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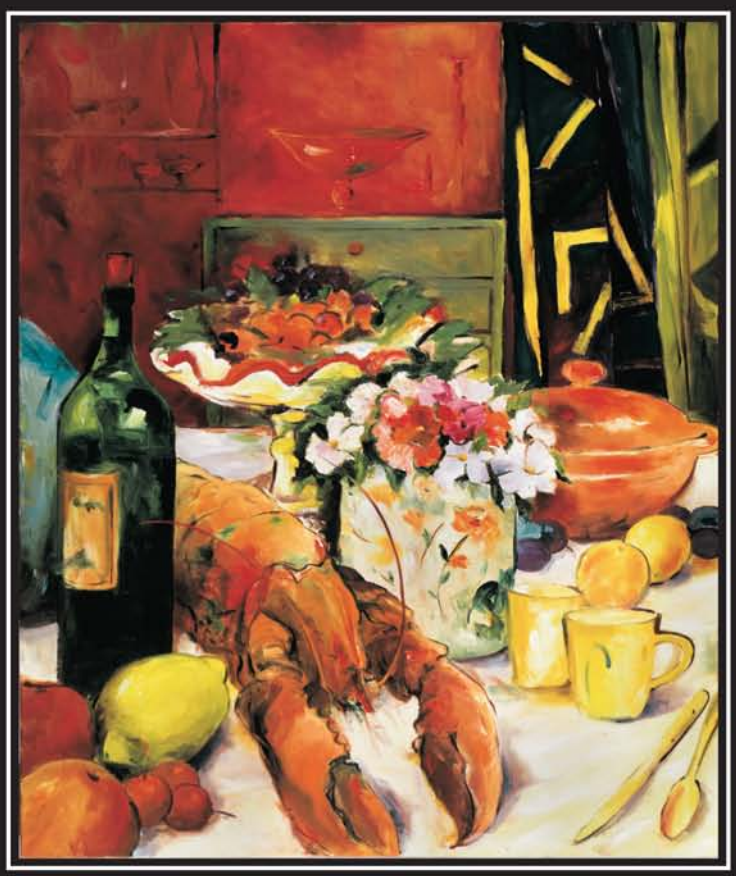
FAT DETECTION

TASTE, TEXTURE, AND POST INGESTIVE EFFECTS



Edited by
Jean-Pierre Montmayeur and Johannes le Coutre

FRONTIERS IN NEUROSCIENCE

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FRONTIERS IN NEUROSCIENCE

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David M. Lovinger, Ph.D., Laboratory of Integrative Neuroscience, NIAAA, Nashville, Tennessee

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Miguel A.L. Nicolelis, M.D., Ph.D., Professor of Neurobiology and Biomedical Engineering, Duke University Medical Center, Durham, North Carolina

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Antonius M. VanDongen, Duke University Medical Center, Durham, North Carolina

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Jerry J. Buccafusco, Ph.D., Alzheimer's Research Center, Professor of Pharmacology and Toxicology, Professor of Psychiatry and Health Behavior, Medical College of Georgia, Augusta, Georgia

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Ron Frostig, Ph.D., Professor, Department of Neurobiology, University of California, Irvine, California

Fat Detection: Taste, Texture, and Post Ingestive Effects
Jean-Pierre Montmayeur, Ph.D., Centre National de la Recherche Scientifique, Dijon, France
Johannes le Coutre, Ph.D., Nestlé Research Center, Lausanne, Switzerland

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Edited by

Jean-Pierre Montmayeur, Ph.D.
Centre National de la Recherche Scientifique
Dijon, France

Johannes le Coutre, Ph.D.
Nestlé Research Center
Lausanne, Switzerland

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Series Preface

Our goal in creating the Frontiers in Neuroscience Series is to present the insights of experts on emerging fields and theoretical concepts that are, or will be, at the vanguard of neuroscience. Books in the series cover topics ranging from genetics, ion channels, apoptosis, electrodes, neural ensemble recordings in behaving animals, and even robotics. The series also covers new and exciting multidisciplinary areas of brain research, such as computational neuroscience and neuroengineering, and describes breakthroughs in classical fields like behavioral neuroscience. We want these books to be the books every neuroscientist will use in order to get acquainted with new ideas and frontiers in brain research. These books can be used by graduate students and postdoctoral fellows when they are looking for guidance to start a new line of research.

Each book is edited by an expