with the *ADH1B* *1/*1 genotype and whose alcohol intake was 30 g or less per day had an OR of 10.6 (95% CI, 4.7, 23.7). The OR increased to 71.9 (95% CI, 22.6, 228.5) for those with *ADH1B* *1/*1 genotype but whose alcohol intake was greater than 30 g per day [42].

A number of studies have consistently shown that subjects with inactive enzyme ALDH2 (encoded by the *ALDH2 *1/*2* genotype) are associated with an increased risk for developing esophageal cancer, with ORs ranging from 12.1 to 16.4, depending on level of alcohol consumption [39–41]. This genetic effect occurs due to the reduced capability of those persons to efficiently metabolize the highly carcinogenic acetaldehyde and in turn experience excessive accumulation of acetaldehyde in their bodies [41].

Conclusions

Most epidemiologic studies have demonstrated that alcohol drinking is a modest to strong risk factor for esophageal cancer, particularly squamous cell carcinoma. Substantial evidence also exists supporting the role of interaction between alcohol drinking and cigarette consumption in the etiology of esophageal cancer. The detrimental effect of alcohol consumption on esophageal cancer risk may be also modified by genetic variability in alcohol metabolism. The association between alcohol intake and esophageal adenocarcinoma is suggestive but inconsistent. These epidemiologic findings are of tremendous public health importance because abstinence from alcohol or avoidance of heavy drinking could lead to a considerable reduction in the incidence and mortality of esophageal cancer especially among cigarette smokers and individuals who are genetically susceptible to alcohol exposure.

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Chapter 36
A Nutritional Approach to Prevent Alcoholic Liver Disease

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Key Points

- Betaine feeding with ethanol prevented the blood alcohol cycle and accelerated the rate of ethanol elimination by increasing SAMe, which increases the rate of metabolism. This generates NAD+, the rate-limiting factor utilized in ethanol oxidation by ADH.
- Betaine feeding prevented ethanol-induced fatty liver and liver injury including elevation of blood ALT levels.
- Betaine feeding prevented the molecular epigenetic cellular memory induced by ethanol feeding.
- Betaine feeding induced methylation of histones that silence the gene expression changes induced by ethanol feeding.

Keywords BAL (blood alcohol level) • BHMT (betaine-homocysteine methyltransferase) • PPARα (peroxisome proliferator-activated receptor) • SREBP-1 (steroid response element binding protein) • Igfbp1 (insulin-like growth factor binding protein) • PGC1a (peroxisome proliferator-activated receptor gamma coactivator 1-alpha) • Sirt1 (sirtuin) • ALDH (aldehyde dehydrogenase) • HAT (histone acetyltransferase) • HDAC (histone deacetylase) • P300 and Pcaf (histone acetyltransferases) • Cth (cystathionase) • Gadd45b (growth arrest and DNA-damage-inducible beta)

Introduction

Clinical trials have largely been unable to significantly reduce the mortality of alcoholic liver disease (ALD) beyond that achieved by placebo and alcohol withdrawal when the liver disease has progressed to the stages of alcoholic hepatitis or cirrhosis. This discouraging treatment outcome has reduced the frequency of clinical trials to treat ALD compared to the ongoing numbers of clinical trials to treat other chronic liver diseases such as hepatitis C (HCV), hepatitis B (HBV), and primary biliary cirrhosis [1]. This is despite the fact that the age-adjusted death rate (per 100,000) of ALD is 25 times higher than for primary biliary cirrhosis, 10.5 times higher than HBV, and 2.7 times higher than HCV [1].

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The treatment for ALD has been quite variable in nature. Recently, the methyl donors, betaine and S-adenosylmethionine (SAMe), have been tried as a nutritional approach. This treatment approach has not succeeded either, because, once ALD has been established, it is only reversible when alcohol abuse is stopped. For example, in a recent clinical trial, feeding SAMe or placebo three times a day for 24 weeks to patients suffering from ALD, there were no differences between the patients treated with SAMe and patients treated with placebos. Comparison of the two groups for both baseline and posttreatment parameters of serum liver biochemistries, methionine metabolites, or liver histopathology scores showed no differences between the groups over time. Likewise, the analysis of interactions showed no differences in the treatment outcomes when controlling for the following: the severity of baseline fibrosis and steatosis, recent alcohol drinking, MELD and Child scores, drinking status at the time of enrollment, baseline vitamin B12 levels, and folate levels, gender, age, and ethnic group [2].

Therefore, if treating patients with ALD does not work, the logical next step would be to try to prevent ALD by feeding protective nutrients like betaine or SAMe before ALD develops. In all the animal studies where animals were fed, alcohol with SAMe or betaine steatosis was ameliorated. When executives of the beer industry, NIAAA officials, hepatologists, or psychiatrists who treat alcoholic patients were asked "why not prevent ALD," they say that alcoholics are too difficult as patients. They will not cooperate. The question is "why are they different from patients who have ALD?"

Even though researchers do not want to do clinical trials to prevent ALD, we need to understand the benefits of using SAMe or betaine feeding to prevent ALD as shown by animal and in vitro studies. These studies follow.

History of Dietary Methyl Donor Feeding with Alcohol in Order to Prevent Experimental ALD

At one time, methyl donors were thought to play a role in the pathogenesis of ALD. However, when choline, a methyl donor, was fed to baboons to prevent ALD, it did not prevent liver fibrosis and it was toxic [3]. Choline is an essential nutrient in humans [4]. Following this, baboons were fed S-adenosylmethionine (SAMe), which attenuated but did not prevent ALD [5]. SAMe fed with ethanol attenuated oxidative liver injury and lipid synthesis in micropigs fed a folate-deficient diet [6, 7]. Betaine, another methyl donor, also prevented and reversed experimental fatty liver in rats fed ethanol in vivo [8–10] and prevented steatosis in vitro [10]. Betaine prevents steatosis by restoring phosphatidylcholine generation by the phosphatidylethanolamine methyltransferase pathway [11]. Betaine reduced fatty liver due to ethanol by reducing ER stress. It did this by reducing homocysteine levels, increasing VLDL export, increasing SAMe, reducing SAH levels, and reducing oxidative stress by restoring GSH levels [12–15]. One study combined an increase in multiple dietary methyl donors including betaine, choline, methionine, and B12 in the diet fed with ethanol to mice [16]. As a result, ethanol-induced fatty liver was attenuated, the reduced glutathione-oxidized glutathione ratio was increased, and the ethanol-induced increase in CYP2E1 was blunted. Caspase levels were increased when the methyl donor diet was fed, as were the level of PPARα, CYP 4a10, and acyl-CoA oxidase activity. The elevation of ALDH activity induced by alcohol was attenuated by feeding the methyl donors. The levels of acetate and citrate were reduced by the methyl donor diet. This would stimulate carbohydrate metabolism rather than fatty acid oxidation. The methyl donor diet increased the elimination rate of ethanol, which lowered the blood alcohol levels achieved in the blood alcohol cycle. The blood alcohol level (BAL) cycle develops in the intragastric tube feeding model that was used in the experiments. Feeding the methyl donors ameliorates the BAL cycle [16].

SAMe Used to Prevent Experimental ALD

Epigenetic Background

The rationale for the use of methyl donor nutritional therapy to prevent ALD is based on the fact that ethanol feeding profoundly alters methylation of histones which then changes the expression of a large number of genes. The changed genes control liver metabolism [17–19]. Theoretically, methylation of these histones by feeding methyl donors would prevent the changes in gene expression caused by ethanol.

Microarray Analysis of SAMe Prevention of ALD

To document this phenomenon, microarray analysis and gene mining of the changes in gene expression and histone methylation in rats fed ethanol with or without SAMe added to the diet have been studied both acutely at 3 h, 12 h, and chronically for 1 month [20–23].

One important observation, which was found in the initial study by ethanol feeding of rats for 1 month using the intragastric tube feeding model, was that the blood alcohol level (BAL) at the time of sacrifice was a major variable determining which changes in gene expression were observed in the liver. This was apparent when the gene expression profiles were compared in the control rats versus rats with peak BAL and trough BAL [18]. When the expression of the genes at the peak BAL and trough BAL was compared, PPARα was increased 20-fold, BHMT was increased 8-fold at the peaks, CXC ligand 1 was decreased 12-fold as was SREBP1 4× at the peaks. This indicates just a few differences in the changes in gene expression when the liver BALs at the peak and trough were compared. Many more differences are cited in the published paper [18].

When an acute bolus of ethanol was given to rats (6 g/kg) and microarray analysis was performed at 3 h and 12 h post bolus, the changes in gene expression were markedly different between the 3-h and 12-h liver samples [21, 23]. The heat maps were quite different when the 3 h and 12 h were compared with controls fed isocaloric dextrose. At 3 h after the bolus, there were 488 genes changed. At 12 h post bolus, the expression of 586 genes was changed. After 1 month of ethanol feeding (13 g/kg/day), the heat maps were quite different at the peak and trough BALs when compared to the controls. At 1 month, the changes in gene expression at the peak BALs were markedly different from those seen at the trough and the controls [19]. The trough and controls were only slightly different. After 1 month of continuous ethanol feeding, 1,300 genes were changed at the peak BALs. The results suggest that the epigenetic memory of the hepatocytes was markedly altered at the peak BAL but not at the trough BAL [19].

Almost all the functional pathways had changes in gene expression at the 3 and 12 h post bolus but the pattern of the changes in gene expression in the various pathways was quite different. Igfbp 1 was increased 18-fold at the peak and trough (18.5- and 20.1-fold) but only a 10.8-fold change at 12 h post bolus and was unchanged at 3 h post bolus [19, 21, 23]. PGC1a and RARb increased eightfold only at the peak BAL [19]. PPARg was downregulated at the peaks, and Sirt1 was upregulated at both the peaks and the troughs [19, 21, 23]. ALDH was increased 20-fold both at the peaks and troughs after 1 month of ethanol feeding but not at 3 h and 12 h post ethanol bolus. This indicates that cellular memory required chronic ethanol feeding in order to induce its overexpression by the liver.

Histone Acetylation and Methylation in Experimental ALD

To try to explain these epigenetic changes, histone modifications were tested for at the 3 and 12 h post ethanol bolus as well as the 1 month of ethanol feeding [19, 21, 23]. H3K9 ac and H3K18 ac were upregulated at 3 h but not at 12 h post bolus. At 1 month of ethanol feeding, H3K18 and H3K9 ac were

upregulated at the peaks of the ethanol cycle [19, 24]. The histone acetyltransferase (HAT) p300 and the histone deacetylase (HDAC) were unchanged at 3 and 12 h post ethanol bolus [19, 21, 23]. P300 (HAT) was upregulated threefold only at the peaks of the ethanol cycle [24]. Nuclear levels of H3K4me3 were unchanged at 3 and 12 h post bolus but were increased after 1 month of ethanol feeding [19, 21, 23]. Likewise, H3K27me3, a gene silencing histone, was increased at both the peak and trough of the ethanol cycle [19]. Nuclear global methylation was downregulated only at 12 h post ethanol bolus [19, 21, 23]. Fox O was upregulated at both the peak and trough of the ethanol cycle after 1 month of feeding alcohol [24]. Levels of phosphorylated proteins involved in cell growth such as phosphorylated c-Jun, Akt, p38, Erk, and Sapk/Jnk were all reduced in the nuclear extracts at the peaks and troughs, whereas nuclear levels of β-catenin were increased at the peaks and troughs [24].

Molecular Changes in Human ALD

The molecular changes in humans who have developed alcoholic hepatitis, a condition which takes years of heavy alcohol abuse to develop, do not resemble those seen in the rats at 3 and 12 h post ethanol bolus or 1 month of ethanol feeding. This is probably due to more advanced damage in human livers and because the liver biopsy used for molecular changes is obtained during the alcohol withdrawal period. At this withdrawal period, the liver, which has adapted to alcohol, is in a state of repair and is returning to normal. Microarray analysis performed on liver biopsies done on steatohepatitis, alcoholic hepatitis, and cirrhosis stages of ALD involved changes in gene expressions in functional pathways of fibrogenesis and immune response/inflammation. Steatosis patients when compared with controls showed 98 differentially expressed genes, 30 upregulated and 68 downregulated [25]. Gene changes were involved in transport, biosynthesis, and lipid-metabolism pathways. Alcoholic patients gene expression changes totaled 211 (100 upregulated, 111 downregulated). Upregulated genes involved cell adhesion, immune response, oncogenesis, signal transduction, and embryogenesis functional pathways. Downregulated expression of genes included protein biosynthesis, cell growth, signal transduction, and transport pathways. RT-PCR confirmation of seven gene changes included osteopontin (involved in inflammation, leukocyte recruitment, and cell survival), IL8, annexin AZ (enhanced fibrinolysis), Tnfrsf 14, and claudin. These were all upregulated. CD 209 (dendritic cell LPS binding) and S-adenosylmethionine synthase (Mat1a) (a major methyl donor which downregulates global gene expression, an important epigenetic mechanism) were all downregulated.

A second study on 23 patients with alcoholic hepatitis was compared with 6 controls where RT-PCR of 46 candidate genes was performed [26]. The liver biopsies showed advanced disease stages of ALD. Genes encoding extracellular matrix 1 and collagen 1 fibrogenesis mediators (Tgfb), inflammation (cytokines), apoptosis regulators (Bcl-2) were upregulated. Fibrogenic regulators such as Timp1, several NADPH oxidase components, and Groa correlated positively with morphologic changes in alcoholic hepatitis. Lymphocytic inflammation correlated with Tgfb. Groa and Duox 12 correlated with severe granulocytic infiltrate. Groa expression exceeded all the other genes (30-fold increased). NADPH oxidases were the next most upregulated genes.

S-Adenosylmethionine (SAMe) Prevention of Experimental ALD

SAMe, the most powerful methyl donor, which globally silences gene expression by methylating histones, is postulated to prevent the upregulation of gene expression which is involved in ALD pathogenesis through epigenetic means. To test this hypothesis, rats were fed SAMe with ethanol at 3 and 12 h post alcohol bolus as well as 1 month ethanol feeding using the intragastric tube feeding model [22] and 3 + 12 h post bolus ethanol feeding model [21].

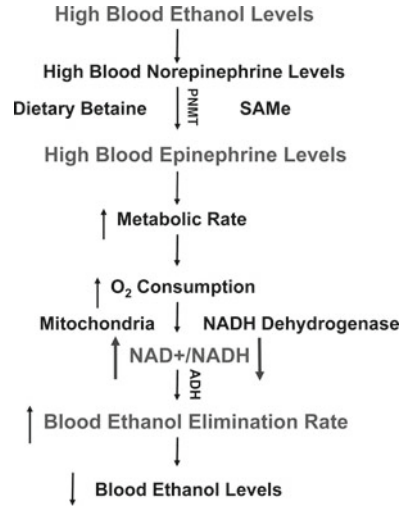

Fig. 36.1 Fluctuations of blood epinephrine levels permit blood alcohol (BAL) to increase when low and decrease when the BAL levels reach a high level during the BAL cycle. At high levels of epinephrine, the metabolic rate is increased which generates NAD + from NADH by mitochondrial NADH dehydrogenase. NAD + then increases the rate of ethanol oxidation by ADH to drive down the BAL levels

SAMe fed with ethanol as a bolus to rats that were then sacrificed 3 and 12 h post ethanol bolus revealed major changes in gene expression when compared to rats fed isocaloric glucose as a control [21]. SAMe reduced the BAL 3 h post ethanol bolus (138 ± 60 vs. 347 ± 68 mg). This increase in the ethanol elimination rate [16] was possibly due to the increase in the metabolic rate as was observed at the peaks of the BAL cycle [27]. SAMe increases the metabolic rate by increasing the epinephrine levels which then increases the generation of NAD [28]. NAD is the rate-limiting cofactor for the oxidation of ethanol by ADH [29]. The metabolic rate is increased by SAMe as a result of the fact that SAMe is an essential cofactor in the conversion of norepinephrine to epinephrine by the enzyme phenylethanolamine N-methyltransferase. Epinephrine is five- to tenfold more potent than norepinephrine in stimulating the metabolic rate [29]. Yuki and Thurman (1980) showed that epinephrine (adrenaline) was responsible for the increased metabolic rate (increased rate of O_2 consumption) caused by ethanol ingestion (SIAM – swift increase in alcohol metabolism) [30]. Figure 36.1 illustrates how the BAL cycle is driven by the conversion of norepinephrine to epinephrine, catalyzed by SAMe as an essential cofactor of PNMT [29].

Microarrays compared the changes in gene expression 3 and 12 h post ethanol bolus with or without SAMe in the bolus of ethanol Venn diagrams showed that SAMe changed the expression of 444 genes that had been changed by ethanol alone at 3 h post ethanol bolus [21, 23]. At 12 h post bolus, SAMe changed the expression of 327 genes. Twenty-five of the twenty-six functional pathways had a large number of genes downregulated when SAMe fed with ethanol 3 h post bolus was compared with 3 h post ethanol bolus. This result was very different when the same comparison was done at 12 h post ethanol bolus. The upregulation of gene expression by ethanol was changed. At this time, an equal number of genes were up- or downregulated in most of the 26 functional pathways. When the effect of SAMe feeding on ethanol-induced changes in functional pathways, which occurred after 1 month of ethanol feeding, was studied, only a few pathways were downregulated by SAMe such as focal adhesions, MAPK, and PPAR signaling pathways [22].

At 3 h post ethanol bolus, SAMe downregulated Aldh, Bal, 1gf2bp3, Bhmt, Cth, Mat2a, Foxn3, Jun, Tnfrsf9, Ahcyl, Tgfbrl, Pcaf, Rxra and Tgfbr2. Some of these are enzymes involved in methionine metabolism. Pcaf is histone acetyltransferase (HAT). At 12 h post ethanol bolus, the expression of the genes downregulated by SAMe were Cth and Lepr. Genes upregulated were Cyp17A, Cycl 1, Gadd45b, Cyp7a1, Gsta2, Gadd45g, Hmox1, Fabp 4, Mknk3, and Adipor 2. SAMe caused marked global downregulation of gene expression only at 3 h post ethanol bolus. It was concluded that SAMe treatment effectively prevented the gene expression changes at 3 h post ethanol bolus but not at 12 h

post bolus. This supported the concept that SAMe's effect on gene expression was short lived. Therefore, lasting epigenetic cellular memories require chronic exposure before they become permanent in the chronic ethanol-induced decrease in DNA and histone methylation. It is likely that these changes in epigenetic memory can be prevented by SAMe treatment [31]. LPS toxicity increases binding of the trimethylated H3K4 to the iNOS and TNFα promoter, and this is blocked by SAMe treatment. SAMe inhibits the H3K4me3 binding to the promoter response element. In this way, SAMe inhibits the proinflammatory response which is seen in ALD [32, 33].

After 1 month of intragastric ethanol feeding with or without SAMe, the urinary alcohol cycle was ablated by SAMe and the BALs were significantly lowered by SAMe (454 ± 148 with ethanol and 153 ± 35 with SAMe) when added to the alcohol diet [22]. Fatty liver was reduced by 50 % when SAMe was added to the diet. The expression of the following genes were upregulated by ethanol but not when SAMe was fed with ethanol: 1l1r2, 1l1r1, Tnfrsf6, Cxcl4, Ccl6, TLR4, Cxcl 12, Ccl4, TLR2, Tgfbr3 Tnf, Igfbp 2, Falp 2, Gadd45b, Hmox1, Ppara, Herpud 1, Col4a1, Klf9, Hgf, Jak2, Prkcb1, F10, Igf1r, Shc1, Rxra, Mapk4, Klf3, Mapk7, F8, and C3. Ethanol increased the levels of H3K27me3 (a gene silencing histone), but SAMe increased its level even higher.

To determine the role played by SAMe in preventing the upregulation of TLR4 and 2 in rats fed ethanol for 1 month, PCR microplate array analysis specific for the TLR signaling pathway was performed [20]. The ethanol-fed rats were sacrificed at peak BALs. TLRs studied included 1, 2, 3, 4, 5, 6, 7, and 9. Downstream in the signaling pathway, MyD88 and Traf6 were studied, and upstream of TLR4, CD14 was also studied. All the TLRs listed were upregulated at peak BALs as was MyD88. In addition, Traf 6, FOS, Jun oncogene, Irf-1, Hspala, Ifna1, Ifng, 1l10, 1l1r1, 1l6, 1l2, 1rak 1 and 2, Nr2c2, Ppara, Inf, Infrsfla, and Tradd were upregulated. The upregulation of MyD88 conflicts with a previous report where MyD88 was not essential for the TLR4 signaling in response to LPS based on the fact that MyD88 knockout mice did not prevent the TLR4 signaling. The latter observation would suggest that the MyD88-independent pathway was the signaling pathway involved in the response to ethanol [34].

Based on the PCR microarray analysis data mining, qRT PCRs were done on livers from rats fed ethanol with or without SAMe added to the liquid diet. The results showed that SAMe completely prevented the upregulation of TLR2 but not TLR4. SAMe downregulated TLR 3 and 9 with or without ethanol feeding. SAMe, with or without ethanol, downregulated the expression of CD14, MyD88, IL1r1, 1rf1, and Tnfrlsf1a. When the protein levels of TLR4 and MyD88 were measured, TLR4 was significantly increased by ethanol, and this was prevented by SAMe. Ethanol significantly increased the levels of MyD88, but SAMe did not prevent this increase. The prevention of the upregulation of TLR4 by SAMe may prevent LPS from the increased permeability of the gut. This would prevent stimulation of the TLR4 signaling pathway, which would prevent generation of cytokines that occur from NFκB activation that, stimulates hepatic fibrogenesis [35].

SAMe feeding with an acute ethanol bolus increased the ADH1 levels and the gene expression of ADH1, 3 h post ethanol bolus. This was associated with a decrease in blood alcohol levels (BAL) [34]. This may in part explain the mechanism involved in lowering this BAL. However, SAMe did not affect the BAL 12 h after the ethanol bolus when the protein levels of ADH 1 were unchanged and the gene expression levels of ADH1 were upregulated by SAMe [23]. CYP2E1 levels were unchanged at 3 and 12 h post bolus despite the marked upregulation of CYP2E1 gene expression at these times. A similar dissociation between the protein levels and the gene expression changes induced by SAMe was observed for ALDH 1 and 2 [23]. These discrepancies supported the conclusion cited earlier where the low BAL caused by SAMe during chronic ethanol feeding was found to be due to an increase in metabolic rate induced by SAMe or betaine [22, 29].

The changes in gene expression at 3 and 12 h post ethanol bolus with or without SAMe feeding were associated with alterations in histone methylation. Ethanol fed without SAMe decreased the level H3K9me2 at 3 h post bolus, and SAMe alone or with ethanol increased the levels of both H3K4 me2 and H3K9me2. By 12 h post bolus, both H3K4me2 and H3K9me2 were unchanged by ethanol

feeding but increased by SAMe with or without ethanol feeding [23]. These findings indicate that the epigenetic alterations in gene expression caused by acute ethanol were short lived but that SAMe induced changes persisted. H3K4me2 activates gene expression [36], which could explain the upregulation of ADH 1, CYP2E1, and ALDH 1 and 2 by SAMe [37]. It is concluded that SAMe prevents the liver from injury to some degree, by causing methylation of histones.

Betaine Used to Prevent Experimental ALD: In Vitro Studies

Betaine promotes the generation of hepatic S-adenosyl methionine levels and protects the liver from developing fatty liver in rats [8] and mice [38]. Betaine prevented lipid peroxidation injury in mice given a bolus of ethanol daily for 5 days [38] and in tissue of HepG2 cells that express CYP2E1 in vitro [39].

Using the in vitro model of Cederbaum [40], betaine was shown to totally prevent lipid peroxidation caused by ethanol. Carbonyl protein formation induced by ethanol was significantly reduced to control levels [39]. Betaine markedly reduced GPX, SOD2 Gadd45b, SESL1, and HSP70 levels to control levels or below [39]. This in vitro response to ethanol + betaine indicates that oxidative stress caused by ethanol is prevented by betaine.

Betaine Prevents Acute Ethanol-Induced Changes in Gene Expression In Vivo

To test the preventive effect of betaine on rats, rats were fed an ethanol bolus with and without the betaine supplement. Rats were then sacrificed 3 and 12 h post ethanol bolus with and without the betaine supplement [41]. Microarray analysis was done to determine the effect of betaine on the ethanol-induced changes in the expression of genes. Betaine supplement decreased the BAL achieved at 3 h post ethanol bolus, probably by the same mechanism as when SAMe was fed with ethanol [22, 23]. Both the 3- and 12-h heat maps of ethanol, betaine, and ethanol plus betaine fed rats differed from the controls and each other. The functional pathways showed a decrease in the changes in the expression of genes when betaine was fed with ethanol 3 h post ethanol bolus. This was most notable for the metabolic pathways. At 3 h post ethanol bolus, 50 % of the genes in the metabolic pathway (out of a total of 95 genes) were downregulated by betaine. At 3 h post ethanol bolus, betaine downregulated several genes in methionine metabolism, i.e., Cth, Gnmt, and Ahcyl 1. Other genes downregulated by betaine included Car 2 previously reported [41], cxcl 13, Prkci, Aldhlal, and 1gfbp2. At 12 h post ethanol bolus, betaine downregulated cxc1, Car 12, Scap, Cth, llgfbp1, and lepr. The gene expression change response seen after both ethanol and betaine boluses was global in nature involving almost all functional pathways. Also the response was changed between the 3 and 12 h post bolus. Betaine modified the effects of ethanol on gene expression at 3 h post bolus but had less effect at 12 h post bolus.

Betaine Prevents Experimental ALD in Rats Fed Ethanol Intragastrically for 1 Month

Betaine fed with other methyl donors protected the mouse liver when ethanol was fed intragastrically [16]. When rats were fed betaine, as a methyl donor with ethanol intragastrically for 1 month, betaine prevented fatty liver, liver enlargement, and liver inflammation [29]. The urinary alcohol cycle was

completely ablated by betaine as it was by SAMe [22]. The BALs were reduced by half (450 vs. 220 mg %) due to the increased elimination rate of ethanol. Betaine prevented the increase in serum ALT levels caused by ethanol ingestion.

Microarray analysis of the livers showed that the gene changes caused by ethanol feeding alone differed markedly from the rats fed betaine with ethanol and the betaine plus isocaloric glucose fed controls. The expression of 397 genes changed by ethanol differed from the livers of the ethanol plus betaine fed rats. Overall, the difference was highly significant (15-fold, $p<0.005$). Betaine prevented most of the functional pathway changes of gene expression caused by ethanol. The genes that were upregulated by ethanol and prevented by betaine included Lbp, Dapk 1, Gadd45b, Wnt 2, Lepr, Tlr 2 and 4, Tfngr 1, 1fngr 1, Tgfgb2, Tnfrs1b, Stat 3, Jak 3, Nos 3, Clh, and FAS. A marked increase in Tlr 4 mRNA and protein has been reported to be increased by ethanol by threefold, and this was prevented by SAMe [42]. In that study, Tnfa and Ifng were upregulated by ethanol and prevented by betaine.

Betaine feeding also induced changes in the metabolites choline, dimethylglycine (DMG), and betaine levels in liver tissue, serum, and urine. Choline levels were increased in the liver tissue in rats fed ethanol and betaine. DMG levels were decreased by ethanol, but adding betaine to the diet did not change this. Serum levels of choline were increased. Betaine prevented this.

Serum betaine levels were reduced by betaine fed with ethanol but not with ethanol alone or betaine alone. Urine levels of DMG were increased by betaine plus ethanol compared to ethanol alone. These changes show the complex nature of betaine metabolism and its effects on ethanol-induced changes.

Conclusion

SAMe and betaine, both methyl donors, are effective in preventing the early stage of experimental ALD. Betaine is more effective and less toxic. High doses of SAMe (4 g/kg) fed to the rat is fatal. One gram per kilogram SAMe increases the ALT after an ethanol bolus [23].

Both SAMe and betaine are antioxidants (Cederbaum, quercetin b). Both inhibit liver cell proliferation [43], SAMe, [44], DDC betaine [45]. Both reduce fatty liver caused by ethanol feeding, betaine more than SAMe. Both inhibit the molecular epigenetic cellular memory induced by ethanol feeding by methylating histones that silenced gene expression. The hope is to have clinical trials in which betaine is fed in order to prevent ALD.

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Chapter 37
Nutraceutical Potential of Indigenous Plant Foods and Herbs for Treatment of Alcohol-Related Liver Damage

Vaishali Agte and Upendra Raghunath Gumaste

Key Points

- It is a well-known fact that ingestion of ethanol from the alcoholic beverages results in formation of acetaldehyde, which is more toxic than ethanol and linked to most of the clinical effects of alcohol including alcoholic liver disease (ALD). Free radical-mediated oxidative stress is a major contributing factor in liver damage by chemical and environmental toxicants including alcohol. Several mono- and polyherbal preparations in the form of decoctions, tinctures, tablets, and capsules are reported in the literature on Ayurveda, an ancient medical science in India. There are also some foods which may have potential for management of ALD. It is of prime importance to explore such plant materials for antioxidant capacity and hepatoprotective action to validate their claims of having nutraceutical potential for ALD.
- We have screened various reported studies from literature during the last two decades covering herbs, foods, and individual molecules for their antioxidant potential as well as hepatoprotective action. Out of the 123 reports, the claims in literature for 45 herbs, 5 foods, and 15 isolated compounds find evidence as hepatoprotective agents through in vitro cell model and rat models and further supported by the levels of phenolics and antioxidant capacity.

Keywords Herbs • Alcoholic liver disease • Hepatoprotective agents • Functional foods

Introduction

Ingestion of ethanol from the alcoholic beverages results in formation of acetaldehyde by the enzyme alcohol dehydrogenase and then into acetic acid by acetaldehyde dehydrogenase. Thus, acetaldehyde is the first metabolic product of ethanol, as well as an intermediate in other metabolic processes,

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which is more toxic than ethanol and linked to most of the clinical effects of alcohol. It has been shown to increase the risk of developing cirrhosis of the liver, multiple forms of cancer, and alcoholism [1]. It is also known that acetaldehyde readily reacts with amines, and in the case of the exocyclic nitrogens of nucleosides, the primary product [R2>N–CH(CH3)–OH] has a very reactive hydroxyl group, which rapidly condenses with alcohols to give stable mixed acetals [R2>N–CH(CH3)–O–C2H5] at ambient temperature [2].

Epidemiological studies reveal that alcohol consumption is a risk factor for the cancer of the mouth, larynx, esophagus, and various other organs**.** Alcohol depletes most of the micronutrients in the body, which are necessary for energy, brain functions, sound nerves, and good digestion. Ethanol-induced oxidative stress appears to play a major role in mechanisms by which ethanol causes liver injury. Liver is an important site for zinc metabolism and also a target organ for alcoholic liver disease (ALD), which more commonly occurs with consumption of illicit liquor [3]. In long-term bioassays, liquor caused 22% total tumor incidence in male BALB/c mice and 28% in male Swiss mice [4].

The main cause of alcoholic liver disease (ALD) is chronic ingestion of alcohol. Major attention has been given to this condition in the last few years due to a wide range of serious illnesses associated with ALD. Many compounds are used experimentally to study hepatotoxicity in vivo and in vitro, among which three extensively studied molecules include acetaminophen (AA), ethanol, and carbon tetrachloride (CCl_4) because of their resemblance in the hepatotoxic effect with high reproducibility. Chronic ethanol consumption results in hepatic lipid accumulation due to utilization of ethanol as the preferred fuel instead of fat. It is also responsible for lipogenesis activated by altered NAD/NADH ratio and excessive formation of acylglycerol [5–7]. However, involvement of regulatory molecules such as PPAR-alpha or sterol regulatory element-binding protein 1 activation due to ethanol-mediated oxidative stress cannot be neglected [8]. Major histopathological changes observed due to intoxication of these molecules are loss of structural integrity of hepatocyte membranes [9], intracellular particles like lysosomes [10], deposition of fat, and enlargement of liver (hepatomegaly), where simultaneously there is also increase in protein content along with lipid [11]. In hepatomegaly, due to chronic ethanol consumption, there is an almost four to tenfold increase in liver volume [12]. Similarly, ethanol causes megamitochondria; alteration in microtubules; increase in microsomes, peroxisomes, lysosomes, and lipid bodies; decrease in surface areas of smooth endoplasmic reticulum (SER) and rough endoplasmic reticulum (RER) [13]; microvascular steatosis; and parenchymatous degeneration leading to atrophy in sinus hepaticus [14]. Moreover, mitochondrial membrane depolarization leads to alteration in permeability responsible for apoptosis [15]. Acetaldehyde (the intermediate metabolite of ethanol) and altered redox status stimulate collagen synthesis. Apart from lipid accumulation, chronic ethanol consumption is reported to cause inflammation, Kupffer and hepatic stellate cell activation and fibrosis [5–7, 16]. High HDL cholesterol, impaired cholesterol efflux capacity of HDL, and reverse cholesterol transport to the liver in individuals with chronic alcohol consumption further leads to higher incidence of cardiovascular disease in heavy drinkers [17]. LCAT is the enzyme which catalyzes formation of cholesterol ester and lysolecithin using cholesterol and lecithin as substrate and thereby maintains cell surface lipoprotein composition. In case of AA intoxication in male Wistar rats, level of LCAT was found to be reduced, leading to the increase in serum cholesterol and triglyceride [18].

Biochemical Changes in ALD

The most common feature of intoxication due to ethanol (EtOH), CCl_4, and AA is involvement of microsomal enzyme which converts these toxic agents into harmful metabolites. The p450 enzyme converts AA into reactive quinone imine (N-acetyl-P-benzoquinone imine), which further reacts with

thiols and depletes reduced glutathione (GSH), a major redox potential containing molecule of the cell [19–21]. This further leads to oxidative stress and activation of poly-ADP-ribose polymerase (PARP), which is responsible for conversion of NAD into ADP-ribose and nicotine. ADP-ribose forms protein-ADP-ribose and causes cells to undergo apoptosis [22, 23]. While in case of ethanol, three main pathways are responsible for its toxicity, which include cytosolic alcohol dehydrogenase (ADH), ER-based ethanol-oxidizing system, and catalase present in peroxisome. These together convert alcohol into acetaldehyde, acetic acid, superoxide, H_2O_2, hydroxyl radicals, and oxygen radicals [24, 25]. Use of transfected HeLa cells, HepG2, and stellate cells to study the genesis of alcohol-related toxicity has been reported [26].

Different toxic levels of EtOH have been observed in different cell culture studies, e.g., 60–80 mM for HepG2, 69–174 mM for human liver cells, and 30 mM for Chang liver cells. These indicate differences in metabolism of EtOH by ADH and/or induction of p450 enzymes [27]. On the other hand, liver p450 converts CCl_4 into trichloromethyl radical, which further reacts with oxygen to yield highly reactive and toxic trichloromethoxy radicals [28].

Even though in vitro experiments using primary human hepatocytes and adipocytes show that alcohol does not directly affect adiponectin release from adipocytes, high serum adiponectin levels (SAL) were found in patients with chronic excessive alcohol intake without having signs of advanced liver damage. It is predicted that mediators that are altered in the serum are responsible for such effect [29]. Similarly, it was also found that rats were not accompanied by inflammation and NF-kB or ALP activity alteration [30], which are chronically exposed to ethanol, and suggests possibility of adaptation and change in steady state of redox state [6]. However, in alcoholic liver disease, liver fibrosis can also occur without having inflammation and both acetaldehyde and transforming growth factor are involved in such process [7, 31].

The levels of IL-10, TNF-alpha, IFN-gamma, TGF-beta1, and VEGF-A were found to be increased, while IL-4 level was found to be reduced in chronic ethanol consumption [32]. One of the major cellular enzymes inhibited by ethanol consumption has been methionine synthase, which is involved in remethylating homocysteine. However, in some species, ethanol increases activity of alternative enzyme betaine homocysteine methyltransferase, which catalyzes same reaction by utilizing hepatic betaine to form methionine and maintain levels of S-adenosylmethionine, the key methylating agent. But chronic ethanol exposure adversely affects this alternate pathway as well, further leading to increase in two toxic metabolites, S-adenosylhomocysteine and homocysteine. Therefore, betaine, by restoring S-adenosylmethionine level, reverses steatosis, prevents apoptosis, and reduces both damaged protein accumulation and oxidative stress associated with alcoholic abuse [33].

Nutraceutical Effect of Herbs

Herbs play a vital role in the management of various liver disorders. Numerous medicinal plants and their formulation are used for liver disorders in ethnomedical practice as well as traditional system of medicine in India, the Ayurveda. In the absence of a reliable liver-protective drug in the modern medicine, a number of medicinal preparations in Ayurveda are recommended for the treatment of liver disorders (Table 37.1).

Most of the in vivo studies for the assessment of hepatoprotective effect of herbal preparations have been conducted in Wistar rats. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), and bilirubin are prominently considered as biomarkers which are expected to change positively for the herb to be an effective hepatoprotective agent. These enzymes are mainly present in liver, and any damage to tissue is responsible for them to appear in serum. At the same time in hepatic tissue, certain parameters like reduced

Table 37.1 List of foods for hepatoprotective action with their active molecules

No	Name of plant/herb	Compounds isolated	Hepatotoxic agent used	Efficacy	Ref no.
1	*Daucus carota* L. (common name "carrot")[a]	α-, β-, and γ-Carotene, lycopene, cryptoxanthin lutein, abscisic acid, trisporic acid, β-apo-carotenals, crocetin, and many common polar carotenoids, e.g., violaxanthin	CCl_4	Pretreatment	[91–93]
2	*Trigonella foenumgraecum* (common name Fenugreek)[m]	Vitexin, tricin, naringenin, quercetin, and tricin-7-O-beta-D-glucopyranoside	EtOH	Simultaneous treatment	[94]
3	Propolis (mixture of gums, resins, and balms)[e]	Cinnamic acid, benzoic acid and their esters, substituted phenolic acid and ester, flavonoid glycones, bee wax, and caffeic acid phenylethyl ester	econazole	Posttreatment	[95]
4	*Murraya koenigii* (commonly known as curry tree/curry leaf)[a]	Carbazole alkaloid and tannin	EtOH	Simultaneous treatment	[96]

[a]aqueous extract, [m]methanol extract, [e]ethanol extract

glutathione (GSH), glutathione peroxidase (GPx), glutathione S-transferase (GST), catalase, superoxide dismutase (SOD), malondialdehyde (MDA), or thiobarbituric acid-reactive substances (TBARS) are considered so as to assess alleviating effect of herb on tissue. These parameters are related to redox potential of tissue and oxidative stress, the major consequences of ethanol toxicity. High dosage of *Niuchangchih* showed a hypercholesterolemic effect and reduced hepatic lipid content, an effect partially attributed to downregulation of 3-hydroxy-3-methylglutaryl-CoA reductase, sterol regulatory element-binding protein-1c, acetyl-CoA carboxylase, fatty acid synthase, malic enzyme gene expressions, and more excretion of cholesterol and bile acid in alcohol-fed rats. There was also an upregulation found with respect to low-density lipoprotein receptor and peroxisome proliferator-activated alpha gene expression [34].

In order to analyze ethanol toxicity, simultaneous treatment of ethanol and extract is usually done. Though hepatoprotective effect of alcoholic extract of *Cassia occidentalis* L. (commonly known as "Kasondi") is evident by serum enzymes, cholesterol, and total lipid levels, this extract failed to restore serum AST [25]. Positive correlation with respect to tissue parameters was found in case *Hemidesmus indicus* extract complementing with negative correlation with respect to serum parameters along with prevention of liver fat accumulation, restoration of liver glycogen content, improvement in weight gain, and liver to body weight ratio indicating its action as normalization mechanism [35]. Other promising herbal extracts include methanol extract of *Phyllanthus amarus*, which also shows upregulation of expression of gamma-glutamylcysteine synthetase, the rate-limiting enzyme in biosynthesis of GSH [36]. There was also an increase in hepatic triglyceride and calcium-dependent phospholipase degradation resulting into increase in free fatty acid due to AA intoxication. Pretreatment of *Premna tomentosa* not only resulted in decrease in serum and liver free fatty acid but also restored serum LDL, VLDL, cholesterol, and triglyceride levels along with improvement in LCAT activity [18].

When CCl_4 intoxication is used as model, usually pretreatment of extract is performed before giving toxic dose of CCl_4. Certain well-reported hepatoprotective herbal extracts include 80% methanol extract of *Artemisia maritima* L. (locally known as "Afsanteen-ul-bahr") [9] which also shows protection against AA intoxication, while others include ethanol water extract (1:1) of *Lawsonia alba* (commonly known as Mehndi) [37] and aqueous extract of *Woodfordia fruticosa Kurz* (common name Dhataki) [38]. Similarly, pretreatment of both aqueous and ethanol extract of *Boerhaavia diffusa Linn*

(common name Pigweed or Hogweed) shows hepatoprotective activity in AA intoxication [21], while extract of *E. fusiformis* shows hepatoprotection in rifampicin intoxication [39]. It has been shown that TNF-alpha and other proinflammatory cytokines are increased in alcoholic liver disease [40–42]. D-galactosamine (D-GalN) intoxication resembles viral hepatitis, and ethanol extract of *Phyllanthus rheedii Wight* (locally known as Kaattukeezharnelli) shows hepatoprotection by downregulating TNF-alpha and TGF-beta [43].

Oxidative stress plays pivotal role in ALD. Therefore, heme oxygenase 1 (HO-1) has received considerable attention because of its key role as an antioxidant enzyme. *Ginkgo biloba* (EGb) extract resulted into significant increase in HO-1 mRNA and protein expression under chronic ethanol exposure in Sprague–Dawley rats. It is supposed that HO-1 may directly scavenge CYP2E1-derived reactive oxygen species (ROS) due to same intracellular location [14]. On the other hand, *Mangifera indica* stem bark aqueous extract (MSEB) showed hepatoprotective activity in hepatocytes isolated from Sprague–Dawley rats in both CCl_4 and EtOH intoxication [44]. When similar studies were performed in vitro, 80% methanol extract of fenugreek showed protective effect and abolished apoptotic nuclei in ethanol-toxicated Chang liver cells [45].

Methanol extract of *Ocimum gratissimum*, aqueous root extracts of Pelargonium reniforme *Curtis* (Geraniaceae), and leaf extract of Phyllanthus niruri have been reported to protect liver against alcohol toxicity and prevent the release of the liver marker enzymes in Wistar rats [46–48]. *P. niruri* also prevented ΔPUFA-induced hyperlipidemia, while extract of *Magnolia officinalis* completely inhibited maturation of sterol regulatory element-binding protein-1c in the liver and provided protection [49]. Methanolic and aqueous extracts of the bark and leaf of *Soymida febrifuga* (Roxb.) *A. Juss*. (Meliaceae) significantly reduced ethanol-induced cytotoxicity in HepG2 cells [50]. Extract of fruit pericarp of *S. mukorossi* (commonly known as Ritha or Aritha) and rhizome of *R. emodi* (commonly known as Indian or Himalayan rhubarb) were tested for their hepatoprotective action on primary hepatocyte culture (isolated from Wistar rats) and in vivo using Wistar rats. Levels of LDH and GPT in medium of hepatocytes were reduced when extract was cotreated with CCl_4. Similarly, serum enzyme levels were also found to be reduced [51].

Certain polyherbal drug preparations were also studied for their hepatoprotective action. Liv52 activated PPAR-gamma and inhibited ethanol-mediated TNF-alpha induction in HepG2 cells, suggesting hepatoprotective potentials [52], while another herbal drug *Normeta*, apart from reducing effect on serum ALT, decreased serum iron level, suggesting iron-chelating activity and hence might be helping in decreasing the toxicity due to increased level of iron occurring due to alcohol consumption [53].

The fat-free ethanol (95%) extract of aerial parts of *Phyllanthus reticulates*, aqueous and ethanol extracts of *Pergularia daemia*, ethanol seed extracts of *S. marianum,* and flowers extract of *Vitex trifolia* (Verbenaceae) showed hepatoprotection against carbon tetrachloride-induced toxic damage [54–57]. Similarly, methanol extracts of *Ficus carica* (leaves and fruits) and *Morus alba* (bark) showed potent antioxidant and hepatoprotective activity in CCl_4-intoxicated rats [58]. Other studies, which show promising hepatoprotective activity against CCl_4-intoxicated Wistar rats, include ethanolic extract of *Hibiscus hispidissimus*, *A. fertilisima*, and *P. daemia* [59–61]. On the other hand, hot water extract of *Taraxacum officinale* showed hepatoprotection in ethanol-intoxicated ICR mice [62], while ethanol extract of *Arachniodes exilis* showed protection in CCl_4-intoxicated Kunming albino mice [63].

Nutraceutical Effect of Foods

Some of the promising fruit/root vegetables (Table 37.1), which are studied in mice and showed hepatoprotective effect, include aqueous extract of carrot in Swiss albino mice [10] intoxicated with CCl_4 and chest nut extract in C57BL/6 mice [64] in ethanol intoxication. Carrot extract also showed

lower level of hepatic acid phosphatase and acid ribonuclease level which indicate improvement in lysosomal integrity, while chest nut extract showed significant reduction in hepatic and plasma triglyceride, cholesterol apart from inhibition of mRNA, and protein expression of CYP2E1, thereby reducing ROS production.

Ethanol extract of propolis (PEE) which is a mixture of gums, resins, and balms showed remarkable anti-lipid peroxidation activity and hepatoprotective effect in male Wistar rats as evidenced by levels of ALT and AST in serum against toxicity induced by acute administration of econazole, an antifungal drug [65]. Fermented sea tangle (FST) showed hepatoprotection against ethanol and carbon tetrachloride-induced toxicity in rats [66], while oral administration of dried earthworm powder (Perionyx excavates) for 42 days reversed tissue antioxidant enzymes towards normalcy, which were reduced due to ethanol toxication [67].

Nutraceutical Effect of Individual Molecules (Nutrients, Metabolites)

Several isolated molecules were also considered for their effectiveness in hepatoprotective action (Table 37.2). Pretreatment with carotenoid as lutein in Wistar rats (3,3′-dihydroxy-beta, eta-carotene) before ethanol, CCl_4, and paracetamol intoxication showed reduction in serum enzyme markers and improved tissue redox potential as compared to respective vehicle controls. U.S. FDA has approved this phytochemical as "generally regarded as safe" for nutritional supplement. Lutein may also be responsible for inhibition of cytochrome p450 enzyme, which acts as major source of ROS [68]. Another promising molecule studied in Wistar rats is ferulic acid (FA), which chemically has 3-methoxy, 4-hydroxyl, and carboxylic acid group adjacent to unsaturated C–C double and provides attack site for free radicals, therefore shows anti-lipid peroxidation activity and hepatoprotective effect when challenged with ethanol and PUFA together [69]. Similarly, coadministration of ethanol and (+)-cyanidanol-3 in CFY male adult rats showed significant reduction in the extent of liver cell enlargement and alteration in cell components of the hepatic lobule [70] and restored mitochondrial morphology [13]. In another study in NMRI female mice, use of polyADP-ribose polymerase (PARP) inhibitor such as nicotinic acid amide has been shown to be effective in preventing GSH depletion and liver damage caused by AA. The main reason for such effect is the prevention of activation of PARP and formation of ADP-ribose and nicotine [20].

Another widely considered and well-studied promising phytoalexin molecule is *trans*-resveratrol (3,5,4′-trihydroxystilbene). It belongs to hydroxystilbene subgroup of polyphenols [71]. Phenolic compounds present in most of the natural ingredients are found to be antioxidants. The hydroxyl phenoxy group of phenolic compound donates their electron to free radicals and quenches them and in turn forms stable quinone methide intermediate which is excreted via bile [72]. Prominent sources of *trans*-resveratrol are berries of grapevine (*Vitis vinifera* and *V. labrusca* L.) and red grape wine [73–75]. Increased deposition of iron, acetaldehyde, and its role in formation of adduct with DNA and inhibition of DNA repair system together contribute in ethanol-mediated DNA damage process. In this context, significant reduction in oxidative stress marker MDA and 8-hydroxy-2′-deoxyguanosine (8-OHdG; marker for oxidative DNA damage) after red wine treatment as compared to ethanol group is a noteworthy fact. However, hepatic conjugation of red wine with GSH could additionally contribute to the lower hepatic content of GSH found in red wine-treated rat resulting in reduced GSH/GSSG ratio [30, 76]. Derivatives of *trans*-resveratrol, *trans*-piceatannol, *trans*-rhapontigenin, and *trans*-deoxyrhapontigenin are reported from *Rheum rhaponticum* L., *R. rhaponticum* [77]. Chronic ethanol administration caused liver damage as evidenced by collagen accumulation, fatty change, and necrosis in naïve male inbred BALB/c/Bkl mice. *trans*-Resveratrol showed remarkable hepatoprotective effect, and the number of Kupffer

Table 37.2 List of herbs for hepatoprotective action with their active molecules

No	Name of plant/herb	Compounds isolated	Hepatotoxic agent used	Efficacy	Ref no.
1	*Premna tomentosa* (common name "Krishnapalai and Pudangainari")[m]	D- and DL-Limonene, β-caryophyllene, cadalene-type sesquiterpene, sesquiterpene tertiary alcohol, and diterpene	AA	Pretreatment	[97]
2	*Lawsonia alba* (common name "Mehndi")[e/a]	β-Sitosterol glucosides, flavonoids, quinoids, naphthalene derivatives, luteolin, betulin, lupeol, garlic acid, coumarins, xanthones and phenolic glycosides, and two pentacyclic triterpenes (hennadiol and 20S)	CCl_4	Pretreatment	[98–104]
3	*Ginkgo biloba*[#]	Terpenes and flavonol heterosides	EtOH	Pretreatment	[14]
4	*Hemidesmus indicus* (Asclepiadaceae)[e]	Hemidesmol, hemidesterol, saponins, and 2-hydroxy-4-methoxy benzoic acid	EtOH	Posttreatment	[35, 105]
5	*Woodfordia fruticosa kurz* (common name Dhataki)[p/e/c/a]	Oenothein B and woodfordin A, B, and C, isoschimacoalin-A, and five oligomers-woofordin E, F, G, H, I, quercetin-3-*O*-(6′′-galloyl)-B-d-galactopyranoside, quercetin-3-*O*-(6′′-galloyl)-B-d-glucopyranoside, quercetin-3-*O*-alpha-L-arabinoside, quercetin-3-*O*-oxylopyranoside, myricetin-3-*O*-6′′-*O*-galloyl)-B-d-galactopyranoside, and myricetin-3-*O*-arbinopyranoside	CCl_4	Pre- and posttreatment	[106–108]
6	*Taraxacum officinale* (known as dandelion)[ha]	Quercetin, luteolin, and luteolin-7-O-glucoside	EtOH	Cotreatment	[109]
7	*Arachniodes exilis*[e]	Aspidin BB, isoaspidin BB, isoaspidin AB, araspidin BB, 4-methyl-2-butyl-3,5-dihydroxyphenol, epicatechin, eriodictyol, arachniodesin A, arachniodesin B, procyanidin B2, miscanthoside, eriocitrin, eriodictyol-7-O-β-d-glucopyranuronide, luteolin, luteolin-4′-O-β-d-glucopyranoside, lutinolin-7-O-rutinoside	CCl_4	Pretreatment	[110]
8	Chestnut (Castanea crenata)[m] inner shell	Scoparon and scopoletin	EtOH	Cotreatment	[64]
9	*Euphorbia fusiformis Buch-Ham. ex D. Don*[e]	Diterpenes, ellagic glycoside, euphol	Rifampicin	Pretreatment	[39]
10	*Ficus carica*[m]	Umbelliferone, caffeic acid, quercetin-3-O-β-d-glucopyranoside, quercetin-3-O-α-l-rhamnopyranoside, and kaempferol-3-O-α-l-rhamnopyranoside	CCl_4	Pretreatment	[58]
11	*Magnolia officinalis*[e]	Honokiol and magnolol	EtOH	Posttreatment	[49]
12	*Hemidesmus indicus*	2-Hydroxy-4-methoxy benzoic acid	EtOH	Cotreatment	[111]

[#]Commercially available

[a] aqueous extract, [m] methanol extract, [e] ethanol extract, [a/e] aqueous/ethanol extract, [ha] hot aqueous extract, [p/e/c/a] petroleum ether/chloroform/ethanol/aqueous extract

cells also increases after treatment of *trans*-resveratrol and *R. rhaponticum*. Kupffer cells play an important role in the normal physiology and participate in the acute as well as chronic responses to toxic compounds [78].

Upregulation of HO-1 expression, a known adaptive response/enhanced resistance against various oxidative stress, occurs through activating nuclear factor erythroid 2-related factor (Nrf2), and naturally occurring quercetin as well as other flavonoids and polyphenols follow this Nrf2-mediated pathway [79, 80]. Quercetin shows concentration-dependent inhibition of LDH and AST leakage from ethanol-intoxicated human hepatocytes. Though quercetin and ethanol evidently promoted Nrf2 translocation into nuclei, ERK pathway is mainly responsible for quercetin-derived HO-1 induction in concentration-dependent manner, and p38 is mainly responsible for ethanol-stimulated HO-1 induction [81]. Quercetin also inhibited H_2O_2-induced NF-kB transcriptional activation and DNA strand breaks. This is important because activation of NF-kB and activator protein 1 in Kupffer cells is responsible for upregulation of fibrogenic cytokine genes, which stimulate hepatic stellate cells [16, 30].

Bilirubin, a distant metabolite formed in HO-1-mediated pathway, is also considered to be a lipophilic antioxidant, which lowered ethanol-induced lipid peroxidation but failed to inhibit GSH depletion, indicating limited cytoprotection. However, CO, another metabolite of HO-1, might mediate defensive action through inactivating CYP2E1 enzyme, and studies on human hepatocytes show that HO-1 induction downregulates ethanol-dependent CYP2E1, suggesting alternative hepatoprotective mechanism of quercetin [82].

Curcumin, another widely studied powerful antioxidant, when analyzed for hepatoprotective effect showed that pretreatment is responsible for increase in GSH and reduction in LDH and AST release from primary hepatocytes isolated from Sprague–Dawley rat. It also inhibited MDA production with dose- and time-dependent induction of HO-1, when enzyme activity reached a peak at 15 uM and at 1 h before ethanol administration [63]. Hepatoprotective effect of whole extract of *Phyllanthus amarus*, when tested on primary hepatocyte culture from Wistar rats, is more prominent as compared to isolated phyllanthin alone, which shows involvement of other phenolic compounds inherent in extract [83]. Ethanol treatment increased GGT level. However, study using HepG2 cells showed that (−)-epigallocatechin (EGCG) improved cell viability and was a strong inhibitor of GGT, which catalyzed extracellular GSH breakdown and appeared to mediate ethanol toxicity. However, EGCG decreased intracellular GSH significantly and also failed to preserve GSH pool upon ethanol exposure which suggests that intracellular GSH depletion may not be the primary cause of cell death [84, 85].

β-Carotene and S-adenosylmethionine supplementation can prevent ethanol-induced liver damage [86, 87] but later exerts protective effect by reducing serum TNF-alpha, TGF-beta1 levels, lipid peroxidation, and their expression in the liver. Kolaviron (KV), a biflavonoid complex from *Garcinia kola* seeds, was responsible for inhibition of hepatic LPO and ameliorated SOD and GST activities in Wistar rats [88]. Alcoholic liver steatosis and damage is mainly attributed to the disequilibrium in NAD/NADH ratio and excessive ROS generated because of chronic ethanol ingestion and ethanol metabolism [89], and another promising molecule, caffeine, significantly reduced serum and tissue inflammatory cytokines, tissue lipid peroxidation, steatosis, immigration of inflammatory cells, and mRNA expression of lipogenic genes and inhibits necrosis of hepatocytes [90].

In the present study, we have reviewed hepatoprotective activities of 24 herbs and 20 foods as evident through in vitro and in vivo antioxidant potential and levels of marker enzymes and molecules of liver function and antioxidant defense (Table 37.3). Further systematic human studies on individuals exposed to various degrees of alcohol intoxication by use of single or multiple herbs as adjunct therapy may be needed.

Table 37.3 Various experimental models used for testing hepatoprotective action

Model used	Dose of alcohol/ toxicant	Herb and dose	Brief findings	Reference
Male Wistar rats	20% 5 g/Kg	*Hemidesmus indicus* 200 mcg/kg	Significant elevation in the activity of enzymic and nonenzymic antioxidants in plasma, erythrocytes, and liver and also increased levels of plasma and liver vitamin C and alpha-tocopherol	[111]
Rats and patients with acute viral hepatitis	Galactosamine	Picrorhiza kurroa 200 mg/kg p.o.	Biological plausibility of efficacy of Pk supported by clinical trial in viral hepatitis, hepatoprotection in animal model	[112]
Rats	CCl_4	*Solanum nigrum* LINN	The ethanol extract showed remarkable hepatoprotective activity.	[113]
Mice		Emblica officinalis Gaertn (Euphorbiaceae)	It may potentially ameliorate the hyperthyroidism with an additional hepatoprotective benefit	[114]
Rats	Carbon tetrachloride	Amalkadi Ghrita (AG) 100 and 300 mg/kg, p.o.	AG prevented CCl_4-induced elevation of levels of serum GPT, GOT, ACP, ALP, and bilirubin	[115]
Male and female Fischer 344 rats	Nil	Flax seeds	Dietary 10% flax chow is without long-term effect on growth, development, and behavior, is nontoxic, and may be hepatoprotective	[116]
Rats	Acetaminophen (APAP)	*Asteracantha longifolia* (AL) seeds	Pretreatment with AL extract prevented APAP-induced acute liver damage	[117]
Rats	CCl_4 (0.7 ml/kg, i.p.)	Haridradi ghrita (50, 100, 200, and 300 mg/kg)	Significant hepatoprotective action of H. ghrita in CCl_4 damaged rats	[118]
Rat liver homogenates		Achyrocline satureioides (Lam.) DC. (Compositae)	Extracts of A. satureioides possess significant free radical scavenging and antioxidant activity in vitro	[119]
Freshly isolated rat hepatocytes and rats	Paracetamol and tertiary-butyl hydro peroxide	Tetracera loureiri	T. loureiri had free radical scavenging properties and may be of potential therapeutic value in some liver disorders	[120]
Mice	Carbon tetrachloride	*Artemisia campestris* extract was given intraperitoneally	A. campestris scavenges radicals formed by CCl_4 treatment resulting in protection against CCl_4-induced liver toxicity	[121]

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Chapter 38
Alcohol and Nutrition as Risk Factors for Chronic Liver Disease

Stefano Bellentani, Claudio Tiribelli, and Giorgio Bedogni

Key Points

- The great burden of chronic liver disease (CLD) in forthcoming years is expected to come from nonalcoholic fatty liver disease (NAFLD) and especially from its progressive form known as nonalcoholic steatohepatitis.
- The burden of NAFLD goes in parallel with the burden of obesity and type 2 diabetes, and NAFLD is emerging as an independent predictor of cardiometabolic disease and liver-related and general mortality.

Keywords Epidemiology • Risk factors • Alcohol • Nutrition • Liver disease • Nonalcoholic fatty liver disease • Nonalcoholic steatohepatitis

Introduction

The liver is the largest gland of the human body and plays a central role in the metabolism of nutrients. Hundreds of biochemical reactions take place in the liver, explaining its susceptibility to metabolic stressors. However, the natural history of metabolic liver disease has started to be unraveled only recently. For instance, it is now known that nonalcoholic fatty liver disease (NAFLD), which has long been considered a benign and nonspecific response of the liver to different inflammatory and metabolic factors, can progress to fibrosis and cirrhosis when associated with necroinflammation [1–3]. The burden of NAFLD goes in parallel with the burden of obesity and type 2 diabetes, so that NAFLD

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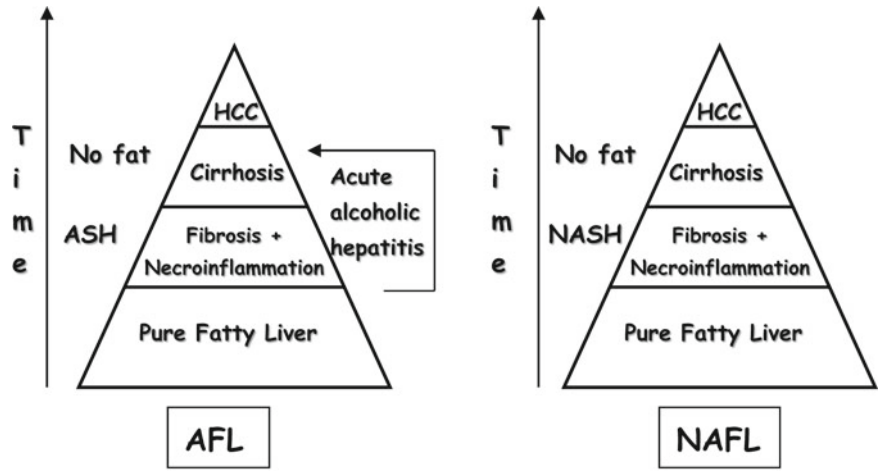

Fig. 38.1 Common natural history pathway of alcoholic fatty liver (AFL; *left panel*) and nonalcoholic fatty liver (NAFL; *right panel*). Liver steatosis (pure fatty liver) induced either by alcohol or other nutritional and metabolic causes may progress to alcoholic or nonalcoholic steatohepatitis (ASH and NASH), then to cirrhosis and hepatocellular carcinoma (HCC)

is currently considered the hepatic manifestation of the metabolic syndrome [1–3]. More importantly, NAFLD is emerging as an independent predictor of cardiometabolic disease and liver-related and general mortality [3–8]. As shown in Fig. 38.1, fatty liver (FL) may progress to fibrosis and cirrhosis both in alcoholic liver disease (ALD; left panel) and in nonalcoholic liver disease (NALD; right panel). Fibrosis leading to cirrhosis can accompany any chronic liver disease (CLD) associated with hepatobiliary distortion and/or inflammation [9, 10]. The main causes of fibrosis, cirrhosis, and hepatocarcinoma (HCC) worldwide are presently hepatitis B (HBV) and C (HCV) virus infections [11, 12]. Alcohol consumption is another important cause of CLD at present but may be a less important risk factor in coming years. Indeed, the great burden of CLD in forthcoming years is expected to come from NAFLD and especially from its progressive form known as nonalcoholic steatohepatitis (NASH) [1].

Alcohol as Risk Factor for CLD

How Much Alcohol Is Safe?

The best known and studied predictor of CLD progression is continued alcohol abuse. Patients with liver fibrosis who continue to drink alcohol can be virtually certain of the progression of their liver disease, but only 6–30% of heavy drinkers will develop CLD [13, 14]. The most likely explanation for this fact is that the relation between alcohol consumption and CLD is multifactorial. Despite the commonly held mantra "No alcohol, no ALD," epidemiological data suggest that alcohol consumption might not be the only determinant of ALD. The search for potential risk factors besides alcohol abuse has been extensive but mostly inconclusive. Moreover, it has been difficult to determine whether a greater number of risk factors predispose heavy drinkers to more severe forms of ALD [15–28]. Clinical observations suggest a wide individual susceptibility to ALD [29–33]. On the other hand, the "safe alcohol dose," that is, the amount of alcohol which separates individuals with no or minimal risk of liver damage from those at higher risk, is highly variable depending from the study population and the study design [29]. Dose–response curves show that that the risk of developing cirrhosis increases exponentially with the amount of alcohol ingested during lifetime [29–35]. In this respect, an important

question that most persons ask their doctor and that often remains answered is: "How much alcohol is safe for me?" When some years ago we tried to determine what is the safe daily dose or the safe lifetime dose of alcohol in a healthy subject, we found estimates ranging from 20 to 80 g of alcohol/day for 10–12 years. This wide range of apparent safety was at least partly due to the fact that most of the studies were retrospective and performed in samples not representative of the general population. Other problems were suboptimal measurement of alcohol intake [36] and lack of control groups. [14, 29–34, 37–40]. Also, to answer the question of the safe alcohol dose, our group started the so-called Dionysos Study in the early 1990s [13, 18, 41], which was followed by a 10-year follow-up [42, 43] and by the Dionysos Nutrition and Liver Study [44, 45]. The Dionysos Study is an ongoing study performed in two towns of Northern Italy, Campogalliano (Emilia Romagna) and Cormons (Friuli Venezia Giulia). These towns were chosen because they had similar demographic and economic features but different drinking and dietary habits. Causes of CLD such as viral-induced and drug-induced liver damage were excluded. Particular care was taken to employ reliable measurements of alcohol consumption: a semiquantitative color-illustrated food questionnaire in the first edition [18, 43] and a food diary in the second edition [45]. ALD was operationally defined as a persistent alteration of blood markers for alcohol abuse or hepatocyte necrosis (alanine aminotransferase, aspartate aminotransferase, γ-glutamyl-transferase, mean corpuscular volume, and platelet count). Patients with any clinical sign of liver disease or an abnormal blood test underwent liver ultrasonography and, when necessary, liver biopsy, to reach a final diagnosis. In the Dionysos Study, the threshold of safe alcohol consumption was 30 g/day for both sexes [18]. No significant risk for CLD was present up to this level, but after this level, the risk of CLD increased with the amount of daily alcohol intake. Alcohol abusers, operationally defined as individuals who drunk more than 120 g/day of alcohol, had a risk of cirrhosis 60 times higher than alcohol abstainers [13]. Thus, according to the Dionysos Study, the safe dose of alcohol that an apparently healthy individual can drink is 30 g/day, that is the equivalent of 3 standard drinks per day or 21 drinks per week. This value is similar to that obtained in the longitudinal Copenhagen City Heart Study (7–13 drinks per week for women and 14–27 drinks per week for men) [39] and is very close to the threshold level conventionally used to separate NAFLD from alcoholic fatty liver [46]. We advise however caution when teaching this to patients because of the high variability in how "drinks" are measured [47]. The Dionysos Study showed also that CLD does not develop until lifetime alcohol ingestion reaches 100 kg and that the effects of alcohol intake on the liver are independent from body mass index (BMI) and the kind of alcoholic beverage (wine, beer, spirits) [13, 18].

Epidemiology of Alcohol-Induced Chronic Liver Disease

The prevalence, incidence, and natural history of alcohol-induced CLD in the general population are largely unknown because most of the available data were obtained from retrospective studies performed in hospitalized patients. Investigating hospital patients not only gives a potentially misleading picture of the "tip of the iceberg" but also carries the risk of inferring a much higher burden of disease if these data are wrongly extrapolated to the general population [43, 48]. In the general population of the Dionysos Study, the prevalence of cirrhosis was 1.1%, that is, three times the value reported by mortality registers and hospital data. Most cirrhotic individuals were asymptomatic. Forty-percent of the cases of cirrhosis were alcohol-related, for an overall prevalence of 0.42%. After exclusion of HBV and HCV infections, prevalent liver damage was estimated to be 17.8%. The prevalence of alcohol-induced liver damage was 1.1%, while that of "pure" alcoholic cirrhosis was 0.5%; of notice, only 10% of these patients were symptomatic. The Dionysos Study allowed to better define the "iceberg phenomenon" of CLD (Fig. 38.2). Starting from a prevalence figure of 500 every 100,000 subjects for alcoholic cirrhosis in the general population, 50 every 100,000 cases are symptomatic and in need of medical support while 11 every 100,000 cases die yearly. Importantly, the prevalence of symptomatic alcoholic cirrhosis in the general population is 45 times higher than the one estimated by mortality registries [18].

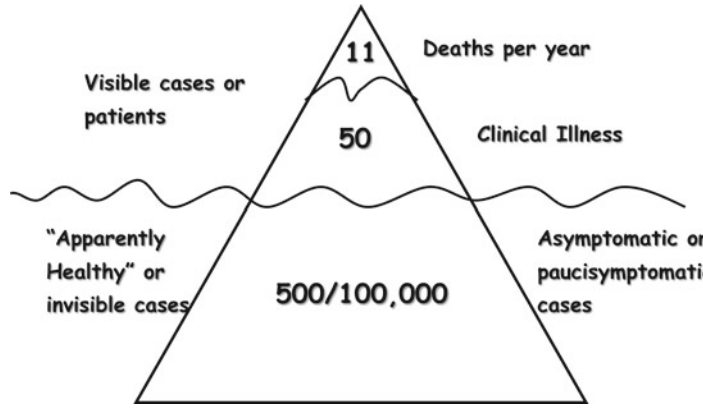

Fig. 38.2 The "iceberg" phenomenon. Prevalence and natural history of alcoholic cirrhosis in the general population of two towns in Northern Italy (Dionysos Study 6,23,38): from healthy subjects to bedside

Drinking Habits and Pattern of Drinking: Do They Influence the Risk of CLD?

Others questions frequently asked from patients to their doctors are "What is the safest time of day to drink?" and "What kind of beverages should I choose?" Some studies have shown that a sustained alcohol intake induces ALD more strongly than binge drinking [33]. This has been attributed to the possibility that alcohol binging might give liver cells a chance to recover at (least in part). However, other studies in rats fed a choline-deficient diet to induce steatosis showed that repeated whiskey binges promote more liver injury [49]. A sustained alcohol intake is more likely to produce inadequate food intake and malnutrition than binging or social drinking, and malnutrition clearly aggravates ALD. A number of studies, derived in part from alcohol abuse treatment programs, suggest that heavy drinkers with cirrhosis have a less severe pattern of alcohol dependency and perhaps less psychosocial stigmata than heavy drinkers without cirrhosis [50, 51]. An interesting observation was made by Gronbaek et al. [52] who, in confirming the known association between alcohol intake and the risk of upper gastrointestinal tract malignancies, noted that there was a carcinogenic effect for beer and liquor but not for wine. This may be partly due to the protective effect of resveratrol, present in wine but not in beer and liquor [53, 54]. Two recent studies confirmed these findings. Roizen et al. evaluated the mortality for alcoholic cirrhosis in the USA during the last 50 years and found a significant association with the consumption of liquor but not with that of other alcoholic beverages [55]. Another study by Becker et al. reported a lower risk of developing cirrhosis in wine drinkers as compared to liquor and beer drinkers [56]. However, these studies are contradicted by others. Guallar-Castillón et al. showed that moderate drinking of beer spirits may be just as "healthy" as wine drinking and that it is the overall quantity of alcohol consumed rather than the type of alcoholic beverage that has the greatest impact on health [57]. More research is needed to reconcile, if possible, these discrepancies. The Dionysos Study showed that, in addition to the total amount of alcohol ingested, the pattern of drinking is a determinant of ALD [13, 18]. For equal amounts of alcohol, individuals who drink at mealtime and outside mealtime had an incidence of ALD (including cirrhosis) three to five times higher than that of the individuals drinking it only at mealtime. The increased risk starts to be significant in heavy drinkers from 50 years of age. Furthermore, while the type of alcoholic beverage *per se* had no apparent effect on the incidence of ALD, the use of multiple kinds of beverages (wine, beer, and liquor) was associated – within the same range of total alcohol consumption – with a higher incidence of ALD and cirrhosis [13].

Genetic Factors: Are They Involved in the Progression of ALD?

Several studies have linked ALD with different genes, such as those encoding for alcohol dehydrogenase (ADH2, ADH3) and aldehyde dehydrogenase (ALDH2) as well as those encoding for the cytochrome P4502E1 (CYP2E1) [19–26]. However, results are often conflicting possibly because of selection bias and absence of a gold-standard diagnosis of ALD. The Dionysos Study helped in shading some light also on this complicated issue. The distributions of nine different polymorphisms in three genes involved in alcohol metabolism (ADH2, ADH3, and CYP2E1) were investigated among drinkers reporting comparably high amounts of ethanol intake (more than 120 g/day for more than 10 years) but differing for the presence or absence of clinical and biochemical signs of liver damage. In the inhabitants of Campogalliano, the C2 allele in the promoter region of the CYP2E1 gene had a frequency significantly higher in heavy drinkers with cirrhosis as compared to healthy heavy drinkers. In Cormons, whose inhabitants have different genetic background, a prominent association between ALD and homozygosity for allele ADH3*2 of ADH3 was observed, with a prevalence of 31% and 7% in heavy drinkers with or without ALD, respectively. These results suggest that the presence of either at least one allele C2 of cytochrome P4502E1 or of the homozygosity for the ADH3*2 allele is a predisposing factor for the development of ALD in the Dionysos population. The identification of two genetic polymorphisms potentially predisposing to ALD reinforces the notion that ALD is a polygenic disorder, as recently shown also for the Danish general population [58].

Gender Differences: Are Women at Greater Risk for ALD?

Previous studies have shown that the risk of alcoholic cirrhosis rises much more steeply in females than in males at increasing levels of alcohol intake [59, 60]. It has also been reported that clinical liver disease develops after a shorter period of alcohol intake in women [61]. Pharmacokinetic studies have shown that blood ethanol levels are higher in women than in men after ingestion of the same quantity of alcohol, and this is attributed to a smaller distribution volume or to a lower activity of gastric ADH [62, 63]. However, the Dionysos Study found that the minimum dose associated with ALD was the same in men and women [13]. In contrast, a recent systematic review showed that the same amount of average consumption was related to a higher risk of liver cirrhosis in women than in men [64].

Chronic Viral Infections as Risk Factor for ALD Progression

Chronic alcoholism is associated to more severe ALD in patients with chronic HBV and HCV infection [65, 66]. Among patients with alcoholic cirrhosis, the risk of HCC is eight times higher in HCV positive than in HCV negative subjects [67]. In a large European cooperative study, an alcohol intake greater than 50 g/day was an independent risk factor for liver fibrosis in subjects with HCV-related chronic hepatitis [68]. In the Dionysos Study, we found that the risk of cirrhosis or HCC in alcohol abusers infected with HBV or HCV was higher than in alcohol abusers without viral infection [13, 18]. Also, ethanol intake was an independent predictor of incident liver cirrhosis in subjects with chronic HCV infection and an independent predictor of death in subjects with either HCV or HBV infection [43]. Owing to the synergistic effect of viral infections and alcohol consumption on the progression of CLD, such patients should be counseled to either completely abstain from alcohol or, less preferably, to reduce alcohol consumption to occasional small amounts.

Nutrition as Risk Factor for CLD

Alcohol Abuse and Obesity as Risk Factors for the Progression of CLD

The relative role of alcohol and obesity as risk factors for CLD has long been difficult to quantify in the absence of studies performed in the general population. In the last years, new data were made available on the prevalence, incidence, and natural history of the most common form of CLD, that is, FL [46]. In the Dionysos Nutrition and Liver Study, nearly 4 out of 10 individuals had FL, and this was attributable to alcohol intake in about 50% of cases [45]. When excessive alcohol intake, presently defined as a value ≤20 g/day in women and ≤30 g/day in men, is excluded [46], the main risk factors for fatty liver are obesity, dyslipidemia, and diabetes [69]. However, this separation is somewhat artificial, and there is substantial advantage in studying the relative effects of alcohol and obesity on FL and its complications [41, 44, 70]. In a nested case–control study of the Dionysos Study, the risk ratio for FL increased progressively in heavy drinkers (2.8), obese individuals (4.6), and obese heavy drinkers (5.8) [41]. This nested case–control study allowed to infer that FL is almost always present in obese subjects drinking more that 60 g/day of alcohol (95%). Most important was the demonstration that steatosis is associated more strongly with obesity (76%) than with heavy drinking (46%), suggesting a greater role for overweight than alcohol consumption in inducing fat accumulation in the liver, a finding which has been confirmed by other studies [60, 71]. Alcoholic patients often show a severe distortion of their diets, but no specific association between low intake of some nutrients and chronic liver disease is usually reported [72]. The interaction between alcohol intake and BMI on the progression of CLD has been confirmed recently by two large prospective population studies [73].

Nutrition as Risk Factors for Nonalcoholic Fatty Liver Disease

As stated above, NAFLD is a condition characterized by a significant accumulation of lipids inside the hepatocytes without a history of excessive alcohol consumption [46]. NAFLD encompasses a wide spectrum of liver injury, ranging from simple steatosis to NASH, fibrosis, and cirrhosis. NASH is a stage of NAFLD characterized by histological lesions similar to those of alcoholic steatohepatitis (ASH) [74, 75]. While simple steatosis has a benign clinical course, NASH may evolve into fibrosis, cirrhosis, and, possibly, HCC. Because NASH cannot be distinguished from ASH on histological grounds, its diagnosis relies heavily on the determination of the quantity of alcohol consumed by the patient. Studies on NAFLD published before 1990 allowed no alcohol consumption, while those published subsequently allowed up to 210 g per week, that is, up to 30 g per day. Hepatic steatosis can however be induced by a quantity of alcohol of 20 g per day, [76] and this is the upper limit employed by recent studies on NAFLD even if values up to 30 g/day may be accepted for men [46]. Obesity, type 2 diabetes, and hyperlipidemia are risk factors for NAFLD; the prevalence of obesity in patients with NAFLD varies between 30% and 100%, that of type 2 diabetes between 10% and 75%, and that of hyperlipidemia between 20% and 92% [69, 77]. In the Dionysos Study, the prevalence of NAFLD was 4.6 times higher in obese than in nonobese individuals [41]. Insulin resistance is common in obesity and hyperlipidemia and is the hallmark of type 2 diabetes [78]. Moreover, it is frequently detected in patients with NAFLD/NASH [79–82], also in those without obesity and diabetes. Thus, insulin resistance has been proposed as the minimum common denominator for most cases of NAFLD/NASH [69, 83]. However, insulin may be necessary but not sufficient as suggested by the lack of

efficacy of insulin-sensitizing medications in most trials [84]. Insulin resistance, impaired fasting glucose, obesity, and hyperlipidemia are all elements of the metabolic syndrome so that NAFLD has been considered another "disease of affluence." In a recent study, Marchesini et al. have assessed the prevalence of the metabolic syndrome in 304 consecutive NAFLD patients without diabetes [82]. Eighteen percent of normal-weight and 67% of obese subjects had the metabolic syndrome. Eighty-eight percent of the patients with NASH had the metabolic syndrome as compared to 53% of those with simple steatosis. Interestingly, the metabolic syndrome was a predictor of fibrosis. All these data point to the conclusion that insulin resistance *per se* may be a risk factor for the progression of simple steatosis to NASH, even if a cause-effect relationship can be disclosed only by prospective studies. Insulin is believed to be the main "hit" in the pathogenesis of NASH by the so-called two-hit hypothesis of NAFLD since insulin resistance is a prerequisite for the development of NASH, although it is probably not sufficient [84]. The "second hit" is supposed to be oxidative stress, mainly in the form of an excessive production of reactive oxygen species from the mitochondria of lipid-laden hepatocytes [83]. Because of studies showing that insulin resistance is a risk factor for NASH [79, 80, 82], insulin itself may however act as a "second hit." [83] However, the "two-hit" theory is presently being supplanted by a "multiple-hit" theory postulating multiple hits working simultaneously [85]. Obesity, type 2 diabetes, hyperlipidemia, and insulin resistance can be considered "nutritional risk factors" in view of their association with nutritional status. Although obesity is clearly an independent risk factor for NAFLD, few studies have investigated whether specific dietary patterns are more frequent in patients with NAFLD. A cross-sectional study performed in a subsample of the Israeli National Health and Nutrition Survey found that after adjustment for age, gender, BMI, and energy intake, the consumption of soft drinks and meat was associated with an increased risk of NAFLD [86]. A study performed in a sample of hospital patients reported a higher fat intake and an excessive intake of n-6 fatty acids in patients with NASH [87]. Fructose may have a role in the pathogenesis of NAFLD because it stimulates triglyceride and its excessive consumption has been linked to various metabolic abnormalities [88, 89]. In this respect, it has been hypothesized that fructose may have similar metabolic and hedonic characteristics to ethanol, but this is to be interpreted as a working hypothesis as there is presently not enough scientific evidence to support this notion [90, 91].

Conclusion

As indicated above, the link among liver disease, alcohol consumption, and nutrition is still vague. This contrasts sharply with the booming dimension of FL due in large part to the current epidemic of obesity. The change of life habits with a weight loss of about 5% has been demonstrated to be effective in reducing the content of liver fat and the NAFLD/NASH burden, but this simple and effective therapy is largely disregarded [92]. On the other hand, there is presently no pharmacological treatment demonstrated to be effective in NAFLD/NASH. Part of this is related to the lack of reliable *in vitro* or *in vivo* experimental models where drugs may be tested without the confounding factors found in humans. Although some advances have been made in this direction [93], we hope that the future will provide us with a better treatment for these potentially serious disorders.

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Chapter 39
Alcohol-Related Liver Disease: Roles of Insulin Resistance, Lipotoxic Ceramide Accumulation, and Endoplasmic Reticulum Stress

Suzanne M. de la Monte

Key Points

- Chronic alcohol-related liver disease with steatohepatitis is associated with hepatic insulin resistance. Insulin resistance impairs major functions in the liver, including protein synthesis, cell survival, cell growth, and energy metabolism, resulting in increased tissue injury, cell death, inflammation, and activation of stress pathways.
- Insulin resistance in the liver perturbs lipid homeostasis, resulting in the breakdown of membrane sphingolipids. The resultant increased generation of toxic lipids, including ceramides, promotes inflammation, insulin resistance, apoptosis, mitochondrial dysfunction, and endoplasmic reticulum (ER) stress.
- ER stress contributes to the progression of alcoholic liver disease by causing DNA damage, oxidative stress, radical injury, and cell death.
- In chronic progressive alcoholic liver disease, a vicious cycle of ethanol-induced hepatocellular injury and degeneration is established whereby insulin resistance dysregulates lipid metabolism, worsens steatohepatitis, increases cytotoxic ceramide generation, and promotes ER stress, while cytotoxic ceramides and ER stress cause hepatic insulin resistance.
- Chronic alcohol-related neurodegeneration is mediated by insulin resistance, proinflammatory cytokine activation, ceramide accumulation, and probably ER stress. However, neurodegeneration may also be caused by cytotoxic ceramides generated in livers with steatohepatitis since cytotoxic ceramides can cross the blood–brain barrier.
- Potential therapeutic approaches for chronic alcohol-related liver and brain diseases include the use of insulin sensitizer agents and ceramide enzyme inhibitor drugs.

Keywords Ceramides • Cytokines • Endoplasmic reticulum stress • Gene expression • Genetic factors • Insulin resistance • Liver-brain axis • Neurodegeneration • Peroxisome-proliferator-activated receptors • Steatohepatitis

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Overview

Alcohol-Induced Liver Diseases

Alcohol dependence and abuse are major public health problems throughout the world. Acute alcohol exposure causes hepatic steatosis and steatohepatitis, which are reversible. However, chronic alcohol abuse causes steatohepatitis to progress through stages of fibrosis, followed by cirrhosis, and finally liver failure. In addition, chronic alcohol abuse contributes to hepatocellular carcinoma development. Major consequences of chronic excessive alcohol abuse include impaired regenerative and remodeling responses to liver injury [1–4], giving way to fibrosis [5]. In addition, chronic ethanol exposure causes continuous liver injury due to inhibition of DNA synthesis [6–11], energy metabolism, insulin responsiveness, and antioxidant defenses [12, 13]. Undoubtedly, progression of alcoholic liver disease (ALD) from inflammatory-fibrotic states to cirrhosis ensues when recurrent injury and cell loss fail to be counterbalanced by adequate repair mechanisms. Aggregate data from multiple sources, including human and experimental animal model studies, suggest that insulin resistance, chronic inflammation, lipid dyshomeostasis, and endoplasmic reticulum (ER) stress are important pathogenic factors because they mediate oxidative stress, DNA damage, lipid peroxidation, and formation of reactive oxygen species (ROS), which together promote hepatocellular injury and death.

Alcohol-Induced Brain Diseases

The brain is the other major target of alcohol-mediated toxicity and degeneration. Acute alcohol exposure causes intoxication, which is reversible, but increases risk of injury and death from traumatic falls, accidents, and behavioral disturbances. Chronic alcohol abuse bears a significant toll on the central nervous system (CNS) due to functional changes the lead to addiction, self-negligence, poor nutrition, and disrupted family and social environments. In addition, chronic alcohol abuse can cause cognitive impairment and dementia, which are associated with permanent structural and degenerative changes in the brain. Although Wernicke-Korsakoff syndrome is one of the most devastating forms of alcohol-associated neurodegeneration, its pathogenesis is related to thiamine deficiency [14, 15]. In contrast, the mechanisms responsible for the much commoner alcohol-related neurodegenerative changes that contribute to cognitive and motor deficits, including white matter fiber loss (leukoencephalopathy), ventriculomegaly, cerebellar degeneration, and neuronal loss in corticolimbic structures [14–16], are still under investigation. However, evidence suggests that like ALD, alcohol-associated neurodegeneration is fundamentally mediated by insulin resistance [17].

Adverse Effects of Ethanol on Insulin and Insulin-Like Growth Factor Signaling

Insulin and Insulin-Like Growth Factor Type 1 (IGF-1) Signal Transduction Mechanisms

Insulin and IGF-1 bind to cell surface receptors and activate very similar signal transduction cascades that promote cell growth, survival, energy metabolism, cell motility, remodeling, repair, and plasticity. Insulin and IGF-1 stimulate autophosphorylation of their own receptors, activating receptor tyrosine

kinases (RTKs) that phosphorylate a major docking protein, insulin receptor substrate, type 1 (IRS-1) [18]. Phosphorylated IRS-1 transmits signals by interacting with adaptor molecules that contain *src* homology domains, such as growth factor receptor-bound protein 2 (Grb2) and the regulatory p85 subunit of phosphatidylinositol-3-kinase (PI3K). PI3K signals downstream by activating 3-phosphoinositide-dependent protein kinase 1 (PDK1), which phosphorylates and activates Akt/PKB, protein kinase C, p70S6K, and the serum – and glucocorticoid-induced (SGK) serine/threonine protein kinase. Akt phosphorylates and inactivates glycogen synthase kinase-3β (GSK-3β) and the proline-rich Akt substrate of 40 kDa (PRAS40); the latter inhibits mTOR, a positive regulator of p70S6K. Net effects include increased mitogenesis, cell survival, gene expression, energy metabolism, and motility, all of which are needed for liver remodeling and repair after injury [18–20]. At physiological concentrations, insulin and IGF-1 selectively bind to their own receptors and differentially mediate various functions in both liver and brain [21].

Insulin and IGF signaling pathways utilized by CNS cells are virtually identical to those present in liver, except IRS-2 instead of IRS-1 is the major docking protein [18]. Insulin, IGF-1 and IGF-2 polypeptides, and receptors are abundantly expressed in neurons and glial cells throughout the brain [18, 22–26], but their highest levels of expression are in the hypothalamus, temporal lobe, and cerebellum [18], which are the major targets of ethanol-mediated neurotoxicity. Because insulin and IGF signaling are critical mediators of survival, plasticity, metabolism, and myelin and neurotransmitter homeostasis [18, 27–30], sustained impairments in their networks have dire consequences with respect to cognitive and motor functions.

Ethanol-Mediated Liver Degeneration Linked to Inhibition of Insulin and IGF-1 Signaling

Chronic ethanol exposure inhibits insulin and IGF signaling in the liver [31–35]. These adverse effects of ethanol are mediated at multiple levels within the insulin/IGF-1 signal transduction cascades (Fig. 39.1), beginning with ligand binding and activation of RTKs. In chronic ALD, the failure to transmit signals downstream, despite ample availability of trophic factors, corresponds to a state of insulin/IGF-1 resistance [36–41]. Attendant reduced activation of Erk-MAPK, which is needed for DNA synthesis, corresponds with the impairments in liver regeneration [6, 9–11]. Inhibition of PI3-kinase-Akt leads to impaired hepatocellular growth, survival, cell motility, glucose utilization, plasticity, and energy metabolism [9, 42–49].

Another consequence of ethanol-induced insulin resistance is liver injury caused by increased DNA damage, oxidative stress, lipid peroxidation, mitochondrial dysfunction, and activation of proinflammatory and proapoptosis mediators [47, 50, 51]. These effects are due to the inhibition of insulin/IGF-stimulated survival and metabolic signaling through Akt and increased activation of proapoptotic, anti-survival mechanisms such as GSK-3β and PTEN phosphatase. In addition, oxidative stress, which promotes inflammation and insulin resistance, is increased by acetaldehyde accumulation and adduct formation [52, 53]. Moreover, ethanol-induced steatohepatitis is associated with increased activation of proinflammatory cytokines [54–57], including tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and IL-1β [12, 13], which themselves cause tissue injury with DNA damage, oxidative stress, mitochondrial dysfunction, and insulin resistance [54, 57]. Inflammatory cascades, once established, can promote energy failure, increased membrane permeability, and cell death. Therefore, ethanol-induced steatohepatitis, oxidative stress, DNA damage, mitochondrial dysfunction, and cell death are all intimately tied to hepatic insulin resistance.

Experiments in rat models of chronic ethanol feeding have provided excellent insight into the causes and consequences of ethanol-mediated insulin resistance in both liver and brain. In an experimental model of chronic ethanol feeding of Long-Evans rats, steatohepatitis was correlated with

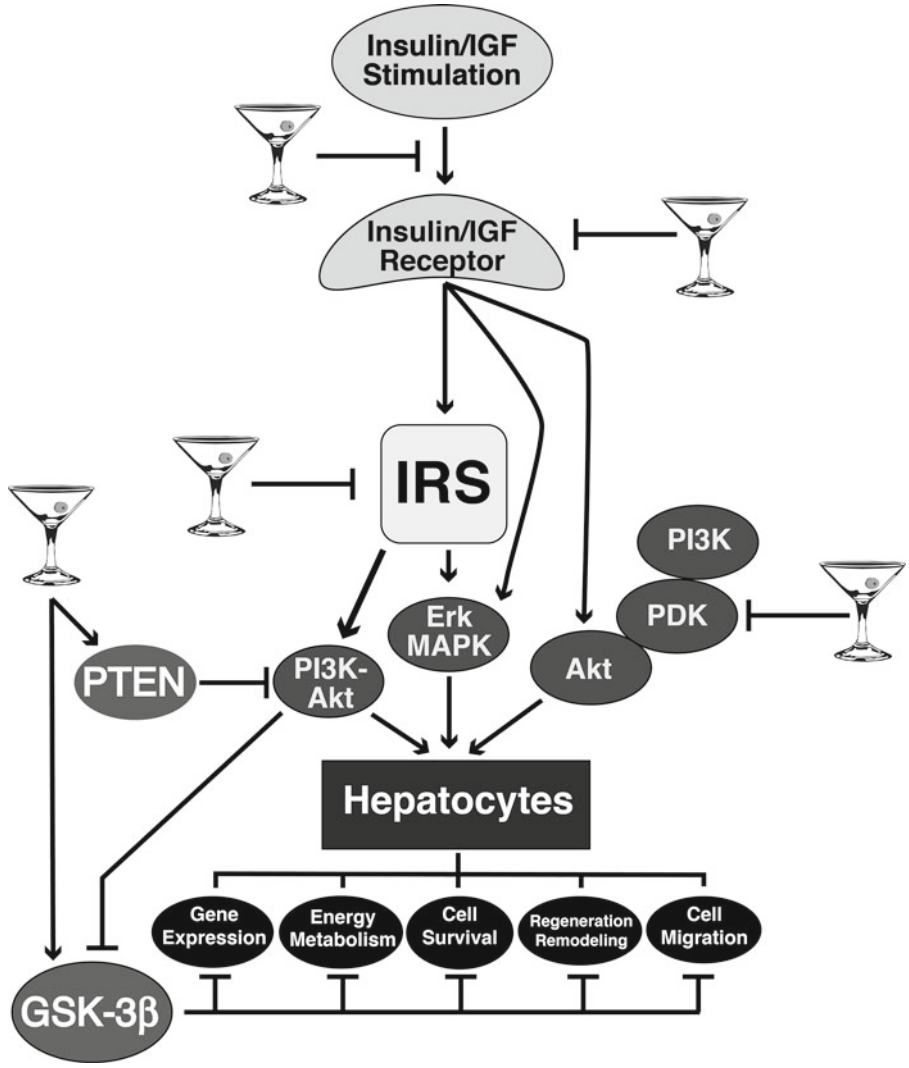

Fig. 39.1 Ethanol inhibition of insulin/IGF-1 signaling. Ethanol inhibits insulin and IGF-1 signaling at multiple levels, beginning at entry points of the cascade. Ethanol impairs ligand binding to cell surface receptors, tyrosine phosphorylation and activation of receptor tyrosine kinases, tyrosine phosphorylation of IRS-1, and downstream signaling through both PI3-kinase-Akt and Erk-MAPK pathways. In addition, ethanol stimulates PTEN, which inhibits PI3K-Akt and activates GSK-3β. The oxidative stress effects of ethanol independently stimulate GSK-3β, which inhibits multiple positive stimulatory effects on hepatocytes

reduced insulin receptor binding and insulin-responsive gene expression and increased caspase-3 activation, DNA adducts, lipid peroxidation, and oxidative stress [12]. However, during the course of our investigations, we discovered that the severity of ALD was not entirely dependent on ethanol dose or duration of exposure and that genetic background can be a major contributing factor. Correspondingly, our analysis of the effects of chronic ethanol feeding in three different rat strains demonstrated that, despite similar blood alcohol concentrations, Long-Evans rats were highly susceptible to alcohol-induced steatohepatitis with insulin resistance, inflammation, and fibrosis, while Fischer 344 rats were relatively resistant, and Sprague–Dawley rats had intermediate degrees of susceptibility [58].

To some extent, these responses could be attributed to differences in baseline and ethanol-induced levels of alcohol metabolizing enzyme gene expression and higher levels of ethanol-induced inflammatory responses and lipid accumulation in Long-Evans compared with the other two strains [58]. In addition, higher levels of p53 activation, hepatocellular death, impaired insulin signaling, and activation of the Tp53-induced glycolysis and apoptosis regulator (TIGAR) were observed in Long-Evans compared with the other two strains, and again, intermediate responses were present in Sprague–Dawley rats [59]. These studies clearly highlight the importance of metabolic derangements in the setting of insulin resistance as a key factor regulating the severity of chronic ethanol-induced liver injury.

Until relatively recently, it has not been feasible to extensively characterize the effects of chronic ethanol exposure on the integrity of insulin/IGF-1 signaling networks using an in vivo model. In fact, most of the published works were either based on studies in cultured cells, or they included limited analyses of the pathways. Due to the present availability of highly sensitive multiplex format assays, we were able to simultaneously examine the effects of ethanol on insulin/IGF-1 signaling, from the receptor through pathways downstream of Akt, in the Tsukamoto and French intragastric feeding model of chronic ethanol feeding in Sprague–Dawley rats [60, 61]. Those investigations demonstrated that besides impairments in ligand-receptor binding, chronic ethanol feeding inhibits signaling through the insulin/IGF-1 receptors, IRS-1, Akt, and p70S6K [62]. Moreover, treatment with anti-inflammatory agents, i.e., N-acetylcysteine, is not sufficient to restore insulin/IGF-1 signaling, despite reduced inflammation [62]. Recently, similar impairments in insulin/IGF signaling were demonstrated in human chronic ALD [63].

Ethanol-Mediated Neurodegeneration: Role of Insulin/IGF-1 Resistance

In the adult CNS, chronic ethanol exposure causes neurodegeneration with atrophy of cortical-limbic structures, including the anterior frontal regions, temporal lobe, hypothalamus, and thalamus, central white matter, the corpus callosum, and the cerebellum, particularly the vermis [15, 16, 21]. Studies in both humans and experimental animals demonstrated that these structural abnormalities correlate with insulin and IGF-1 resistance with reduced ligand-receptor binding [17, 21, 31, 64]. Moreover, similar to the findings in liver, alcohol-associated neurodegeneration is associated with constitutively reduced expression of insulin/IGF-1 responsive genes, increased oxidative stress, lipid peroxidation, mitochondrial dysfunction, DNA damage, and cell (neuronal and oligodendroglial) loss [17, 21, 64]. Of note is that the neurodevelopmental abnormalities produced by chronic prenatal exposure to ethanol are also mediated by inhibition of insulin and IGF signaling in the brain [31, 49, 65–67].

In the CNS, ethanol disproportionately impairs signaling through PI3-kinase-Akt [33, 35, 65, 68]. Consequently, major adverse effects of ethanol on CNS neurons include reduced survival and plasticity, increased apoptosis [33, 35, 69], and mitochondrial dysfunction with deficits in energy metabolism and acetylcholine homeostasis [33, 49, 65, 66, 68, 70]. The lopsided inhibition of PI3K-Akt and attendant activation of glycogen synthase kinase 3β (GSK-3β) in neurons and brain are partly due to ethanol's activation of phosphatases such as PTP-1b and PTEN [66], although other factors are also likely involved. Further studies of brains from the three rat strains described above demonstrated that ethanol-induced neurodegeneration of the cerebellum and temporal lobes was most pronounced in Long-Evans, followed by Sprague–Dawley, and they were subtle or nondetectable in Fischer 344 rats [21]. Therefore, increased susceptibility to alcohol-induced neurodegeneration with brain insulin/IGF-1 resistance correlates with inherent genetic differences in susceptibility to alcohol-induced steatohepatitis and liver insulin/IGF-1 resistance.

Insulin Resistance, Dysregulated Lipid Metabolism, and Toxic Lipid-Mediated Injury

Steatohepatitis and Lipotoxicity

Insulin stimulates lipogenesis, which results in increased triglyceride storage in liver [71, 72]. While this process is generally benign and well tolerated, disturbances in homeostasis can shift the balance toward a state of insulin resistance [71, 73]. This concept has been well documented in chronic ALD [12] but also has relevance to steatohepatitis caused by other disease states including (1) diet-induced obesity [74], (2) chronic high-fat diet (HFD) feeding without obesity [75], (3) nitrosamine-mediated injury [76–78], and (4) constitutive overexpression of the hepatitis B virus X gene (HBx) in transgenic mouse livers [79]. In essence, it appears that steatohepatitis, irrespective of cause, can be associated with decreased insulin receptor (IR) binding, IR gene expression, IR tyrosine kinase activation, signaling through IRS-1, and insulin-responsive gene expression, and increased oxidative stress and adduct (DNA, protein, and lipid) accumulation. Therefore, steatohepatitis plays a pivotal role in the pathogenesis of hepatic insulin resistance, which itself promotes lipolysis [80]. Lipolysis generates toxic lipids, i.e., ceramides, which further impair insulin signaling, mitochondrial function, and cell viability [73, 81, 82].

Clear demonstrations of how alcohol-induced steatohepatitis promotes insulin resistance and ceramide accumulation in liver were provided by two distinct experimental rat models of chronic ethanol feeding. After chronic pair-feeding with isocaloric control (0%) or ethanol-containing (37% by caloric content) liquid diets, the ethanol-exposed livers exhibited conspicuous micro- and macrovesicular steatohepatitis with apoptotic bodies, disorganized hepatic chord architectures, and chicken wire (perihepatocyte) fibrosis [12, 13, 62]. Biochemical and molecular assays demonstrated that steatohepatitis was associated with (1) increased levels of hepatic neutral lipids and triglycerides, (2) reduced insulin receptor binding and signaling downstream through the Akt pathway, (3) increased expression of several genes that regulate ceramide production via biosynthetic or catabolic mechanisms, (4) increased acid sphingomyelinase activity, and (5) increased ceramide levels in both liver and serum [12, 13, 21]. These observations are especially of interest in light of the recent finding that similar abnormalities occur in human chronic ALD [63].

Ceramides, Lipotoxicity, and Insulin Resistance

Ceramides are lipid signaling molecules that can promote positive or negative cellular responses including increased proliferation, motility, plasticity, inflammation, apoptosis, and insulin resistance [83]. Ceramides are generated during biosynthesis and degradation of triglycerides and sphingomyelin [81, 84–87]. Ceramides are generated biosynthetically from fatty acid and sphingosine [83, 88, 89] through ceramide synthase and serine palmitoyltransferase activities [90–92] and catabolically from sphingolipid through activation of neutral or acidic sphingomyelinases [89, 92] or the degradation of complex sphingolipids and glycosphingolipids localized in late endosomes and lysosomes [88]. Interest in characterizing ethanol dose effects and severity of steatohepatitis on mediators of ceramide accumulation stems from data showing that ethanol-induced steatohepatitis results in increased proceramide gene expression and ceramide levels in liver and plasma [21, 62] and that the severity of chronic ALD seems to correlate with severity of neurodegeneration [21].

Disease-associated lipolysis is a feature of insulin resistance and initiated by critical levels of endoplasmic reticulum (ER) stress and mitochondrial dysfunction [93–96]. Ceramides generated in disease states can themselves cause insulin resistance by activating proinflammatory cytokines and

inhibiting signal transduction through PI3-kinase-Akt [97–100]. With regard to diet-induced obesity, hepatic insulin resistance is mediated by two mechanisms: enhanced ceramide production in adipocytes with secondary effects on hepatic insulin signaling [83, 86, 87, 101–103], and steatohepatitis with endogenous hepatic production of cytotoxic ceramides. Correspondingly, recent studies showed that (1) exogenous cytotoxic ceramide exposures cause hepatic insulin resistance [104], (2) chronic ethanol exposure and other models of steatohepatitis lead to increased proceramide gene expression in liver [62], and (3) hepatic steatosis and steatohepatitis lead to increased ceramide levels (immunoreactivity) in liver and serum [21]. Moreover, in vitro experiments showed that hepatocytes treated with C2 or C6 synthetic ceramides exhibit reduced viability, mitochondrial function, insulin signaling, and insulin-responsive gene expression [105], indicating that exogenous ceramide exposure is hepatotoxic and causes insulin resistance. Furthermore, the finding of increased serum ceramide levels in chronic ethanol-fed rats with steatohepatitis [21] suggests that ceramides produced in liver can leak into peripheral blood (following hepatocellular injury or death) and thereby exert toxic and metabolic insults to distant organs, including brain.

The Liver-Brain Axis of Alcohol-Mediated Neurodegeneration

Steatohepatitis, Ceramides, Insulin Resistance, and Neurodegeneration

Steatohepatitis caused by alcohol, obesity, or viral hepatitis (hepatitis C) can all be associated with cognitive and neuropsychiatric dysfunction [106–112]. Previous studies demonstrated histopathologic and biochemical evidence of neurodegeneration, with deficits in learning and memory in various models of steatohepatitis, including chronic ethanol feeding, diet-induced obesity, high-fat diet feeding, and nitrosamine exposure [74, 113–115]. Importantly, steatohepatitis was consistently associated with insulin resistance in both liver and brain [74, 78, 113–115], increased expression of multiple proceramide genes in liver [74, 116], and increased ceramide levels in liver and peripheral blood. Further studies showed that severity of ethanol-mediated steatohepatitis, rather than blood alcohol levels, correlated with severity of neurodegeneration and brain insulin resistance [21].

The Liver-Brain Axis of Neurodegeneration Hypothesis

As discussed, alcohol-induced steatohepatitis promotes hepatic insulin resistance, oxidative stress, and injury with attendant increased generation of ceramides that could further increase insulin resistance, inflammation, and injury. Since toxic lipids, including ceramides, readily cross the blood–brain barrier and cause insulin resistance by interfering with critical phosphorylation events [88, 117, 118] and activating proinflammatory cytokines [83, 119, 120], we conducted experiments to address our hypothesis about the potential role of extra-CNS (liver)-derived ceramides as mediators of neurodegeneration. In vitro and in vivo experiments demonstrated that C2 or C6 cytotoxic ceramide exposures cause neuronal insulin resistance with increased oxidative stress, DNA damage, lipid peroxidation, and impaired neuronal viability, neurotransmitter function, and mitochondrial function [104]. In addition, in vivo ceramide exposures cause cognitive-motor deficits that mimic features of chronic alcohol exposure [121]. Therefore, ceramides generated or delivered from extra-CNS sources can cause brain insulin resistance and attendant neurodegeneration. Correspondingly, liver-derived cytotoxic lipids entering the circulation and capable of penetrating the blood–brain barrier may mediate CNS insulin resistance, oxidative stress, proinflammatory cytokine activation, and neurodegeneration in the context of chronic alcohol exposure. We postulate that chronic moderate – to high-level alcohol exposure

leads to neurodegeneration in part, via a liver-brain axis mediated by the trafficking of toxic sphingolipids (ceramides) from liver through blood to brain. This concept opens an exciting new chapter on disease mechanisms and strategies for developing noninvasive tools to monitor proneness and progression of alcoholic neurodegeneration.

Alcohol-Mediated Insulin Resistance and Endoplasmic Reticulum Stress

Endoplasmic Reticulum (ER) and ER Stress

The ER is an intracellular organelle that mediates a broad array of functions, including protein synthesis, folding, maturation, and trafficking, i.e., posttranslational protein processing and transport [122]. In addition, the ER is critical for Ca^{2+} homeostasis and triglyceride synthesis. ER stress is caused by perturbations in homeostatic mechanisms that cause unfolded proteins to accumulate and reactive oxygen (ROS) and reactive nitrogen (RNS) species to form, exacerbating oxidative stress [122]. Normally, the ER adapts to stress by activating the unfolded protein response (UPR) [123, 124], which results in increased levels of three major ER stress sensor proteins: inositol-requiring enzyme 1 (IRE-1α), PKR-like ER-localized eIF2α kinase (PERK), and the activating transcription factor 6α (ATF-6α; ER membrane-anchored transcription factor). PERK and IRE1 transmit stress signals in response to protein misfolding or unfolding and thereby activate ER stress signaling networks. In the unstressed state, the luminal domains of PERK and IRE1 are stably complexed with the ER chaperone BiP. ER stress induced by UPR reversibly dissociates BiP from the luminal domains of PERK and IRE1. BiP translocation to the cytosol correlates with activation of PERK or IRE1 [123–125]. In addition, Bim, a proapoptotic member of the Bcl-2 family, is normally sequestered by Bcl-xL, preventing apoptosis. However, with ER stress, Bim dissociates from Bcl-xL, translocates to the ER, and activates a caspase-12-mediated prodeath cascade [126].

ER Stress and Alcoholic Liver Disease

Insulin resistance contributes to ER stress because vital ER functions such as protein synthesis, modification, and folding, calcium signaling, and lipid biosynthesis utilize glucose as the main source of energy to drive these processes, and insulin resistance impairs glucose uptake and metabolism. Therefore, ethanol-induced signaling can promote hepatocellular injury and death via activation of ER stress pathways [94–96, 127]. Ethanol's effects on ER stress signaling are broad-based and mediated by activation of the three major ER stress sensor cascades: PERK, IER-1a, and ATF6, as well as ER resident sterol regulatory element–binding proteins (SREBP)-1c and 2, with attendant upregulation of fatty acid/triglyceride synthesis, beta oxidation (SREBP-1a), and cholesterol synthesis (SREBP2) [128].

Increased ER stress is an important feature of alcohol-related insulin resistance states [54, 128, 129] because it marks lipid dyshomeostasis and may reflect activation of proceramide and proinflammatory pathways with increased generation of toxic lipids [54, 128, 129]. Correspondingly, ceramide immunoreactivity and ER stress gene expression were significantly increased in the ethanol-exposed relative to control livers [21]. ER stress leads to activation of PERK, and then the growth arrest and DNA damaging, and GADD34/PP1 phosphatase complex, which dephosphorylates EIF2α, promoting apoptosis. In addition, our recent studies showed that proapoptotic targets of ER stress, i.e., Fas, p53, and Bax, were upregulated by chronic ethanol exposure. Correspondingly, ceramide immunoreactivity and ER stress genes are significantly upregulated in livers of chronic ethanol-fed rats [62] and in humans with chronic progressive ALD [63].

ER Stress in Alcoholic Brain Disease

Thus far, there is little information about the role of ER stress in alcoholic brain disease. As in other organs, ER stress in the CNS is triggered by the accumulation of unfolded or misfolded proteins in the ER lumen. This abnormality is a recognized feature of several major neurodegenerative diseases, including Alzheimer's and Parkinson's, in which misfolded cytoskeletal proteins accumulate, aggregate, and become ubiquitinated, and thereby promote ER and oxidative stress [130–132]. Using a cell culture model, short-term ethanol exposure resulted in increased expression of GP78, CHOP, ATF4, ATF6, and phosphorylated PERK and eIF1α, but only after induction by tunicamycin or thapsigargin [133]. This suggests that the ER stress response associated with acute ethanol neurotoxicity is driven by calcium release from the ER together with oxidative stress and possibly mitochondrial dysfunction. A subsequent study using a late gestation equivalent binge ethanol exposure model in mice showed that short-term effects of the ethanol increased ER stress-inducible proteins including ATF6, CHOP/GADD153, GRP78, and phosphorylated eIF2α, caspase-12, and CHOP [134]. Therefore, despite relatively limited information, ethanol exposure appears to mediate CNS neuronal injury and death via activation of ER stress pathways, similar to the findings in liver. However, more information is needed about long-term effects of in vivo ethanol exposure in relation to neurodegeneration.

Hypothesis: Insulin Resistance Precipitates and Propagates Chronic Progressive Alcohol-Related Degeneration of Liver and Brain

Chronic alcohol misuse causes progressive liver injury and degeneration (Fig. 39.2). The aggregate findings from multiple studies suggest that hepatic insulin resistance is the critical initiating factor governing ALD progression, although oxidative injury caused by ethanol itself or its chief toxic metabolite, acetaldehyde, contributes to the process. Persistent injury with inflammation and metabolic dysfunction ultimately precipitates a cascade marked by dysregulated lipid metabolism with increased ceramide production. Intrahepatocyte accumulation of cytotoxic ceramides promotes ER stress which exacerbates insulin resistance, inflammation, and oxidative stress. Consequences include increased DNA damage, mitochondrial dysfunction, energy depletion, ROS production, and eventually the formation of lipid, protein, and DNA adducts, which impair cellular functions at multiple levels. Finally, a reverberating cascade of malsignaling and insulin resistance gets established, and progressively impairs cell survival [21], and mediates the transition from reversible alcohol-induced liver injury to chronic progressive ALD. The implications for therapy are that (1) inhibition of ceramide generation and accumulation in liver and blood may reduce the severity of ALD and alcohol-related neurocognitive deficits and (2) agents that restore insulin responsiveness could correct the disorders in lipid metabolism that lead to cytotoxic lipid accumulation, ER stress, and liver degeneration.

Hypothesis Testing 1: Ceramide Inhibitor Treatments

To begin testing this hypothesis, we treated liver precision-cut slice cultures (PCSCs) [135–137] from control and ethanol-fed adult rats with ceramide inhibitor drugs and examined the effects on cytotoxicity, histology, and steatohepatitis. The liver PCSCs were generated with freshly isolated livers that were cut at a thickness of 150 μm with a McIlwain Tissue Chopper. Cultures were maintained for up to 96 h at 37 °C in a standard CO_2 incubator with gentle platform agitation [135–137]. The cultures were treated with myriocin, a de novo ceramide synthesis inhibitor; apocynin, an NAD(P)H oxidase

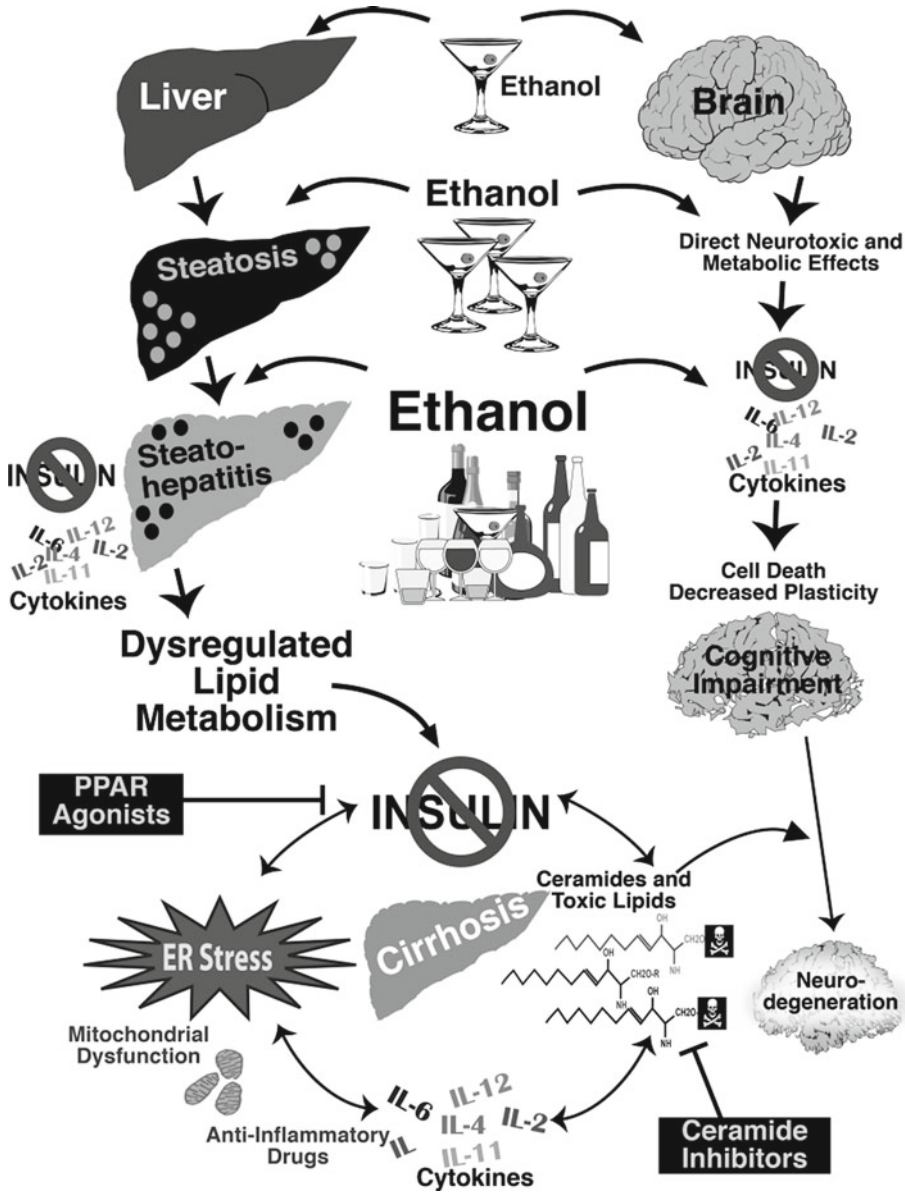

Fig. 39.2 Pivotal role of insulin resistance in alcohol-mediated liver and brain degeneration. Limited and low levels of alcohol exposure cause reversible injury and metabolic disease states in liver and brain. Higher levels and more chronic ethanol exposures cause hepatic steatosis to progress to steatohepatitis. In addition, insulin resistance and proinflammatory cytokine activation lead to increased cellular injury and death in both liver and brain. Persistent, high levels of ethanol exposure establish a path toward progressive injury and degeneration of liver and brain. In liver, dysregulated lipid metabolism leads to increased toxic lipid (ceramide) generation, ER stress, with further activation of proinflammatory cytokines, and increased insulin resistance. Each of these pathophysiological processes worsens the others, furthering hepatocellular degeneration, DNA damage, adduct formation, energy failure, and cell death, which favor fibrogenesis and cirrhosis. In the brain, chronic, high levels of ethanol exposure cause direct toxic injury that leads to neuronal dysfunction and loss with cognitive impairment and neurodegeneration. In addition, toxic lipids from livers with steatohepatitis can exacerbate alcohol-induced brain injury, resulting in "second-hit"-mediated neurodegeneration. Potential therapeutic strategies for reducing or reversing chronic progressive alcoholic liver and brain disease include treatment with insulin sensitizer drugs (e.g., PPAR agonists; metformin), enzymatic inhibitors of ceramide generation (particularly those that function via the degradation pathway), and anti-inflammatory agents (alone, not sufficient)

inhibitor of sphingomyelin hydrolysis; or desipramine, an inhibitor of acid sphingomyelinase, for 48 h. Ceramide inhibitor treatments significantly reduced hepatic lipid content, LDH release (cytotoxicity), and ceramide immunoreactivity, and they restored the normal hepatic chord architecture [138].

Hypothesis Testing 2: Peroxisome-Proliferator-Activated Receptor (PPAR) Agonist Treatment to Prevent or Reduce ALD and Alcohol-Related Neurodegeneration

PPAR α,δ, and γ are expressed in liver [13] and brain [113]. Signaling through these nuclear receptors regulates lipid metabolism, inflammation, glucose utilization, and insulin-responsive gene expression [139, 140]. To examine the effects of PPAR agonist treatments in vivo, during the last 4 weeks (total 8 weeks) of control (0% ethanol) or ethanol-containing (37% ethanol by caloric content; 9.2% v/v) isocaloric liquid diet feeding, rats were administrated twice weekly (Monday and Thursday) i.p. injections of vehicle (saline), a PPAR-α (GW7647; 25 μg/kg), PPAR-δ (L-160,043; 2 μg/kg), or PPAR-γ (F-L-Leu; 20 μg/kg) agonist. The results demonstrated that despite continued high ethanol (37% diet) consumption, rats treated with PPAR-δ >>PPAR-γ>PPAR-α agonists had reduced severities of alcoholic steatohepatitis and insulin resistance, corresponding with reports by other groups [36, 40, 141]. In addition, the PPAR agonist treatments reduced the severity of alcohol-induced neurodegeneration and proceramide gene expression and ceramide immunoreactivity in both liver and brain. Furthermore, PPAR-δ agonist treatments restored the regenerative capacity of the liver [13] and normalized cognitive performance on Morris water maze tests [67] in ethanol-exposed rats.

Summary and Conclusions

Ethanol-induced insulin resistance dysregulates hepatic lipid metabolism, worsens steatohepatitis, and increases cytotoxic ceramide generation and ER stress. In turn, cytotoxic ceramides and ER stress promote hepatic insulin resistance, thereby establishing a vicious cycle of hepatocellular injury and degeneration. This sequence of events establishes a reverberating loop of progressive hepatic dysfunction that could evolve toward end-stage liver disease and also contribute to neurodegeneration. Insulin sensitizer agents or ceramide enzyme inhibitor drugs could be used to abrogate alcohol-mediated ER stress and insulin resistance and thereby help to restore normal liver structure and function.

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Chapter 40
Nutrition and Alcoholic and Nonalcoholic Fatty Liver Disease: The Significance of Cholesterol

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Key Points

- The feedback system controlling intracellular lipids levels is disrupted in NAFLD and AFLD.
- Excess cholesterol intake is an appropriate stimulant for the development of fatty liver, and excess cholesterol intake alone can induce liver steatosis.
- The accumulation of cholesterol rather than triglycerides plays a critical role in the progression from simple steatosis to steatohepatitis.
- Cholesterol management is considered to be a promising treatment target for NAFLD and AFLD.

Keywords NFLD • AFLD • Cholesterol • Ezetimibe • NPC1L1

Introduction

Fatty liver is a typical feature of alcoholic liver disease (ALD) and nonalcoholic fatty liver disease (NAFLD). Alcoholic fatty liver disease (AFLD) is considered to be a subtype of ALD in the initial stage, and the histological manifestations of AFLD include micro- and macrovesicular steatosis, the formation of Mallory bodies, hepatocellular ballooning, apoptosis and necrosis, and inflammation [1, 2]. These histological changes are apparent in over 90% of liver biopsy samples following the consumption of alcohol for just 2–4 weeks at a dose of 60 g/day in males or 30 g/day in females [3, 4]. Although it is possible to recover from AFLD by avoiding alcohol intake and adequate nutrition supports [5], chronic alcohol intake induces marked liver damage and fibrosis and eventually leads to cirrhosis and hepatocellular carcinoma (HCC) [6, 7]. NAFLD, which occurs in people consuming less

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than 20 g/day of ethanol, shows almost identical histological features [8]. NAFLD includes nonalcoholic steatohepatitis (NASH) in 10–20% of patients, which can develop into cirrhosis and HCC [9–12]. The main cause of NAFLD is excess nutrition intake and is often accompanied by obesity, insulin resistance, hypertension, and/or dyslipidemia [13]. Therefore, nutritional management and therapeutic exercise are important components of the treatment of NAFLD.

To explain the pathogenesis of NAFLD and NASH, the "two-hit theory" has been widely adopted [14]. The two-hit theory is also thought to underlie the pathogenesis of AFLD and alcoholic steatohepatitis (ASH) [15]. The first hit consists of simple accumulation of fatty acids/triglycerides (i.e., steatosis) in the liver, while the second hit involves oxidative stress, mitochondrial dysfunction, and inflammation, which ultimately cause the liver damage in NASH and ASH. Considering the similar histological findings and lipogenetic disturbances, AFLD and NFLD are essentially differentiated by the history of alcohol consumption according to the definition of these diseases. However, in practice, it is difficult to differentiate these diseases because chronic alcohol consumption and excess nutrition intake occur simultaneously in many patients with fatty liver. Moreover, the presence of alcohol consumption in NAFLD patients or the presence of excess nutrition intake in AFLD patients is associated with the progression of fibrosis [16, 17]. There may also be important links between inflammatory cytokines, insulin resistance, and fatty liver during the progression of these diseases. Although lipid metabolism has received much attention in the context of AFLD and NAFLD, dysregulation of cholesterol metabolism has received much less attention. In this chapter, we discuss the role of cholesterol and its metabolites on pathogenesis of AFLD and NAFLD. We also discuss the importance of cholesterol management as a component of their treatment.

Lipid Metabolism in AFLD and NAFLD

Hepatic lipid homeostasis reflects a balance between lipid synthesis, catabolism (oxidation), and secretion. AFLD and NAFLD are characterized by steatosis caused by disordered lipid metabolism, such as inhibition of fatty acid oxidation and enhanced lipogenesis. The hepatic expression profiles of lipid metabolism-associated genes and proteins have been examined in AFLD and NAFLD patients. Even though the precise cellular mechanisms involved remain to be elucidated, both diseases share the basic network of lipogenesis. Figure 40.1a, b summarizes the accepted changes in the liver of AFLD and NAFLD patients, respectively. The expression pattern of genes and proteins is essentially similar between AFLD and NAFLD, and the accumulation of triglycerides, free fatty acids, and cholesterol is a characteristic observation of both diseases.

Excess alcohol and fatty acids levels are considered to be the main factors involved in the disordered hepatic lipid metabolism in AFLD. In the ethanol–acetaldehyde–acetate metabolic pathway, the activity of two NAD-dependent enzymes, alcohol dehydrogenase and aldehyde dehydrogenase, increases the NADH/NAD ratio, which impairs gluconeogenesis and the tricarboxylic acid cycle, thus upregulating fatty acid synthesis and inhibiting mitochondrial fatty acid oxidation [18–21]. Because NADH suppresses the NAD-dependent action of dehydrogenases, the level of glycerol triphosphate, the substrate of triglyceride synthesis, increases.

Although fatty acids are used for β-oxidation in mitochondria and peroxisome under the regulation of peroxisome proliferator-activated receptor α (PPARα), chronic alcohol consumption and its metabolite, acetaldehyde, inhibit the transcriptional activity of PPARα [22]. Fatty acids are ligands for PPARα, which transactivates the expression of genes involved in the transport, oxidation, and export of free fatty acids, including carnitine palmitoyltransferase-1 (CPT-1), which is the rate-limiting enzyme in fatty acid β-oxidation. Therefore, these suppressive effects of alcohol aggravate steatosis [23, 24].

It has been reported in chronic ethanol-fed rats that the activity of AMP-activated protein kinase (AMPK) is decreased in hepatocytes [25]. AMPK is a metabolic master switch and its activity is regulated

by adiponectin and tumor necrosis factor-α (TNFα). Inhibition of AMPK results in the activation of sterol regulatory element-binding protein-1c (SREBP-1c), which upregulates enzymes involved in fatty acid synthesis including acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS), enhances fatty acid synthesis and the overproduction of triglycerides, and leads to liver steatosis [25]. Abnormal homocysteine/methionine metabolism in the liver and adipose tissue is also associated with the pathogenesis of AFLD [26, 27]. Chronic ethanol feeding inhibits methionine synthase, which reduces the synthesis of S-adenosylmethionine and causes hyperhomocysteinemia, which was recently suggested to be a regulator of adiponectin levels. Adiponectin regulates hepatic fatty acid uptake and de novo lipogenesis. Hence, ethanol-induced hyperhomocysteinemia contributes to the reduction of serum adiponectin levels and increases the levels of TNFα, which activates SREBP-1c. Ethanol also decreases the expression of sirtuin 1 (SIRT1) and forkhead box protein O1 (FOXO1), which are associated with insulin sensitivity, and thus reduces adiponectin levels [28, 29]. Furthermore, downregulated expression of hepatic adiponectin receptors has been demonstrated in ethanol-fed animals [27, 28].

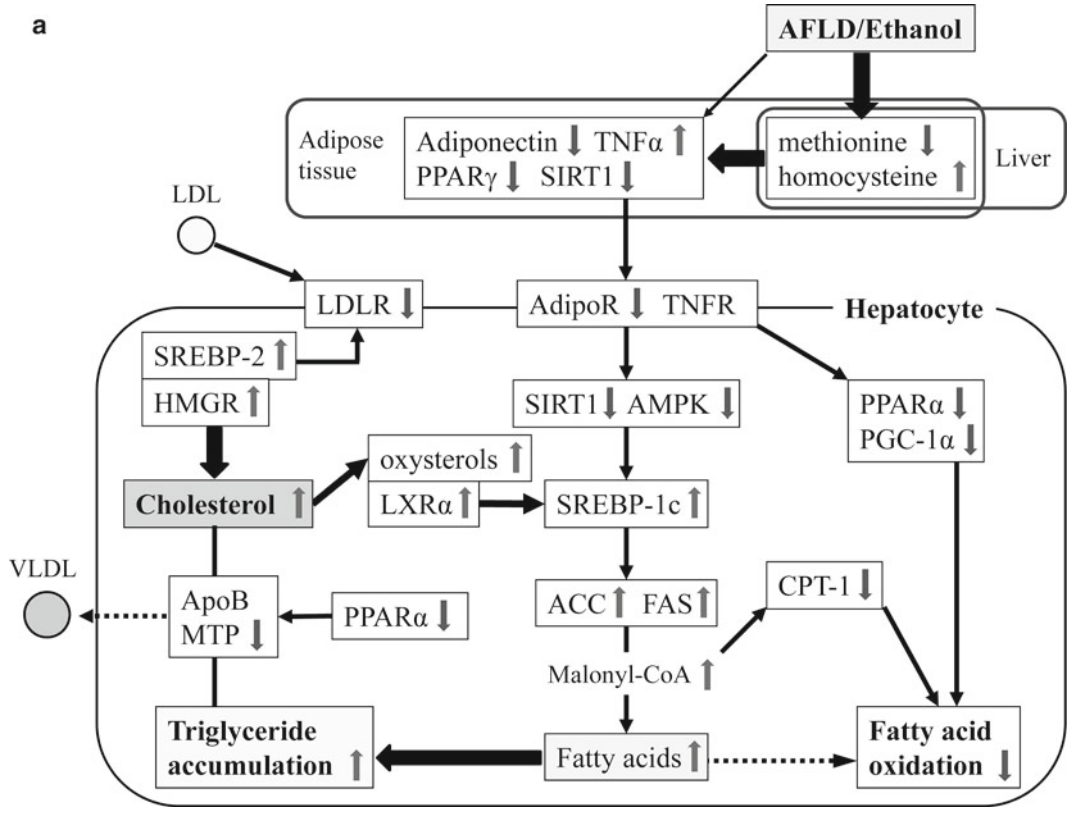

Fig. 40.1 Lipid metabolism and the expression of lipid metabolism-associated factors in hepatocytes. (**a**) Alcoholic fatty liver disease appears to involve increased fatty acid and cholesterol synthesis, impaired secretion of VLDL, and decreased fatty acid oxidation. (**b**) The established pathophysiological pathways in nonalcoholic fatty liver disease involve increased delivery of fatty acids to the liver and increased SREBP-1c signaling because of cholesterol overload and insulin resistance. *ACC* acetyl-CoA carboxylase, *AdipoR* adiponectin receptor, *AFLD* alcoholic fatty liver disease, *ABCG5/G8* ATP-binding cassette G5/G8, *AMPK* AMP-activated protein kinase, *ApoB* apolipoprotein B, *CPT-1* carnitine palmitoyltransferase-1, *FAS* fatty acid synthase, *HMGR* HMG-CoA reductase, *IR* insulin receptor, *LDLR* LDL receptor, *LXR* liver X receptor, *MTP* microsomal triglyceride transfer protein, *NAFLD* nonalcoholic fatty liver disease, *NPC1L1* Niemann–Pick C1-like 1, *PGC-1α* PPARγ coactivator-1α, *PPAR* peroxisome proliferator-activated receptor, *SIRT1* sirtuin 1, *SREBP* sterol regulatory element-binding protein, *TNFα* tumor necrosis factor-α, *TNFR* TNF receptor

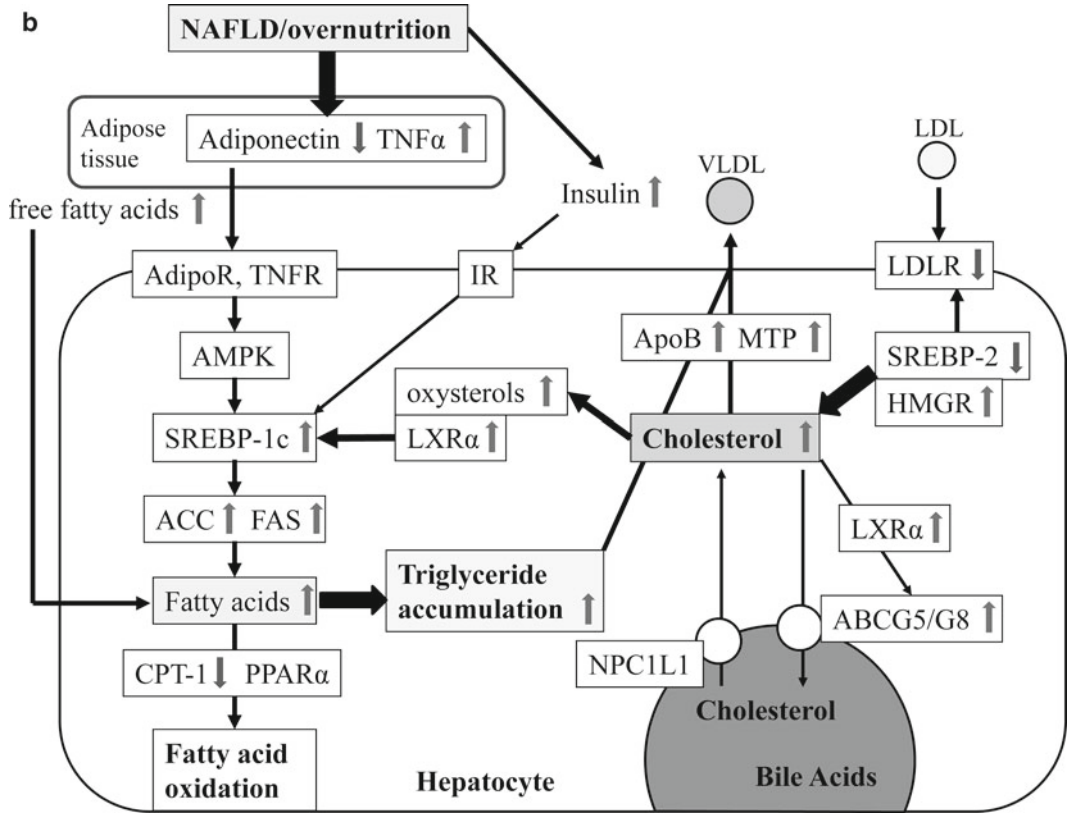

Fig. 40.1 (continued)

In particular, negative regulators of fatty acid accumulation (e.g., adiponectin, AMPK, SIRT1, and PPARγ) are downregulated while positive regulators (e.g., TNFα, SREBP-1c, ACC, and FAS) are upregulated by chronic ethanol ingestion.

In NAFLD, hepatic steatosis develops because of upregulated fatty acid synthesis but it is questionable whether downregulation of fatty acid oxidation is also involved [30–33]. Although the possible role of hyperhomocysteinemia has not been determined, reduced adiponectin production has been demonstrated in NAFLD patients because of increased visceral fat accumulation. Adiponectin levels are inversely proportional to insulin resistance and hepatic steatosis in NAFLD patients [34]. Moreover, insulin resistance, which is common in NAFLD, causes fatty liver, while increases in hepatocyte fatty acids levels cause hepatic insulin resistance [35]. Disturbed insulin signaling in hepatocytes leads to steatosis associated with the activation of SREBP-1c and induction of fatty acid synthesis [36]. The severity of insulin resistance is correlated with the severity of NASH. The relationship between NAFLD and lipid metabolism has been extensively investigated in studies that analyzed the hepatic gene expression profile in animals fed a high-fat diet [37] and in liver biopsy samples from NAFLD patients [38–42]. The gene expression profile was generally similar to that in AFLD. However, fluctuations in PPARα, a regulator of fatty acid oxidation, and AMPK may be conflicting in NAFLD [24, 39, 42], and their roles in steatosis may be less important than in AFLD. Therefore, fatty acid oxidation, through the changes in AMPK, PPARs, and mitochondrial function, may be significantly altered in ALD, although studies suggest that activation of this fatty acid oxidation pathway may improve NAFLD.

Recent findings suggest that the cannabinoid system also plays an important role in the development of fatty liver [43–45]. In animal studies, ethanol and high-fat diet upregulated the activity of cannabinoid 1 (CB1) receptors by increasing the synthesis of endocannabinoid, 2-arachidonoylglycerol or anandamide. CB1 receptor activation upregulated several lipogenic factors, including SREBP-1c, ACC, and FAS, and downregulated CPT-1, which increased de novo fatty acid synthesis and decreased fatty acid oxidation. Conversely, administration of a CB1 receptor antagonist suppressed the lipogenic effect in these animals and CB1 receptor-knockout mice were resistant to ethanol- or high-fat-induced fatty liver. CB1 receptor agonist treatment induced the expression of lipogenic genes in wild-type mice. However, the cannabinoid receptor signaling pathway in the context of lipid metabolism has not been understood well.

Cholesterol Absorption and Metabolism in AFLD and NAFLD

In humans, cholesterol is absorbed from the diet and synthesized by cells in various tissues. A human male weighing 60 kg contains approximately 140 g of cholesterol in the body, and about 1% of the total cholesterol is involved in a dynamic metabolic cycle [46]. Although the levels of dietary cholesterol intake vary between countries and depend on individual eating habits, the estimated daily dose (300–500 mg/day) aggregates into micelles with biliary cholesterol (800–1,300 mg/day) in the duodenum [42]. Then, approximately 50% of the cholesterol is absorbed through Niemann–Pick C1-like 1 (NPC1L1), a cholesterol transporter expressed on the brush border membrane of the jejunum. After reconstruction into chylomicrons, the cholesterol is transported to the liver [47]. There are also transporter pump systems in the intestine and liver that use ATP-binding cassette (ABC) G5/G8 to excrete cholesterol into the intestinal lumen [48]. In humans, NPC1L1 is abundantly expressed on the canalicular membrane of hepatocytes and may facilitate the hepatic accumulation of cholesterol, although the exact functions of hepatic NPC1L1 remain unknown.

The main metabolic pathways of cholesterol in healthy human hepatocytes are as follows: (1) cholesterol de novo synthesis (acetyl-CoA–mevalonate–cholesterol), (2) cholesterol uptake in the form of LDL and chylomicron remnant, (3) cholesterol excretion into the blood in the form of VLDL, (4) cholesterol excretion and uptake through bile via ABCG5/G8 and NPC1L1, and (5) synthesis of bile acids and their excretion. These pathways are involved in the maintenance of cholesterol levels with a specific range. However, in AFLD and NAFLD patients, these regulation systems are disorganized. SREBPs act as sensors of hepatic cholesterol levels and activate genes involved in the synthesis of cholesterol and free fatty acids [49]. In the activation of SREBPs, SREBPs are first translocated to the Golgi apparatus by SREBP cleavage activating protein (SCAP). SCAP has a cholesterol sensing domain and its activity is controlled by intracellular cholesterol levels. Next, SREBP undergoes proteolytic cleavage in the Golgi apparatus and the activated form is released to the nucleus. Under normal circumstances, when intracellular cholesterol levels are high, SCAP activity and SREBP activation is suppressed. However, in AFLD and NAFLD, the regulatory loop of SREBP is disturbed, even if the intracellular levels of cholesterol and/or fatty acids are high [22]. In our earlier study using biopsy samples from NAFLD patients, despite excess cholesterol accumulation in hepatocytes, de novo cholesterol synthesis remained greatly upregulated despite the downregulation of SREBP-2 [50]. In their livers, as evidence of excess cholesterol accumulation, cholesterol uptake was downregulated because the expression of LDL receptor (LDLR) was significantly downregulated. Although cholesterol excretion was enhanced via overexpression of ABCG5/G8, apolipoprotein B, and microsomal triglyceride transfer protein (MTP) [50], it was considered that the secretion of VLDL is increased and the secretion level reaches a plateau in NAFLD patients. In contrast, MTP expression is decreased in the livers of ethanol-fed animals [51]. Nevertheless, the excretion of cholesterol may be impaired in both AFLD and NAFLD. However, even in this condition, cholesterol was still being synthesized, as demonstrated

by upregulation of HMG-CoA reductase and synthase, farnesyl P-P synthase, and squalene synthase [50–52]. Excess levels of cholesterol and its oxysterol metabolites, which are agonists for liver X receptor-α (LXRα) [52], lead to excessive fatty acid synthesis and steatosis through the activation of the LXRα–SREBP-1c pathway. LXRα expression was also upregulated in the liver of NAFLD patients [51, 52]. In animals with chronic alcohol consumption, hepatic cholesterol levels were increased via the activation of SREBP-2 and HMG-CoA reductase, while LDLR levels were decreased [53]. As shown in Fig. 40.1, the gene expression profile in hepatocytes is generally similar in NAFLD and AFLD. Accordingly, cholesterol uptake in the form of LDL is limited by the intracellular accumulation of fatty acid and cholesterol, while fatty acid synthesis and cholesterol synthesis are upregulated in the NAFLD and AFLD liver. These findings suggest that the feedback system controlling intracellular lipids levels is disrupted in these diseases.

The Nutritional State in AFLD and NAFLD Patients

In the field of dietetics, it is well known that patients with severe ALD lapse into absolute malnourishment as compared with healthy individuals. However, the nutritional state in patients with AFLD, which is considered to be an early stage of ALD, is unclear because their intake of specific nutrients has not been precisely determined. This may be because clinicians can recognize fewer than 30% of significant drinkers within their patients and it is difficult to determine the extent of alcohol consumption [54]. In a recent study in Finland, the percentage of AFLD patients with metabolic syndrome or type 2 diabetes was similar to that in NAFLD patients [55]. Accordingly, it is now accepted that excess nutritional intake is a synergistic steatotic factor in many AFLD patients. Obese patients with AFLD have recently become a focus of research and were included in our analysis of Japan individuals. Drinking alcohol in-between meals reduces fat oxidation in the liver at 30% [56]; therefore, alcohol intake will increase fat accumulation unless the effects of alcohol are offset in some way. It has been hypothesized that an equivalent amount of fat to the amount of alcohol consumed should be removed from the meal to maintain the lipid metabolism balance [57, 58]. Since alcohol consumption enhances the accumulation of abdominal fat and is associated with hypertension and dyslipidemia, it may be a risk factor for metabolic syndrome [59]. However, in several recent epidemiological studies, the typical features of metabolic syndrome have not usually been shown and the risk of fatty liver does not increase in mild to moderate alcohol consumers [60–63]. In this way, the associations among alcohol, fatty liver, and metabolic syndrome are complicated and differ between individual patients.

On the other hand, some nutritional analyses of NAFLD patients have suggested that high-fat, high-fat plus low-protein, high-carbohydrate, and/or high-cholesterol diets are the main causes of NAFLD [64–67], although definitive conclusions have not been reached. Of course, many NAFLD patients show excess nutrition intake, obese, and/or insulin resistance; however, some NAFLD patients do not show these features. In our nutritional analysis, nonobese NAFLD patients had some features that differed from those of obese patients [68]. For example, the dietary intake of total energy, fat, and carbohydrate was markedly higher in obese NAFLD patients with insulin resistance than in nonobese NAFLD patients without insulin resistance. In contrast, cholesterol intake was significantly higher in nonobese NAFLD patients than in obese NAFLD patients. We have compared the hepatic expression of lipid metabolism-associated genes between nonobese and obese NAFLD patients and found that LXRα expression levels were significantly higher in nonobese patients than in obese patients [41]. Of note, cholesterol overload upregulates LXRα expression via the increase of oxysterols, metabolites of cholesterol, which act as agonist of LXRα. These results indicate that excess cholesterol intake (cholesterol supply) is an appropriate stimulant for the development of fatty liver similar to excess nutrition intake and that excess cholesterol intake alone can induce liver steatosis, even though the total calorie intake may be within the normal range. Furthermore, recent reports in model animals support

our findings in nonobese NAFLD patients. Fatty liver without obesity can be established in animal models by feeding them the hypercholesterolemic but normal calorie diets [69–71]. However, this animal model showed marked hypercholesterolemia, which was not observed in our patients. This may be because the diet for animals contains extremely high levels of cholesterol (0.2–1.25%). It may also explain why serum cholesterol levels are preserved in NAFLD patients because dietary cholesterol is promptly taken up into the hepatocyte cholesterol pool.

Cholesterol Management as a Treatment for Steatosis/Steatohepatitis

As described above, it seems that cholesterol overload may be an initiation/basic factor for the development of fatty liver. Although the progression from simple steatosis to steatohepatitis usually involves the second hit, such as oxidative stress and inflammation, studies of nutritional animal models show that the accumulation of cholesterol rather than triglycerides and fatty acids plays a critical role in this progression, possibly because of increased susceptibility to oxidative cell death [72]. Moreover, it has been suggested that the regulation of cholesterol can control C-reactive protein levels and insulin sensitivity [72]. Conversely, the progression of triglyceride accumulation and suppression of fatty acid oxidation was not hepatotoxic and actually protected against worsening liver damage [73]. Therefore, cholesterol management is considered to be a promising treatment target for NAFLD and AFLD.

Ezetimibe, a NPC1L1-specific inhibitor, is used to lower blood cholesterol levels by selectively inhibiting cholesterol absorption from the intestine. It blocks cholesterol and plant sterol absorption from the diet and bile acids in humans and in animals [74]. Ezetimibe is quickly absorbed, undergoes glucuronidation, and enters the enterohepatic circulation. Its half-life is 24 h and it does not inhibit the activity of enzymes involved in drug metabolism. Clinically undesirable drug interactions have not been found between ezetimibe and inhibitors of cholesterol synthesis (statins). It is nutritionally important that ezetimibe does not inhibit the absorption of fat-soluble vitamins.

In our clinical study, we treated nonobese NAFLD patients showing excess intake of dietary cholesterol with ezetimibe [75]. As a result, their serum ALT levels decreased by $49.33 \pm 16.09\%$ and $45.25 \pm 24.19\%$ at 6 and 12 months, respectively, after starting ezetimibe therapy, while ultrasonography showed reductions in steatotic features in some patients. In other reports, NPC1L1-knockout mice with excess nutrition intake were resistant to fatty liver, and ezetimibe had significant therapeutic effects in animal models of NAFLD [76, 77]. These findings suggest that overintake and hepatic accumulation of cholesterol, as well as the activation of the cholesterol–LXRα–SREBP1c pathway, play an important role in the development of NAFLD. Furthermore, inhibiting cholesterol absorption with ezetimibe, for example, and reducing dietary cholesterol intake may offer a reliable therapeutic strategy for NAFLD. It was also reported that HMG-CoA reductase inhibitors (i.e., statins) decrease serum ALT levels in NAFLD patients [78–80].

Hence, reducing intrahepatocytic accumulation of cholesterol seems to be a fundamental treatment strategy for NAFLD [81]. To establish treatments associated with cholesterol management, the following questions should be assessed in future studies. (1) Is a cholesterol-restricted diet really effective against NAFLD and AFLD? (2) Is ezetimibe effective for obese and insulin-resistant patients with NAFLD and AFLD? Because the dietary intake of cholesterol is significantly higher in these patients than in healthy volunteers [68], ezetimibe may be effective in NAFLD patients with obesity and insulin resistance. However, other factors associated with obesity and insulin resistance are involved in the development of fatty liver and these factors may mask the effect of ezetimibe. (3) Can long-term cholesterol management therapy with ezetimibe and/or statins really improve steatosis in the NAFLD livers? In some previous studies, cholesterol lowering with HMG-CoA reductase inhibitors for 1–2 years decreased ALT levels but did not significantly improve steatosis [78, 80]. (4) Does the therapeutic effect of statin in combination with ezetimibe surpass that of monotherapy?

(5) It is important that the clinical effect of cholesterol management therapy should be assessed separately for patients with simple steatosis and those with steatohepatitis. (6) Finally, is there a synergistic/additive effect of cholesterol management therapy in combination with antioxidant therapy or liver protection therapy?

Conclusions

Lifestyle modifications offer simple therapeutic targets for AFLD and NAFLD. Nutritional support and behavioral and cognitive therapies that are aimed at reducing and avoiding overeating, particularly excess cholesterol intake, should be developed alongside pharmaceutical treatments to prevent the progression of these diseases to cirrhosis and HCC. According to previous clinical and nutritional studies, strategies targeting cholesterol accumulation offer basic therapeutic approaches for NAFLD patients. Considering the hepatic expression profiles of lipid metabolism-associated factors in ALD/AFLD patients, similar therapeutic approaches may also be effective in these patients. The potential clinical benefit of cholesterol management therapy with respect to hepatic steatosis and injury remains to be established in appropriately designed trials for AFLD and NAFLD patients. Large-scale clinical studies using cholesterol-restricted diets as nutrition therapy or pharmacotherapy with ezetimibe and/or statins are now urgently needed.

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Chapter 41
Dietary Fatty Acids and Alcoholic Liver Disease

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Key Points

- Provide evidence of abnormal lipid profiles in the blood and organs of alcoholic patients and discuss the relationship between alcoholic liver biochemistry and pathology
- Define fatty acids and their dietary sources and describe the current intake of dietary fatty acids
- Provide a review of the research on the interaction between fatty acid metabolism (including elongation/desaturation, catabolism, and eicosanoid production) and alcohol exposure
- Discuss the benefit and detriment of dietary saturated and polyunsaturated fatty acid supplementation on alcoholic liver disease
- Provide perspectives on dietary fatty acid intake in alcoholic liver disease

Keywords Alcoholic liver disease • n-3 fatty acids • n-6 fatty acids • Fatty acid composition • Prostaglandins • Fatty acid supplementation

Introduction

Alcoholic liver disease (ALD), such as fatty liver, hepatitis, or fibrosis, is frequently observed in patients with a long history of excessive alcohol intake. These types of ALD are considered alcohol-associated lifestyle diseases and involve both genetic and environmental factors [1]. Interactions between alcohol and nutritional status, which are one of the secondary risk factors, may also be important. Indeed, the presence and extent of protein-calorie malnutrition have important roles in determining the outcome of patients with ALD. Micronutrient abnormalities, such as hepatic vitamin A depletion or depressed vitamin E levels, may also potentially aggravate liver disease [2]. Additionally,

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obesity and excess body weight have been associated with an increased risk of ALD [3, 4]. Alcoholic and nonalcoholic fatty liver each begin with the accumulation of lipids in the liver, which, although a reversible condition, is understood to play an important role in the development of advanced liver disease. With continued alcohol intake, the development of steatosis may progress to hepatitis and fibrosis and might lead to liver cirrhosis. Excessive alcohol intake adversely influences the liver through the production of toxic products such as acetaldehyde and potentially highly reactive oxygen molecules, which are generated by alcohol dehydrogenase and the microsomal ethanol oxidizing system. These products can directly and indirectly interfere with the normal metabolism of other nutrients, particularly lipids, contributing to liver cell damage [5].

Abnormal lipid profiles of various blood cells and organs have been frequently observed in severe alcoholics [6] or reported in animal studies [7–10]. One of the most significant and consistent effects of alcohol on lipid metabolism is the change in the long-chain polyunsaturated fatty acid (LC-PUFA) composition of phospholipids in liver and other tissues. These alterations in membrane fatty acid composition are considered to affect erythrocyte membrane fluidity [11] and enzymatic function [12]. Additionally, ethanol-induced liver injuries involve the interaction of eicosanoids and other lipid peroxides derived from membrane PUFA [13–16]. Loss of n-3 and n-6 LC-PUFA may be associated with the pathogenic mechanism of liver disease.

On the other hand, over the past four decades, the amount and type of dietary fatty acid supplementation have been studied in the context of potentiating or preventing alcoholic liver injury [17]. Animal studies demonstrated that dietary unsaturated fatty acids (e.g., corn oil or fish oil) exacerbated damage by increasing oxidative stress, while saturated or middle chain fatty acids were protective in experimental models of alcoholic liver injury [13, 18–20]. However, PUFA function as structural elements involved in membrane integrity and as precursors for bioactive signaling molecules, contributing to the maintenance of hepatic function and regeneration. Regarding n-3 fatty acids, several studies suggest n-3 fatty acid supplementation alleviated hepatic steatosis in alcoholic [21, 22] and nonalcoholic liver disease [23], while decreased inflammatory response was noted in an acute hepatitis animal model [24]. Thus, continued discussion regarding the pros and cons of dietary PUFA supplementation on alcoholic liver is required.

Recent cellular, molecular, and clinical studies of saturated and unsaturated fatty acids or their derivatives have provided insights into their role in alcoholic liver pathology. In this chapter, we will review the biological function of PUFA and their derivatives, the possible role of PUFA loss on the development of hepatopathology, and finally review the current knowledge regarding dietary PUFA, mainly the role of n-6 and n-3 PUFA in alcoholic fatty liver and disease progression.

Role of Fatty Acids and Their Derivatives in Alcoholic Liver: Impact on Disease Progression

Abnormality of Tissue Fatty Acid Composition in Alcoholic Liver Disease

As mentioned above, abnormal plasma fatty acid composition has been observed in alcoholic patients [6, 25, 26] and patients with end-stage liver disease [27–29]. Several researchers observed that alcoholic patients, especially those with liver injury [26], showed low PUFA levels in plasma and tissue phospholipids. Additionally, a lower concentration of long-chain n-3 fatty acids was observed in alcoholic livers [30] and animal studies [31, 32]. An alcohol-induced decrease in tissue PUFA may be the result of several processes including reduction or unbalanced intake of dietary EFA, increased fatty acid synthesis, decreased elongation/desaturation reaction, upregulated fatty acid catabolism, and utilization or other derivative production.

Dietary Fatty Acids and Alcoholic Patients

Dietary fatty acids are derived from acylglycerols, free fatty acids, phospholipids, and sterol esters and are stored primarily in adipocytes as triacylglycerol. The fatty acids present in various lipid molecules are the major components of dietary fats. Researchers in the alcohol field use various dietary fats including tallow, palm oil, and cocoa butter as saturated fatty acids and corn oil and fish oil as unsaturated fatty acids [12, 13, 22, 33]. There are two types of unsaturated fatty acids, omega-6 (n-6) series derived from linoleic acid (LA; 18:2n-6) and omega-3 (n-3) fatty acids derived from α(alpha)-linolenic acid (ALA; 18:3n-3). LA and ALA are essential fatty acids (EFA) for higher animals since they are not synthesized in the body and must be obtained from the diet. Among unsaturated fatty acids, LC-PUFAs, such as arachidonic acid (AA; 20:4n-6), are found in animal meat, fish oil, egg yolks, human milk, and some seaweeds. Eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) are found mainly in fish oil [34]. The general dietary LA intake in Western society is approximately 8–20 g/day [35], which has increased during the last century primarily due to the consumption of soybean oil [36]. AA intake is much less than LA, approximately 100–130 mg/day, while intake of n-3 PUFA, mainly ALA (1–3 g/day), is far less than n-6 PUFA [37]. Dietary intake of EPA and DHA is approximately 130–900 mg/day [38], with the occurrence of regional variations due mainly to habitual fish consumption. There is evidence that regionally associated plasma fatty acid profiles are largely due to difference in food intake, as was shown in a large cross-sectional European multicenter study [39]. Ecological correlations were observed between fish intake and long-chain n-3 PUFAs, and olive oil intake and oleic acid [39]. On the other hand, using a National (US) Health and Nutrition Examination Survey 2001–2002 of 4,168 adults, Kim et al. reported on the relationship between self-reported alcohol consumption and dietary fatty acid intake. Among men, an inverse relationship existed between frequency of binge drinking and total PUFAs, LA, ALA, and EPA [40]. Alcohol consumption may affect the proper dietary intake of nutrients and fatty acids.

Alteration of Fatty Acid Metabolism in Alcoholic Liver and Possible Role of PUFA Deficiency in Disease Progression

PUFA status may be compromised by alcoholic pathology, in which there is a promotion of fatty acids synthesis or reduction of oxidation. Due to the accumulation of reducing equivalents in the cytosol following ethanol and acetaldehyde metabolism, the rates of saturated/monoene fatty acid biosynthesis and subsequent esterification into triglycerols are markedly increased [41, 42]. A number of studies on the mechanisms of alcoholic steatosis have been undertaken in the last decade [43, 44].

Essential fatty acids can be converted through elongation or desaturation primary in liver and brain [45]. LA is converted to AA via dihomo-γ(gamma)-linolenic acid (DGLA; 20:3n-6), while ALA is also converted to EPA and DHA by the introduction of a double bond and extension of its chain length, as shown in Fig. 41.1. It has long been suggested that these alterations, such as a decrease in arachidonate and other highly unsaturated fatty acids induced by ethanol, are caused by a reduction in delta-6 and delta-5 desaturase activity [8, 10, 46, 47], while Pawlosky and Salem showed that the concentration of several PUFAs, including 20:4n6 and 22:4n6, as well as 22:5n6, which is the product of delta-6 and delta 5 desaturase, in total liver lipids of patients with primary biliary cirrhosis was not altered compared to control [30]. They also examined the direct effect of ethanol consumption on EFA metabolism using in vivo isotope tracer studies in primates [48–50] and felines [30]. They found increased incorporation of deuterated 18-carbon EFA into AA and DHA over short periods rather than inhibition [48]. Prolonged periods of moderate alcohol consumption had no effect on the uptake of either LA or ALA into the plasma and led to an increased incorporation of these deuterated precursors

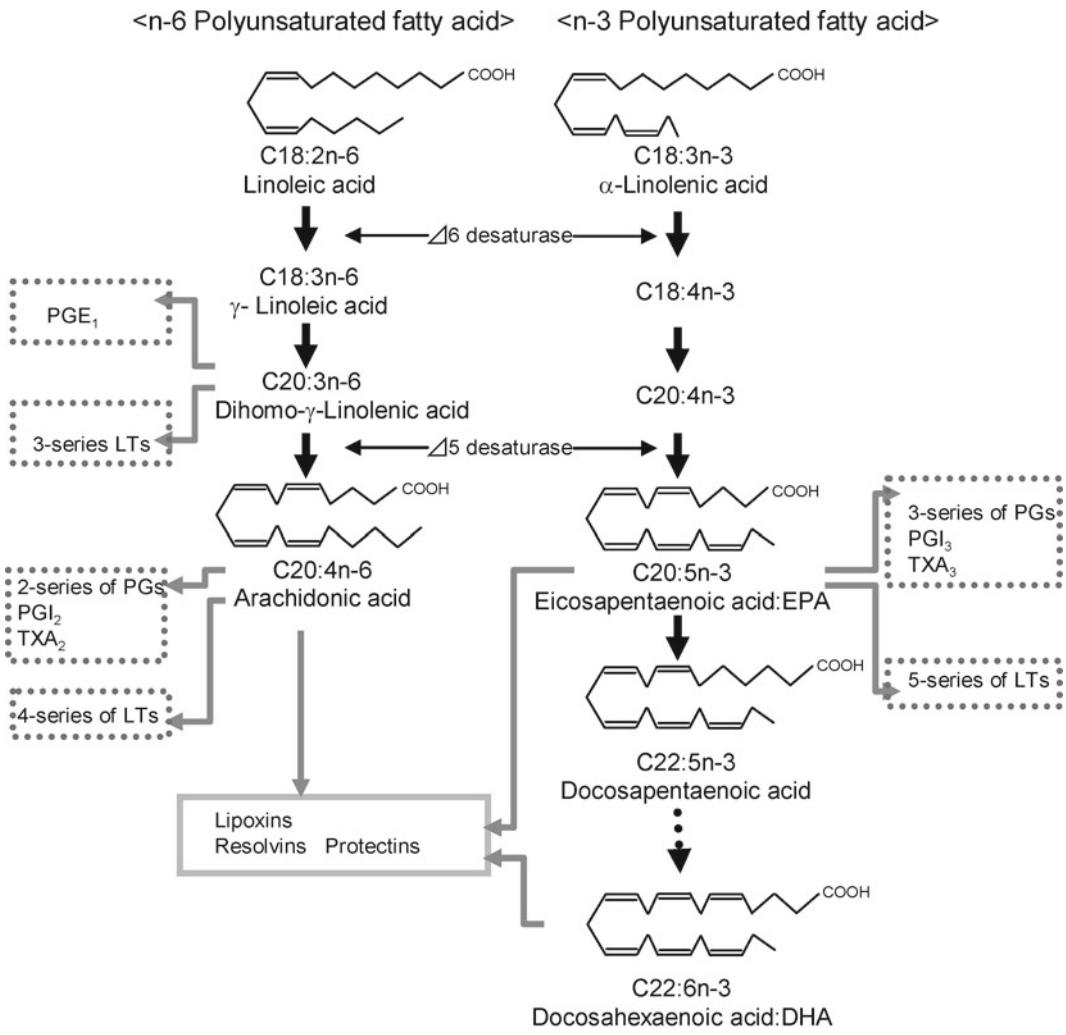

Fig. 41.1 Metabolic pathways for n-6 and n-3 polyunsaturated fatty acids

into AA and DHA. Their major finding showed that fatty acid desaturation was not affected by alcohol ingestion, whereas the reactive oxygen species and metabolized products generated by ethanol likely increased PUFA catabolism [30].

Numerous studies suggest that alcohol has prooxidant effects, which highly interact with cytochrome P450 2E1 and form hydroxy radicals that react with protein, lipids, and nucleic acids [51, 52]. Therefore, highly unsaturated fatty acids, such as AA and DHA, may be susceptible to this reaction with molecular stimulation of lipid degradation/peroxidation [30]. Indeed, urinary excretion of 4-hydroxynonenal (4-HNE) was observed at higher levels in alcoholics relative to controls [15, 53]. Additionally, isoprostanes, such as F2-isoprostanes, which are produced in vivo by nonenzymatic free-radical-induced lipid peroxidation and are markers of oxidative stress, also increased [54]. From these findings, alcohol-stimulated lipid accumulation or degradation/peroxidation and partial decrease in EFA intake likely influenced PUFA status in plasma and tissue phospholipids. It has been reported that these alterations might play a major role in cell membrane fluidity and integrity [55] in both hepatocyte membranes and erythrocyte membranes [11, 25, 56]. Moreover, alcohol-induced PUFA deficiency probably modifies the induction of LC-PUFA-derived metabolites, such as eicosanoids.

The cell membranes of most tissues contain phospholipids and are characterized by predominantly having PUFA, such as LA, AA, and EPA, esterified in position 2. On activation of phospholipase A_2, AA is normally released and oxidized by both lipoxygenase and cyclooxygenase (COX). Some of these long-chain metabolites not only form precursors to respective prostaglandins (PGs), thromboxanes (TXs), and leukotrienes (LTs) but also lipoxins (LXs) and resolvins that have potent anti-inflammatory actions [57]. The noted protective effects of n-3 PUFAs (EPA, DHA)have been attributed not only to eicosanoid inhibition but also to the formation of novel biologically active lipid mediators (i.e., resolvins and protectins). Prostanoids formed in the liver have several functions in hepatocytes, including glycogenolysis and DNA synthesis [58, 59]. Liver nonparenchymal cells, such as sinusoidal endothelial cells [60] and Kupffer cells (KC) [61, 62], are known to produce significant amounts of PGI_2 and PGE_2. KC are also known to produce LTs and COX in response to various stimuli [61]. KC play multiple roles in initiation and progression of alcoholic steatohepatitis [63] and are activated via a mechanism dependent on gut-derived endotoxin in an alcoholic liver model. KC may release active mediators such as proinflammatory cytokines and eicosanoids. Enomoto et al. reported that alcohol induced fatty liver-associated upregulation of PGE_2. They observed that alcohol induced hepatocyte fat accumulation and PGE_2 production by KC [64], when rats were given a single large dose of ethanol intragastrically. This increase was attenuated by inactivation of KC and administration of antibiotics and a COX-2 inhibitor. It was suggested that an ethanol-induced increase in PGE_2 production from upregulation of COX-2 in endotoxin-activated KC may increase triglyceride accumulation [64]. On the other hand, PGI_2 and TXA_2 are involved in vasoactive function. The decreased production of the vasodilator prostanoid PGI_2 enhanced liver injury and portal hypertension [65]. Nanji et al. also found reduced PGI_2 production by liver nonparenchymal cells obtained from ethanol-treated rats and suggested that decreased PGI_2 production may have contributed to the hepatotoxic effect of ethanol [66]. TXA_2 is the major eicosanoid produced by platelets. TXA_2 is a potent proaggregant and a powerful vasoconstrictor of vascular smooth muscle cells. It is also reported that the severity of liver injury was negatively correlated with plasma PGE_2 and positively correlated with plasma LTB_4 in experimental rats fed a liquid diet with corn oil, including a high dose of ethanol constituting 42% of total calories, for up to 2 months [67]. They proposed the importance of the altered TX/PGE_2 balance in the development of fibrosis and cirrhosis [67]. Alcohol ingestion seems to have an influence on eicosanoid production, and the effect might be dependent on the disease stage, such as formation of fat deposition, hepatic microcirculation, and activation of immune responses.

Other lipid mediators, endocannabinoids and N-acylethanolamines, which are derivatives of PUFA, play important functional roles both in the central nervous system and in peripheral organs via interaction with cannabinoid receptor 1 and 2 (CB1-R and CB2-R) [68]. Two main endocannabinoids, 2-arachidonoylglycerol (2-AG) and arachidonoylethanolamide (AEA, also called anandamide), have been demonstrated to be involved in various functions such as the regulation of food intake, neurotransmitter release, bone formation, and pain. Intriguingly, consumption of a high-fat diet or alcohol induces fatty liver, increases the hepatic expression of CB1 receptors, and upregulates endocannabinoids in the liver. Several studies showed that chronic alcohol consumption stimulates hepatic stellate cells (HSCs) through CB-1R by production of 2-AG and expression of lipogenic genes, including sterol regulatory element-binding protein 1c (SREBP-1c) and fatty acid synthetase [69–71].

HSCs are pivotal in the fibrotic response to liver injury, as these cells undergo activation with an increase in extracellular matrix deposition during fibrogenesis. Induction of collagen type I gene expression is a key component of increased fibrogenesis by HSCs [72]. HSCs are activated by various stimuli, such as cytokines and free radicals produced by neighboring cells, such as KC and apoptotic body of hepatocyte. Reactive oxygen species and lipid peroxidation have emerged as important stimuli to collagen gene induction in HSCs [73]. Malondialdehyde and 4-HNE can increase collagen expression [74].

AA, as a component of cell membranes, is a known target for autoxidation and is susceptible to lipid peroxidation and lipid peroxidation-derived products. Cubero and Nieto observed that in vitro-cultured HSCs isolated from rats fed with an ethanol diet proliferated faster and exhibited increased

activation and increased collagen production compared with HSCs from rats fed a control diet. When HSCs from control rats were cocultured with KC from ethanol-treated rats, activation and collagen production of HSCs were upregulated compared with HSCs only. With addition of AA in the culture medium, HSCs and KC were affected synergistically, which was associated with oxidative stress. Interestingly, HSCs and KC cocultured from ethanol-treated rats showed decreased levels of collagen I secretion. This suppression of the fibrogenic effect, which is concomitant with increased levels of tumor necrosis factor-α(alpha) and glutathione, was restored with addition of AA to the culture medium. They proposed that two "hits," synergism with chronic ethanol consumption and PUFA (e.g., AA), activate KC, which likely associate with reactive oxygen species and modulate the fibrogenic response of HSCs even if chronic ethanol sensitizes HSCs to an anti-fibrogenic status [75].

Taken together, these results suggest that alcohol seems to stimulate lipid peroxidation and degradation and generate hydroperoxy or aldehyde compounds, and the loss of PUFA is probably attributable to the catabolism of PUFA, low dietary EFA intake, and antioxidative substances. The decrease in cellular fatty acid composition is likely to contribute to organ pathology. However, these alcohol effects are probably dependent on the dose and duration of ethanol administration.

Effect of Dietary Fatty Acid Supplementation on Alcoholic Liver Disease

Saturated Fatty Acids

Interestingly, it is well documented that the relative proportion of fatty acids in various tissues is influenced by both total caloric intake and the fatty acid composition of the diet [76]. In animal models, for example, diets containing saturated fatty acids are protective against alcohol-induced liver injury [19, 77, 78]. At the molecular level, saturated fatty acids are thought to attenuate ALD progression [33] via downregulation of Cox-2 and TNF-α(alpha) in a rat alcoholic liver model [79]. These effects possibly occur through increased membrane resistance to oxidative stress, partially mediated through the induction of adiponectin [80, 81]. Molecular models of sirtuins 1 and hepatic SREBP-1 suggest suppressed expression of genes encoding lipogenic enzymes and decreased synthesis of hepatic fatty acids [82].

Polyunsaturated Fatty Acids

It has been suggested that polyunsaturated fatty acids such as corn oil or fish oil are a requirement for the development of alcoholic liver disease [13, 19, 77, 78, 83, 84]. In these experiments, animals were fed a nutritionally adequate to high-fat diet (25–35% calories as fat, LA 2.5–59%) with excess ethanol. Continuous intragastric feeding with high-unsaturated fat diets was shown to cause liver fibrosis in rats, possibly through increased membrane resistance to oxidative stress. LA is known to be essential for the development of alcoholic liver disease in this model. It has been suggested that PUFA from fish oil (with the exception of menhaden oil) worsen alcohol-induced liver injury with markedly increased CYP2E1 induction and lipid peroxidation [85]. However, these studies were undertaken using a concentration of fatty acids that far exceeds physiological levels.

On the other hand, Goheen reported in an earlier study on rats fed ad libitum liquid diets containing 34% of the calories as ethanol and 35% as fat, with a small amount of AA (29 mg/day) and without AA, for 4 weeks. The liver TG content of rats in the AA(+) group was reduced ca. threefold over that of rats in the AA(−) group [86, 87]. Our laboratory previously reported on ethanol-treated rats fed lard (10% fat content) with AA ethyl ester (AA: 3% of total weight) [88] or AA-rich oil (AA: 2.4% of total

weight) [89]. Ethanol-treated rats (administered a single daily dose of 3 g/kg body weight) were fed lard or AA oil for 2 weeks. A small but not significant decrease in liver triglyceride was observed in the AA oil-fed rats. In histological observation, hepatocytes containing small to large vacuoles were seen in the periportal area in the ethanol-lard group and showed improvement in the AA oil-fed compared with the lard-fed rats after ethanol treatment [89]. These observations imply that AA decreases triglyceride levels in the liver.

Lakshman and colleagues examined the effect of low n-3 PUFA levels (2.8% of energy) in a rat model. They observed alcohol-mediated hyperlipidemia, and hepatic steatosis was inhibited by n-3 diet [90]. Intriguingly, recent studies also demonstrated the anti-steatogenic and protective effect of PUFA, including fish oil and AA/DHA oil, under certain experimental conditions.

Pawlosky and colleagues examined the effect of low n-3 EFA levels (ALA 0.08% of energy) but with an adequate level of LA (1.4% of energy) using a rhesus monkey, chronic ethanol consumption (mean consumption 2.4 g/kg/day) model. Liver PUFA content and histopathology showed that a marginal intake of n-3 fatty acids was a permissive factor in the induction of alcoholic liver fibrosis or cirrhosis in primates [50]. Wada designed a study in which mice were fed either safflower oil or fish oil (each 30% of total energy) prior to a single shot of ethanol administration (3 g/kg body weight). In the mice fed safflower oil, ethanol increased liver triglyceride threefold, with activation of SREBP-1c and carbohydrate response element-binding protein, which promote de novo lipogenesis, and increased PPAR-γ (gamma) and acyl-CoA diacylglycerol acyltransferases, mRNA expression, which promote triglyceride synthesis. When mice were fed fish oil, ethanol-induced fatty liver was reduced by 73%. Fish oil decreased SREBP-1c activity and increased PPAR alpha activity. They concluded that the prior ingestion of fish oil effectively prevents ethanol-induced fatty liver, at least in part by decreasing basal SREBP-1c activity [22]. Thus, habitual intake of fish oil may prevent fatty liver in acute alcoholics.

Song et al. showed that the consumption of a diet including PUFA prevents alcohol-induced fatty liver and mitochondrial dysfunction in an animal model [21]. Rats were fed an ethanol or control liquid diet containing 11% energy from fat. The basal diet had low but adequate levels of EFA (LA and ALA; each 0.3% energy), while the PUFA diet was identical except for the addition of low levels of AA and DHA (0.56 g/L each) in a nutritionally adequate liquid diet. Alcohol caused increased levels of ethanol-inducible CYP2E1, nitric oxide synthase, nitrite, and mitochondrial hydrogen peroxide. Interestingly, the elevated CYP2E1 and iNOS activities returned to basal levels, while the suppressed 3-ketoacy-CoA thiolase activity was restored in rats fed the alcohol-DHA/AA-supplemented diet. Their findings indicate the beneficial effects of physiologically relevant amounts of PUFA on the incidence of alcoholic fatty liver in this model. However, the mechanism of the protective effect of small amounts of LC-PUFA against steatosis remains unclear.

Some discrepancy exists with previous studies that showed detrimental effects of dietary polyunsaturated fatty acid supplementation on alcoholic liver disease. The contribution of dietary PUFA to alcoholic liver development is likely to be affected by fatty acid metabolism and de novo synthesis, which is influenced by the amount and duration of ethanol ingestion or dietary fat content. Whereas, it is postulated that it is potentially important to distinguish between dietary PUFA and their precursor EFA, such as LA and ALA. Sealls reported that lard and canola oil (rich in EFA: LA and ALA) diets showed high levels of hepatic triglycerides and cholesterol as well as elevation of lipogenic gene expression [91]. In comparison, the livers of mice fed a fish/fungal oil (rich in highly unsaturated LC-PUFA; EPA, DHA and AA) diet had low levels of lipid accumulation and more closely resembled the livers of mice fed standard laboratory chow. SREBP-1c and PPAR- γ(gamma) gene and protein expression were high in the livers of animals fed diets containing lard or canola oil compared to fish/fungal oil. Hepatic fatty acid analyses indicated that dietary PUFA was efficiently converted to LC-PUFA regardless of the source. Differences in hepatic lipid levels and gene expression between dietary groups were probably due to exogenous LC-PUFA rather than endogenous pools. These results may suggest that highly unsaturated LC-PUFA from an exogenous source rather than their precursor can suppress hepatic lipogenesis.

Moreover, the proper intake of n-6/n-3 fatty acids in alcoholic liver is still unclear. A decrease of n-3 PUFA in Western diets influences the risk of cardiovascular and mental illness. Generally, a lower intake of n-6 PUFA and higher intake of n-3 PUFA relative to common dietary levels is recommended for proper health and disease prevention. Schmocker recently reported on the inflammation-dampening effects of n-3 PUFA in the liver of transgenic fat-1 mice. These mice endogenously express a *Caenorhabditis elegans* desaturase. Therefore, the mice are able to form n-3 PUFAs from n-6 PUFAs. Feeding the fat-1 mice a diet rich in n-6 PUFAs resulted in significant enhancement of hepatic function and alleviation of chemically induced acute hepatitis compared with their wild-type littermates, which is associated with reduced TNF-α(alpha), IL-1β(beta), IFN-γ(gamma), and IL-6 gene expression [24]. Given the low n-3 intake of PUFA in alcoholics, supplementation with a permissive amount of dietary n-3 fatty acids may be protective. Further study on the effects of dietary fatty acids in alcoholic liver disease using a relevant model and their underlying mechanisms should be undertaken.

Summary

It appears that chronic alcohol consumption leads to an increase in PUFA utilization or catabolism. Concomitantly, a decrease in dietary EFA intake and antioxidative substances may contribute to the loss of LC-PUFA in the tissues and cells of alcoholics. In animal studies, since there is an interaction between fatty acid metabolism and de novo synthesis, experimental conditions among researchers may influence the effect of lipids on alcohol ingestion, such as differences in cells or animals used, dietary fat composition, route of administration, the dose and duration of alcohol exposure, dietary composition, and lipid class. Therefore, dietary modification remains the basic therapy for liver disease in alcoholics. Additionally, supplementation with physiologically relevant levels of dietary n-3 and n-6 LC-PUFA in antioxidative food substances might be protective against alcoholic liver injury. Determination of the ideal n-3 to n-6 ratio should be the focus of a future study.

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Chapter 42
Nutrition in Alcoholic Steatohepatitis

Juan Caballeria, Javier Michelena, and Jose Altamirano

Key Points

- Protein-calorie malnutrition is a common finding in patients with alcoholic steatohepatitis and correlates with the severity and prognosis of the disease.
- Adequate protein-calorie intake and replacement of nutritional deficiencies (vitamins and trace elements) is mandatory in the management of alcoholic steatohepatitis.
- Nutrition support in patients with alcoholic steatohepatitis improves nitrogen balance and liver function tests but does not enhance survival. Thus, nutrition support could be beneficial when administered with other treatments.
- Specific nutrients require further evaluation before being recommended in the treatment of alcoholic steatohepatitis.

Keywords Alcoholic steatohepatitis • Protein-calorie malnutrition • Enteral nutrition • Parenteral nutrition

Alcoholic steatohepatitis (ASH) is characterized by hepatocellular necrosis, ballooning degeneration, inflammatory reaction with polymorphonuclear leukocyte infiltration and fibrosis [1]. The severity of ASH ranges from asymptomatic cases to severe forms identified by the presence of encephalopathy or a discriminant function greater than 32. More recently, other severity scores such as the ABIC (age, bilirubin, INR and creatinine) identified patients with mild, moderate and severe ASH. The risk of death within 2 months after diagnosis is 40–50% in patients with severe ASH [2]. Corticosteroids are the recommended treatment in patients with severe ASH, but a significant percentage of patients do not respond to steroid treatment or have severe complications, especially bacterial infections [3]. Therefore, the search for alternative therapeutic options is mandatory.

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Only 20–30% of chronic alcoholics develop severe alcoholic liver disease (ALD), suggesting that other factors such as nutritional, genetic, hormonal or environmental play a role in the pathogenesis [4]. The role of nutritional status in the pathogenesis of ALD has been a matter of discussion for decades [5]. During many years, nutritional deficiencies were considered responsible for liver disease in chronic alcoholics. In the 1960s, Lieber clearly demonstrated in experimental models the direct toxic effect of alcohol and its metabolites to the liver [6]. Since the early1990s, the role of nutrition in the pathogenesis of ALD has been reevaluated, and there is no doubt that malnutrition and chronic alcohol consumption have a synergistic effect in the development of ALD as well as in favouring damage of other organs.

Causes of Malnutrition in Alcoholic Steatohepatitis

Deficiencies of nutrients are very common in alcoholic liver disease (ALD), and protein-calorie malnutrition has been associated with the morbidity and mortality of patients with ASH. The aetiology of malnutrition in ASH is multifactorial and includes anorexia and inadequate dietary intake, abnormal digestion and absorption of several macro – and micronutrients, increased protein catabolism, decreased hepatic uptake and storage of vitamins and trace elements and increased faecal and urinary looses of some micronutrients (Fig. 42.1) [7].

Alcohol is a source of calories and provides 7.1 kcal/g. Regular alcohol consumers are often overweight because of added calories from alcohol consumption to normal diet. By contrast, chronic alcoholics replace nutrient-derived calories by alcohol, resulting in weight loss and malnutrition. Furthermore, a substantial part of the energy is used in the microsomal ethanol metabolism pathway, synthesizing lactate and glycerophosphate. The inflammatory response of ASH leads to a catabolic state with depletion of muscle and visceral protein and increased resting energy expenditure that promote negative nitrogen balance [8].

Anorexia is a common feature in patients with ALD, leading to a diminished food intake and primary malnutrition. Anorexia may be partly due to elevated proinflammatory cytokines such as tumour necrosis factor-alpha [9] and leptin [10], which inhibit appetite and food intake. Anorexia may be partly related to the damage of upper gastrointestinal mucosa, aesophagitis and gastritis, secondary to heavy drinking.

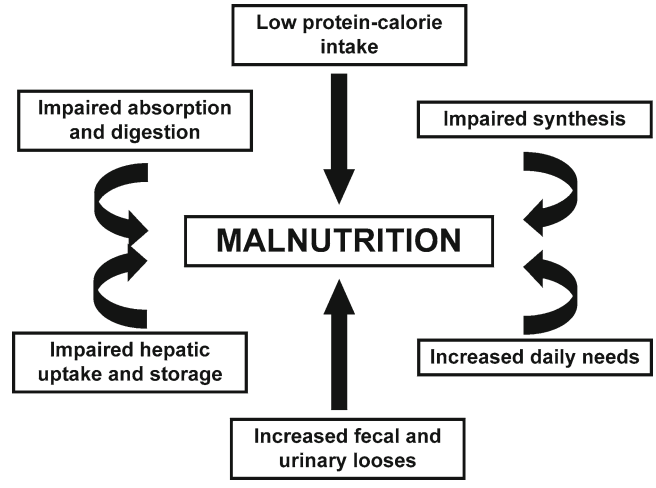

Fig. 42.1 Causes of malnutrition in alcoholic steatohepatitis

Malabsorption of dietary fat and proteins is also very frequent and is a consequence of decreased bile secretion and impaired secretion of pancreatic enzymes. Malabsorption contributes significantly to protein-calorie malnutrition and weight loss [11].

Low serum folate and red blood cell folate levels can be found in many patients with ALD. Folate deficiency in alcoholics is due to poor intake, impaired absorption, altered storage and increased urinary excretion [5, 12]. Vitamin B1 levels are decreased in most alcoholics, as well as pyridoxal-5′-phosphate, the biological active coenzyme of vitamin B6 [13], as a result of an inadequate intake but also of interactions between alcohol and pyridoxal-5′-phosphate metabolism [14]. Chronic alcoholism affects several aspects of vitamin A metabolism, including retinol absorption, enhanced degradation in the liver and a higher mobilization of retinol from the liver to other organs. Hepatic vitamin A levels are markedly decreased in ALD, even in the early stages of the disease [15]. Alcohol consumption may also enhance vitamin A hepatotoxicity since the induction of the cytochrome P450 2E1 isoenzyme favours the formation of toxic polar metabolites from retinoids. The consequences of vitamin A metabolism changes are alterations in hepatocyte regeneration and proliferation and enhanced hepatocarcinogenesis [16]. Deficiencies of other vitamins (C, D, E, K, riboflavin and cobalamin) and trace elements such as zinc, selenium, copper and magnesium are also frequent but less prominent [7]. Zinc deficiency is a cause of liver fibrosis [17].

Assessment of Malnutrition in ASH

It is important to have sensitive and easily applicable methods to assess the prevalence and degree of malnutrition in patients with ASH. The most available techniques are anthropometric measurements such as body mass index, mid-arm muscle area and triceps skinfold thickness. Twenty-four-hour creatinine excretion has been considered an indirect measurement of body muscle mass, as 1 g of excreted creatinine was related to 18.5 kg of muscle mass. The creatinine-height index has also been used. Other useful approach is the determination of resting energy expenditure using the Harris-Benedict equation that included sex, age, body weight and height [18] or other similar equations. Mendenhall et al. [19] described a protein-calorie malnutrition score, combining anthropometric (percentage of ideal body weight, skinfold thickness, mid-arm muscle area, creatinine-height index), biochemical (albumin, transferrin, prealbumin, retinol binding protein) and immunologic data (total lymphocyte count, CD4 lymphocytes, CD4-CD8 ratio, skin test response to a battery of antigens). It has to be taken into account that most of these parameters could be altered by the liver disease (protein synthesis, immunological status) or its complications (ascites and oedema can influence the value of some anthropometric parameters).

Role of Malnutrition in the Development and Progression of ASH

Several studies have demonstrated that patients with ASH had a low intake of non-alcohol calories than alcoholics with less advanced liver disease. In a study performed in chronic alcoholics without cirrhosis, classified as normal liver, steatosis and alcoholic hepatitis, we found that the daily ethanol intake and the duration of alcoholism were similar among the three groups of patients as well as the amount of alcohol-related calories (50–60%). On the contrary, the daily intake of non-alcoholic calories was significantly lower in patients with ASH than in patients with normal liver. The reduced non-alcoholic calories intake in patients with ASH was particularly caused by lower protein and carbohydrate intake (Fig. 42.2) [20]. In this study, we also observed that the consumption of vitamins was, in general, lower than the Recommended Dietary Allowances of the National Academy of

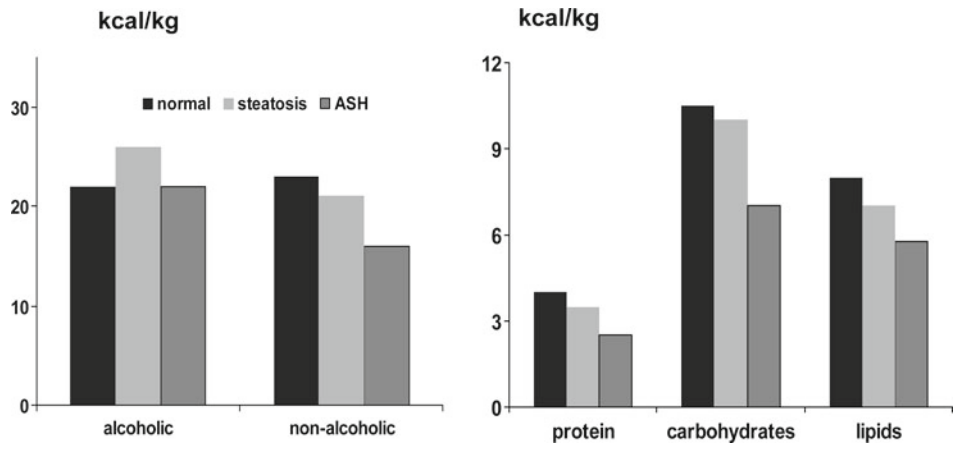

Fig. 42.2 Malnutrition in chronic alcoholics. Relationship between the daily intake of non-alcoholic calories, especially protein and carbohydrate, and the severity of the disease (Based on data from [20])

Sciences, USA. Daily intake of minerals, excepting iron, was also below the recommended. Among alcoholics, the lowest intake of vitamins and minerals was observed in the group of patients with ASH. These findings suggest that protein, carbohydrate and mineral malnutrition could play an important role in the development of ASH.

The relationship of protein-calorie malnutrition and ALD was analysed by Mendenhall et al. in two large VA cooperative studies [19]. According to the protein-calorie malnutrition score, some degree of malnutrition was present in 62% of patients with normal liver or early liver damage and in 100% of patients with ASH.

The intensity of malnutrition closely correlated with the development of liver disease complications, jaundice, ascites, encephalopathy and hepatorenal syndrome. There was also a clear association between the prognosis of ASH and the degree of protein-calorie malnutrition. One-month mortality correlated significantly with the protein-calorie malnutrition score. Furthermore, 6-month mortality was also significantly higher in patients with ASH and severe malnutrition than in those with moderate malnutrition. Although malnutrition in ASH is multifactorial, 6-month mortality was significantly associated to 1-month calorie intake.

Finally, protein-calorie malnutrition influenced the therapeutic response. In the VA studies, the efficacy of corticosteroids was independent of the intensity of malnutrition. By contrast, the beneficial effects of oxandrolone, an androgenic anabolic steroid, were only observed in patients with ASH and moderate malnutrition, and the response was even better when oxandrolone administration was accompanied with nutrition replacement.

These studies confirmed the role of malnutrition in the pathogenesis of ASH, its influence in the prognosis and in the response to some specific treatments.

Nutrition in the Treatment of ASH

Patients with mild to moderate ASH usually recovered in few weeks with alcohol abstinence and an adequate diet. Moreover, improvement of nutritional status is one of the most important supportive measures for hospitalized patients with severe ASH (Table 42.1). In this regard, it is essential to assure the necessary daily intake of calories and proteins and to correct vitamin and mineral deficiencies. Those patients need a daily intake of 1–1.5 g/kg of protein and 35–40 kcals/kg. Administration of

Table 42.1 Treatment of alcoholic steatohepatitis

Alcohol abstinence
Nutritional support
35–40 kcal/kg
1–1.5 g protein/kg
Vitamin supplements (B complex, folate, vitamin K)
Prevention and treatment of complications
Specific treatments

Table 42.2 Effects of parenteral or enteral nutrition in severe alcoholic steatohepatitis

Trials	12
Patients (mean; range)	36 (15–64)
Improvement	
Liver function tests	8/12
Histology	2/2
Nutritional status	6/8
Mortality	2/12

vitamin B1 (750 mg/day), B6 (750 mg/day), B12 (1,200 mg/day) and folate (15 mg/day) is also recommended [3]. When patients are too ill to achieve these requirements with the hospital diet, hypercaloric and hyperproteic supplements must be administered or, if necessary, total enteral or parenteral must be introduced.

The correction of nutritional deficiencies in ASH is not only a supportive measure, but it has been considered as a specific treatment for these patients. In fact, nutritional therapy is, after corticosteroids, the treatment most frequently assayed in ASH [12, 21]. At least 12 studies have been performed (Table 42.2). These studies have wide variations that make difficult its comparison, for example, the severity and the type of patients. Some of them focused in alcoholic cirrhosis, whereas others included patients with ASH, although most of them had underlying cirrhosis. The composition of nutrients as well as the way of administration, the duration and compliance to treatment was also different [22].

Parenteral Nutrition

Intravenous amino acid therapy was first assessed in 1980 by Nasrallah and Galambos in a randomized controlled trial enrolling 35 patients with ASH [23]. The administration of 70–85 g/day of standard amino acids during 4 weeks was associated with a greater improvement of liver function tests as compared by controls and a significantly lower short-term mortality. These results, with regard to mortality, were not confirmed in other studies [24, 25].

The effects of total parenteral nutrition, including amino acids, dextrose and intralipid, were compared with those of conventional diet [26]. Patients were stratified according to the severity of the ASH. Beneficial effects were only observed in patients with more severe ASH, although these effects were a more rapid improvement in biochemical and nutritional parameters, with no changes in short-term mortality.

Similar results were found in a randomized, controlled trial including patients with severe ASH from two US and Spanish hospitals [27]. Patients received an intravenous amino acid solution or dextrose during 4 weeks. Intravenous amino acid administration resulted in a significant improvement of nitrogen balance and liver function tests with no changes in short-term mortality or in 2-year mortality. On the other hand, treatment was well tolerated, and an increase of the episodes of hepatic encephalopathy or a greater difficulty in the control of ascites was not observed.

Enteral Nutrition

Enteral nutrition has also been evaluated in the treatment of ASH. Several studies have compared enteral feeding with oral conventional diet and conventional diet alone for 4 weeks. These studies, independently of the type of diet and the way of administration, showed modest and inconclusive effects on liver function with no changes in short-term mortality [28, 29].

The possible beneficial effects of enteral nutrition as a specific treatment of ASH were evaluated in a Spanish multicentric, randomized, controlled trial, comparing the short-term and long-term outcome of patients with severe ASH, treated with 2,000 kcal/day of a tube-fed total enteral nutrition or 40 mg/day of prednisolone for 4 weeks [30]. There were no differences in the short-term mortality between groups. Nine out of thirty six patients randomized to steroid therapy died during the first 4 weeks, as compared with 11 out of 35 patients receiving enteral feeding, although deaths occurred significantly earlier with enteral nutrition, median 7 versus 21 days. After hospital discharge, patients were followed for a maximum of 1 year. Ten out of twenty seven survivors of steroid group died during the follow-up, compared with only 2 out of 24 patients treated with enteral nutrition. Furthermore, seven of the ten deaths in the steroid group occurred within the first 6 weeks after discharge, and in nine of them, deaths were related to bacterial infections. The results of this study suggest a synergistic beneficial effect of corticosteroids and enteral nutrition in the treatment of severe ASH. A pilot study in which 13 patients were treated with both steroids and nutritional support resulted in a mortality of 15% at 1 year, lower than expected [31]. Unfortunately, a randomized controlled trial comparing this combining therapy versus corticosteroids alone has not yet been done.

Antioxidants

Oxidative stress plays an important role in the pathogenesis of ALD [32]. Many attempts have been done to investigate the role of different combinations of antioxidants in the treatment of ASH. An early trial of vitamin E, selenium and zinc in 56 patients with moderate or severe ASH showed 6.5% mortality in the antioxidant group compared with 40% in the placebo group [33]. However, a second study of 51 patients with mild to moderate ASH found no benefits with the administration of 1,000 mg/day of vitamin E [34]. Two additional trials in patients with severe ASH also showed negative results. In the first trial, Philips et al. compared the standard corticosteroid therapy with an antioxidant cocktail (beta-carotene, selenium, vitamins C and E, methionine, allopurinol, desferrioxamine and N-acetylcysteine), being the 30-day mortality significantly higher in the group of patients treated with antioxidants, although the better survival rate in corticosteroid-treated patients was lost after 1 year of follow-up [35]. In the second study, antioxidant therapy (n-acetylcysteine, vitamins A and E, biotin, selenium, zinc, manganese, copper, magnesium, folic acid and coenzyme Q), alone or in combination with corticosteroids, did not improve 6-month survival in patients with severe ASH [36].

Alcohol consumption results in a depletion of endogenous antioxidant capacities. ALD causes a selective deficiency in the availability of reduced glutathione in mitochondria. N-acetylcysteine (NAC) restores the glutathione mitochondrial stores and reduces oxidative stress, having an excellent tolerance and safety profile. Moreover, GSH inhibits apoptosis and proinflammatory cytokine production. For all these reasons, NAC is a potential therapeutic agent in the treatment of ASH. Nguyen-Khac et al. in a recent study compared the association of corticosteroids and NAC versus corticosteroids alone and found an increased in survival at 2 months in patients treated with the combination therapy [37]. By contrast, the administration of high doses of NAC with adequate nutrition showed neither additional survival benefits nor better biological improvement in patients with severe ASH [38]. The role of NAC in ASH needs further investigation in controlled trials.

Several so-called supernutrients with antioxidant properties have been assayed in the treatment of ALD, mostly patients with alcoholic cirrhosis, although in many cases with associated ASH. In ALD, there is impairment in methionine metabolism due to a difficulty to convert methionine to S-adenosylmethionine (SAMe), leading to a depletion of mitochondrial glutathione and oxidative stress [39]. These effects can be reverted by the exogenous administration of SAMe [40]. A multicentric, controlled trial showed that long-term treatment with SAMe decreased mortality in alcoholic cirrhosis [41], although confirmatory trials are needed before recommending this treatment [42]. Silymarin has antioxidant effects in experimental models of ALD. The studies in patients with ALD have shown contradictory results [43, 44], and a systematic review failed to detect a benefit in liver histology or mortality. However, the role of silymarin is now being review in ongoing clinical trials. Phosphatidylcholine prevents lipid peroxidation associated to oxidative stress in ALD. In experimental models, phosphatidylcholine deleted the development of fibrosis and progression of liver disease [45]. However, a long-term multicentre trial in patients with ALD and biopsy-proven mild fibrosis failed to demonstrate a beneficial effect of this drug on progression of fibrosis compared with patients taking placebo [46].

Summary

Protein-calorie malnutrition is very common in patients with ASH. Malnutrition plays an important role in the pathogenesis, severity and outcome of ASH. Sufficient nutritional repletion altogether with other supportive measures may be effective in reducing complications and mortality in patients with severe ASH. Nutritional therapy is well tolerated, and its association with other treatments such as corticosteroids could increase their beneficial effects. The usefulness of specific nutrients needs further evaluation.

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Chapter 43
Alcoholic and Nonalcoholic Fatty Liver Disease and Vitamin A

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Key Points

- Know the metabolism of vitamin A and its changes in liver diseases of alcoholic and nonalcoholic etiology.
- Identify the determinants of changes in the metabolism of vitamin A in liver diseases.
- Point the prevalence of vitamin A deficiency in patients with liver disease and its consequences.

Keywords Vitamin A • Nonalcoholic fatty liver disease • Oxidative stress • Insulin resistance • Toxicity • Cirrhosis

Vitamin A

According to global estimates by Canadian organization – The Micronutrient Initiative – the control and eradication of vitamin A deficiency (VAD) continue to pose a challenge for researchers because some two billion individuals are affected worldwide, thus compromising socioeconomic development in affected countries. Besides being the most common cause of preventable blindness, it also has a significant impact on to the rise in morbimortality rates associated with infectious processes, given its role in the immune system [1].

Vitamin A plays a part in several key functions in human health, such as visual acuity, cell proliferation, and differentiation, as well as antioxidant and immune activity [1].

Vitamin A is a generic term which designates to any compound possessing the biological activity of retinol and encompasses retinol and carotenoid forms. Among the various forms of carotenoids found in nature, only a few are vitamin A precursors in humans, and retinol activity equivalents data is only available for three carotenoids: β-carotene, α-carotene, and β-cryptoxanthin [1].

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Retinol has a molecular weight of 286.46 kDa and a functional hydroxyl group at carbon 15, which can be esterified with long-chain fatty acid, usually palmitate and stearate, which makes retinol very stable. Within the intestinal lumen, the retinyl esters derived from the diet (mainly retinyl palmitate) are emulsified with bile salts and hydrolyzed to retinol by several pancreatic enzymes and retinyl ester hydrolases (REH), prior to absorption [2].

Within the enterocytes, retinol binds to cellular retinol-binding protein II (CRPBII) and complexed retinol is esterified by the enzyme lecithin-retinol acyltransferase (LRAT). The retinyl esters are incorporated into chylomicrons (CM), which enter the lymphatic circulation and migrate to the bloodstream, where a number of biochemical processes such as triacylglycerol hydrolysis and apoprotein exchange occur, resulting in chylomicron remnants (CMR) [2].

Absorbed β-carotene can be converted into vitamin A within the enterocyte by the β-carotene 15, 15′ monooxygenase enzyme, formerly known as β-carotene 15, 15'dioxigenase. The liver, lungs, adipose tissue, and other tissues also carry this enzyme, suggesting conversion of β-carotene into vitamin A may occur once it has already been taken up by the liver and extrahepatic tissue [3].

The liver is the organ most involved in storing, metabolizing, and distributing vitamin A to the peripheral tissues. Besides serving as a site for vitamin A storage, the liver can use retinol to perform normal functions, like cell proliferation and differentiation. The liver is composed of several different cell types, of which two types – parenchyma cells (or hepatocytes) and stellate cells – are directly involved in vitamin A metabolism [4].

CMR uptake by parenchymal liver cells can be mediated by the presence of low-density lipoprotein (LDL) receptors, LDL receptor-related protein (LRP), and lipoprotein lipase (LPL). Apolipoprotein E on the CMR surface is also required for this uptake to occur. Within hepatocytes, the retinyl esters are hydrolyzed by the REH enzyme in the plasma membrane or in the endosomes, resulting in the formation of retinol [1].

Once the retinol has been formed, it can take several different routes: (1) it can bind to retinol-binding protein (RBP) and be released into the bloodstream; (2) it can be oxidized to retinoic acid; (3) it can be metabolized, like retinoic acid, to more polar forms by the cytochrome P450 enzyme system (CYP26) and combined with bile salts for excretion in bile; (4) or it can be transported to stellate cells, where it will be stored. One's vitamin A nutritional status determines the path it will take [5].

Although the mechanism whereby retinol is transferred to stellate cells has not yet been fully elucidated, it is accepted that it is cellular retinol-binding protein I (CRBPI) that is involved in this intercellular transport, not RBP. CRBPI drives the esterification of retinol and then its oxidation to retinal and retinoic acid [6].

Stellate cells, which under normal circumstances contain around 90 % of the retinol in the liver, are responsible for retinol uptake, storage, and release. In these cells, retinol bound to CRBPI is esterified by the LRAT enzyme, and the resulting retinyl esters are stored in lipid droplets. When released into the blood, the RBP-retinol complex combines with transthyretin, a protein also synthesized by the liver, forming holo-RBP. The retinol is then removed from the bloodstream and used by the target cells where it serves as a precursor to its bioactive metabolites, which are produced intracellularly by two enzymatic reactions: the retinol is converted to retinal or retinaldehyde and then, irreversibly, to retinoic acid [6].

The World Health Organization [7] now prescribes the use of indicators capable of detecting subclinical vitamin A deficiency. These subclinical indicators diagnose VAD at moderate or marginal stages of deficiency and include functional, biochemical, and histological indicators. Among the biochemical markers are serum retinol levels, vitamin A concentrations in the liver, vitamin A concentrations in human milk, and relative and modified relative dose response (RDR and MRDR) and serum 30-day dose response (S30DR) tests. Nevertheless, serum retinol quantification is the most widely used method of vitamin A nutritional status assessment, and international committees have recommended it as being a satisfactory means of identifying those who are at risk of VAD.

The Institute of Medicine (IOM) considers dietary vitamin A intake to be adequate when it is greater than or equal to 900 μg RAE/day for men and 700 μg RAE/day for women. It is worth pointing out that vitamin A is highly bioavailable, whereas bioavailability and bioconversion in carotenoids with provitamin A activity in vitamin A are influenced by liver disease and a number of other factors, like meal composition and preparation, fat intake, and changes in bowel habits [1].

Vitamin A nutritional status is an organic condition whereby serum levels of retinol are maintained to meet the demands of the target tissues. The groups traditionally at risk of this deficiency are pregnant women, nursing mothers, newborns, infants, and preschool children [7]. However, studies show a drop in serum retinol levels in those suffering from diseases that involve changes in the absorption or transport of lipids, in the synthesis of retinol carrier proteins, as well as in those suffering from disorders involving an increased metabolic rate, such as thyroid, liver and kidney diseases, and diabetes mellitus [8].

Chronic liver disease is often accompanied by poor nutritional status, which can come in the form of protein-energy malnutrition and/or deficiencies in micronutrients, including vitamin A. The liver is the organ that does most of the body's storing, oxidizing, and catabolizing of vitamin A. It is also responsible for controlling the release of retinol to other tissues. As a result, liver disease may induce extrahepatic manifestations of vitamin A deficiency due to changes in the metabolism as well as in the synthesis of retinol carrier proteins. Although patients with chronic liver disease are not part of the group most commonly at risk of vitamin A deficiency, this group has been described as showing inadequate levels of serum retinol [9].

Alcohol Liver Disease and Vitamin A

The liver is responsible for approximately 90 % of the ethanol oxidized, as it is the organ containing the greatest quantity of enzymes capable of oxidizing it. Ethanol metabolism by the liver can take place via a primary enzymatic pathway and two ancillary pathways that occur in different cellular compartments. In the main pathway, ethanol oxidation proceeds in two stages: first, it is converted into acetaldehyde by the alcohol dehydrogenase enzyme in the cytoplasm of liver cells, then it is transformed into acetate by the activity of the aldehyde dehydrogenase enzyme. Acetaldehyde is a substance more hepatotoxic than the ethanol itself. Acetaldehyde can form stable acetaldehyde-protein complexes, which are immunogenic and can cause inflammation of the liver [9].

The alcohol dehydrogenase (ADH) oxidizes some physiological alcohols like retinol, hydroxides of steroids, and ω-hydroxy fatty acid [10].

The ancillary pathways are composed of the microsomal ethanol-oxidizing system (MEOS), located in the endoplasmic reticulum, and the catalase action, located in the peroxisomes. The common product of the three pathways is acetaldehyde [11]. The catalase enzyme pathway is insignificant in a person in good physiological condition, becoming more evident when hydrogen peroxide production increases.

Ethanol oxidation by the alcohol dehydrogenase enzyme and CYP2E1 is reliant on the cofactors NAD^+ and $NADP^+$, respectively. This reliance produces excess reduced equivalents in the cytoplasm, resulting in an imbalance in redox potential, which causes a number of metabolic abnormalities, such as the accumulation of triglycerides in the liver. Thus, consumption of alcoholic beverages can cause the following types of liver damage: fatty liver disease, alcoholic hepatitis, cirrhosis, and hepatocellular carcinoma [12].

A number of studies have assessed the relationship between chronic alcohol consumption and levels of β-carotene and retinol in the liver and blood. A drop in concentrations of vitamin A in the livers of chronic alcoholics has been noted, particularly in the most severe form of alcoholic liver disease, both in lab animals and in humans. In a study where rats were given alcohol for 4–6 weeks,

vitamin A deposits in the liver dropped by 60 %, and following vitamin A supplementation five times the usual dose, the amount of vitamin A stored in the liver remained low [13].

Vahlquist et al. [14] noted that alcoholic liver disease is associated with a severe drop in hepatic vitamin A, even when liver injury is moderate, describing ten times lower concentrations of vitamin A in patients with alcoholic hepatitis and 30 times lower in patients with cirrhosis compared to normal.

Ethanol and retinol are two alcohols that compete for the same enzyme pathways and both are converted to their corresponding aldehydes in reactions catalyzed by cytosolic alcohol dehydrogenase isoenzymes. Ethanol, through its hepatotoxic product acetaldehyde, activates stellate cells, which become myofibroblast cells, which secrete fibrous tissue. Following the activation of hepatic stellate cells, the loss of the characteristic stored intracellular vitamin A occurs [13].

So far, not many mechanisms behind the consequences of chronic alcohol consumption on vitamin A nutritional status have been described. It has been noted that chronic alcoholism leads to vitamin A in the liver being mobilized to peripheral tissues and other organs. Alcohol abuse interferes with the production and metabolism of retinoic acid, an important regulator of hepatocyte cellular differentiation and proliferation. Ethanol impairs ADH-mediated oxidation of retinol in rat, mouse, and human livers, as it is the rate-limiting step for retinal and, subsequently, retinoic acid synthesis. Furthermore, alcohol induces cytochrome P450 enzymes, which increase retinoic acid catabolism, due to it converting into polar metabolites, which are hepatotoxic and contribute to the progression of liver disease [15].

Wagnerberger et al. [16] demonstrated another mechanism for chronic alcohol consumption interfering with vitamin A nutritional status. These authors showed a drop in RBP saturation, resulting in a reduction in the availability of retinol to peripheral tissues while still in the early stages of alcoholic liver disease.

In contrast with retinol, of which stores in the liver are depleted in ALD, liver β-carotene is increased. In baboons fed ethanol chronically, concentrations of plasma β-carotene were elevated, with a striking delay in the clearance from the blood following a β-carotene load. The combination of an increase in hepatic β-carotene and a relative lack of a corresponding rise in hepatic retinol stores suggests a blockage in the conversion of β-carotene to retinol by ethanol. The nature of this putative block is unclear [13].

Research has shown that plasma vitamin A is not a good marker for hepatic vitamin A reserves in alcoholics because concentrations of plasma vitamin A have been found to be adequate, even when the reserves in the liver are low, especially during the early stages of the disease. In fact, similar concentrations of plasma retinol and RBP have been described in the plasma of alcoholics and control groups [17]. However, prior studies have come up with contrasting findings, showing decreases in vitamin A and RBP concentrations in the plasma of patients with alcoholic liver disease and alcohol-induced cirrhosis [18].

Regarding plasma concentrations of provitamin A, while chronic alcoholics tend to have low plasma concentrations of β-carotene – probably reflecting low dietary intake – recent ingestion of alcohol can raise them. Alcohol may increase the plasma concentration of β-carotene lost through biliary excretion [19].

The perpetuation of vitamin A deficiency can lead to some consequences to the health of individuals with ALD, as described below.

Xerophthalmia encompasses a series of signs and symptoms according to the severity of the deficiency, including night blindness, the first sign of functional deficiency of the vitamin. It stems from lowered rates of rhodopsin regeneration and is characterized by impaired vision at night or in dim lighting, and it can thus pose health risks and greater chance of injury. In the initial stage, night blindness is reversible by returning serum vitamin A levels to normal [7].

For its antioxidant activity, vitamin A depletion may have to do with the greater need for the vitamin in the oxidative process, as this reduction can throw off the cellular redox balance.

The formation of reactive oxygen and nitrogen via the release of electrons from the enzyme system (CYP450 2E1 and mitochondria) is proposed as key factors in mediating the effects caused by chronic

alcoholism. To combat the action of the free radicals involved in the clinical manifestations of liver disease, the body uses enzymatic and nonenzymatic defenses. Of the nonenzymatic defenses, vitamin A stands out. For being fat soluble, it defends against oxidative damage in the cell membranes. It has been suggested that lipid peroxidation is associated with activation of stellate cells. Furthermore, the increases in hepatic fibrosis observed in patients with low concentrations of antioxidants in the liver suggest that the severity of the disease may hinge on antioxidant depletion caused by oxidative stress or the decline in stocks in the liver due to the process of fibrosis [20].

There is a growing body of evidence suggesting that vitamin A plays an important role in hepatic proliferation and differentiation and that low concentrations of vitamin A may play a part in the development of liver tumors. In cirrhosis or chronic hepatitis, hepatocytes are in a state of intense regenerative activity, and losing vitamin A, which helps regulate hepatocytes and maintain their differentiation, may result in the formation of mutant hepatocytes that are potentially progenitors of HCC cells. Thus, serum retinol levels have been suggested as an indicator for those at greater risk of developing HCC [21].

Although ALD patients are often found to suffer from vitamin A deficiency, caution is recommended when taking in supplement form, as alcohol intensifies the effects of an overdose of this vitamin and may trigger hepatotoxicity.

β-Carotene supplementation has been considered as an alternative for ALD patients. For being a retinol precursor, it is regarded as being less toxic, not to mention its greater antioxidant potential. In tests where rats were given alcohol, β-carotene supplementation reduced the accumulation of fat in the liver, inhibited the reduction of glutathione peroxidase activity, and maintained the plasma concentration of glutathione, when compared to the control group [22]. However, it is not known whether β-carotene can actually compensate for alcohol-induced lipid peroxidation without producing signs of toxicity, especially in individuals who persist in consuming alcohol during supplementation. In baboons, consumption of ethanol together with β-carotene resulted in a more striking hepatic injury than did consumption of either compound alone. This toxic interaction in baboons occurred at a total dose of 7.2–10.8 mg β-carotene/J diet, which is common in subjects taking supplements. In rats, the well-known hepatotoxicity of ethanol was potentiated by large amounts of β-carotene, and the concomitant administration of both β-carotene and alcohol resulted in striking liver lesions [23].

In contrast, in rats that were previously subjected to consuming alcohol, supplementation with low doses of ATRA reduced the formation of polar metabolites of retinol, increased serum and liver concentrations of AR and completely reestablished retinyl and palmitate concentrations in the liver, and lowered the transaminases when compared to the group not given supplementation. Furthermore, upon histological examination, it notably alleviated hepatocellular swelling, steatosis, swelling of mitochondria, and proliferation of smooth endoplasmic reticulum [24].

Models of hepatic toxicity involving alcohol and vitamin A were for the most part carried out in animal testing, making it difficult to determine a safe dose of ALD with which to supplement humans.

Another factor that should be taken into account when considering vitamin A supplementation for patients with chronic liver disease is a possible decline in vitamin A transport due to a drop in RBP production by liver according to the severity of the disease. Supplementation in patients who are unable to release the vitamin supplement for circulation could trigger liver toxicity.

Nonalcoholic Fatty Liver Disease and Vitamin A

Nonalcoholic fatty liver disease (NAFLD) is characterized by the accumulation of fat in the liver when it exceeds 5–10 % of liver weight. It presents an ample histological aspect that results from triglycerides being deposited in hepatocytes. It comprises a spectrum of pathological changes similar to those observed in alcoholic liver disease but occurring in nonalcoholics. These changes range from simple steatosis to nonalcoholic steatohepatitis (NASH), fibrosis, and cirrhosis [25].

The number of NAFLD cases has been on the rise around the world, which has been associated with the increasing prevalence of obesity. The real rate is probably greater than assumed since the course of the disease is clinically silent, changes found in laboratory testing are unspecific, and liver biopsies and/or ultrasound are not performed in the early stages in those belonging to the group at risk of the disease. In the United States, the estimated prevalence in class III obesity is 30–90 % [26]. These are classic features of the disease associated with obesity, type 2 diabetes mellitus, and hyperlipidemia.

The most widely accepted hypothesis explaining the NAFLD pathogenesis mechanism is proposed by Day and James [27] and dubbed "Two Hits," in which the first step in developing the disease ("First Hit") is fat accumulating in hepatocytes – specifically fatty acids and triglycerides – characterizing simple fatty liver. At this stage, the disease does not progress, unless additional cellular events occur ("Second Hit"), provoking inflammation, cell death, and fibrosis, which are the histological markers of NASH. The factors involved in the disease progressing – once the onset of fatty liver is underway – can be grouped into two categories: factors that cause an increase in oxidative stress (OS) and factors that promote proinflammatory cytokine expression. IR is involved in both stages of the development of fatty liver disease, and steatosis on its own can exacerbate the insulin resistance (IR), perpetuating a cycle of aggression upon itself.

Changes in the synthesis, uptake, and degradation of lipid molecules, as a result of IR, are the first metabolic abnormalities, resulting in the accumulation of triglycerides in liver tissue. The increase in free fatty acids (FFA) supply and synthesis by the liver, reduction in β-oxidation in the liver, and/or reduction in synthesis and secretion of very-low-density lipoprotein (VLDL) are a key part of the association between steatosis and lipid metabolism in the liver. Typically, triglycerides are removed from the liver by the VLDL, which is formed by the microsomal triglyceride transfer protein, which attaches to apolipoprotein B (Apo B). Hyperinsulinemia leads to a reduction in this protein's activity, and Apo B synthesis and secretion – which occurs in NAFLD – hinders the export of lipids from the liver and causes triglycerides to accumulate in hepatocytes [25].

The development of NAFLD is directly related to a drop in the tissue sensitivity to insulin. Adipocytes and hepatocytes are influenced by elevated levels of insulin in different ways. In adipocytes, IR mobilizes FFAs and increases uptake by the liver. In hepatocytes, it stimulates synthesis and inhibits oxidization of FFAs. Due to the decrease in FFAs being released by the liver, as an aftereffect of hyperinsulinemia, there is greater degradation of Apo B, which prevents the release of triglycerides from the liver, causing it to accumulate in the hepatocytes [28].

The prooxidant substances most prevalent in NAFLD are singlet oxygen, superoxide anions, hydrogen peroxide, and hydroxyl radical molecules. FFA oxidation is an important source of reactive oxygen species (ROS) production in livers afflicted with steatosis. Chronic OS leads to the depletion of natural antioxidant compounds, resulting in the production of excess ROS in the hepatocytes. High concentrations of ROS not only lead to the lipid peroxidation of cell membranes but also stimulate IR and the production of cytokines, especially tumor necrosis factor-α (TNF-α) in hepatocytes, Kupffer cells, and adipose tissue. The ROS have short half-life; however, once lipid peroxidation in the cell membranes has begun, they result in the formation of such products as malondialdehyde (MDA) and trans-4-hydroxy-2-nonenal. The half-lives of these molecules are longer than that of the ROS and are able to spread out from their places of origin to reach farther-off targets inside and outside the cell, thus aggravating the effects of OS; besides their being harmful to the functions of cellular organelles, these aldehydes formed by the peroxidation of polyunsaturated fatty acids hamper protein and nucleotide synthesis, deplete the natural antioxidant glutathione peroxidase, boost TNF-α production, bring about the influx of inflammatory cells to the liver, and activate stellate cells, leading to collagen deposition, fibrosis, and the perpetuation of inflammatory response. These effects directly induce hepatocyte death, necrosis, inflammation, and liver fibrosis [29].

There is a dearth of research assessing vitamin A nutritional status in NAFLD. Yanagitani et al. [30], in a study using an experimental model performing testing on transgenic mice with defective

retinoic acid receptors in the liver, noted the onset of NASH at 4 months of age and hepatocellular carcinoma at 12 months, suggesting retinoic acid has a protective effect in the development of hepatocellular carcinoma. Bahcecioglu et al. [31] found an increase in serum retinol levels in patients with simple steatosis and NASH, when compared with healthy individuals. The author suggested that the rise in serum retinol levels could serve as an indicator for the increase in lipid stored in hepatocytes and stellate cells in the liver. It may be that stellate cells are activated by the stimulus from a number of cytokines that lead to the fibrogenesis process and cause the release of vitamin A into the circulation.

Chaves et al. [32], in researching vitamin A nutritional status in sufferers of class III obesity and fatty liver disease, found a significantly lower β-carotene average in the group with the disease. The same was not noted for serum retinol values, probably due to the greater antioxidant capacity of β-carotene.

In a study undertaken to evaluate the serum concentration of carotenoids in 350 people sorted according to the degree of fat accumulation in the liver (healthy liver, degree of steatosis moderate or severe), serum β-carotene concentrations were found to decrease significantly according to increases in the lipid content of the liver. The same was not found for other carotenoids studied [33].

More recently, a positive correlation was found between serum retinol values and concentrations of AST and ALT in the grade III obese with NAFLD. Serum retinol was the only biochemical variable that could predict AST and ALT concentrations in these patients. IR assessed by HOMA-IR could also predict ALT concentration [34]. Chaves et al. [32] too correlated liver function and liver damage tests with retinol levels in NAFLD and found a significant positive correlation with albumin and a negative correlation with BT, two liver function markers. No association was found for liver damage markers. Other studies have demonstrated the relationship between liver function and liver damage markers and serum retinol in patients suffering from advanced chronic liver diseases of different etiologies, pointing to retinol as a potential marker for liver damage [35].

The lack of studies assessing ENVA in NAFLD in humans shows a clear need for further research to shed light on the relationship between vitamin A and NAFLD. However, some hypotheses can be postulated as follows:

(a) Considering OS's role in NAFLD pathogenesis and the potency of vitamin A in the fight against ROS, it is likely that these patients bear lower levels of the vitamin since consumptions of substances with antioxidant functions increase with OS.

Retinol and β-carotene are highly efficient, nonstoichiometric free radical scavengers, and their main action is to deactivate singlet oxygen involved in oxidative attacks on nucleic acids, amino acids, and polyunsaturated fatty acids. The mode of inactivation of this reactive oxygen occurs by way of a physical and not chemical mechanism. These retinoids display geometric cis-trans-type isomers. Singlet oxygen is an energy molecule that can transfer its energy in isoprenoid-chain isomerization process of vitamin A and β-carotene. Thus, retinoids can be converted from *cis*- to *trans*-form by the energy of singlet oxygen and, conversely, by the energy of another singlet oxygen in a continuous cycle. A large number of this active type can therefore be deactivated by a single retinoid molecule. Due to its peculiar mode of action, such substances may be termed *isomeric scavengers*. Retinol and carotenoids also acts as inhibitors of gene transcription nitric oxide synthase (iNOS), composed of oxygen that stimulates the production of other free radicals, especially the nitric oxide variety [36].

Musso et al. [37], when comparing patients with NAFLD and a control group matched according to severity of IR, degree of adiposity, and metabolic syndrome, found that reducing vitamin A intake independently correlated with the severity of liver disease and that OS, evaluated according to by nitrotyrosine concentrations, is present at all stages of the disease, even in patients not suffering from IR.

Therefore, adequate intake of vitamin A, particularly carotenoids, is important in protecting against oxidative attacks on cell membranes by free radicals, as it reduces oxidative damage and, thus, prevents the onset of chronic diseases.

(b) An association has been found between vitamin A and insulin resistance. In the grade III obese with NAFLD, the HOMA-IR index, used to assess insulin resistance, showed a significant negative correlation with β-carotene deficiency. Furthermore, almost all the patients with low levels of plasma β-carotene and retinol had IR [32]. Sugiura et al. [38] noted an inverse association between plasma concentrations of carotenoids and IR by using HOMA-IR method of estimation, which supports the hypothesis that carotenoids may have a protective effect on IR pathogenesis, probably for its role as a protective agent in OS, since it has been suggested that an OS increase implies diminished insulin action.

(c) It is suggested that supplementation with all-trans retinoic acid (ATAR) brings about triglycerides oxidation in the liver. The proposed mechanisms are as follows: (1) There is an increase in hepatic expression of genes codifying proteins that promote fatty acid oxidation (PPAR-α, RXR-α, liver-type carnitine palmitoyltransferase 1, carnitine/acylcarnitine carrier, uncoupling protein 2), and (2) there is a reduction of hepatic expression of genes that codify proteins involved in lipogenesis (SREBP-1c, fatty acid synthase). This reduction in liver fat stocks may be a contributing factor to the already-demonstrated improvement in insulin sensitivity in rats treated with ATAR, pointing to the role of vitamin A as a protective agent in steatosis development in situations where there is an increase in the influx of FFA to the liver, as is the case with fat-rich diets, abdominal obesity, and rapid weight loss [39].

(d) The increase in the gene expression of proteins and enzymes related to retinol metabolism has been demonstrated in NAFLD, suggesting the process of retinol oxidizing to ATRA is accelerated with the disease. Also noted was an increase in the expression of CYP26A1, which is most responsible for the degradation of ATRA, which may represent an important mechanism in the disease's progression. Moreover, further degradation of ATRA leads to a reduction in vitamin A stocks in HSC, which is related to loss of retinoid signaling, which results in increased OS and consequently contributes to disease progressing [40].

Supplementation with antioxidant nutrients has been tested. However, its validity in recuperation from the disease is still under debate. There is no existing research that has tested the efficacy of vitamin A supplementation alone in the treatment of NAFLD.

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About the Series Editor

Dr. Adrianne Bendich has recently retired as Director of Medical Affairs at GlaxoSmithKline (GSK) Consumer Healthcare where she was responsible for leading the innovation and medical programs in support of many well-known brands including TUMS and Os-Cal. Dr. Bendich had primary responsibility for GSK's support for the Women's Health Initiative (WHI) intervention study. Prior to joining GSK, Dr. Bendich was at Roche Vitamins Inc. and was involved with the groundbreaking clinical studies showing that folic acid–containing multivitamins significantly reduced major classes of birth defects. Dr. Bendich has coauthored over 100 major clinical research studies in the area of preventive nutrition. Dr Bendich is recognized as a leading authority on antioxidants, nutrition and immunity and pregnancy outcomes, vitamin safety and the cost-effectiveness of vitamin/mineral supplementation.

Dr. Bendich, who is now President of Consultants in Consumer Healthcare LLC, is the editor of ten books including *Preventive Nutrition: The Comprehensive Guide For Health Professionals*, Fourth Edition coedited with Dr. Richard Deckelbaum, and is series editor of *Nutrition and Health* for Springer/Humana Press (www.springer.com/series/7659). The series contains 40 published volumes – major new editions in 2010–2011 include *Vitamin D*, Second Edition edited by Dr. Michael Holick; *Dietary Components and Immune Function* edited by Dr. Ronald Ross Watson, Dr. Sherma Zibadi, and Dr. Victor R. Preedy; *Bioactive Compounds and Cancer* edited by Dr. John A. Milner and Dr. Donato F. Romagnolo; *Modern Dietary Fat Intakes in Disease Promotion* edited by Dr. Fabien DeMeester, Dr. Sherma Zibadi, and Dr. Ronald Ross Watson; *Iron Deficiency and Overload* edited by Dr. Shlomo Yehuda and Dr. David Mostofsky; *Nutrition Guide for Physicians* edited by Dr. Edward Wilson, Dr. George A. Bray, Dr. Norman Temple, and Dr. Mary Struble; *Nutrition and Metabolism* edited by Dr. Christos Mantzoros; and *Fluid and Electrolytes in Pediatrics* edited by Leonard Feld and Dr. Frederick Kaskel. Recent volumes include: *Handbook of Drug-Nutrient*

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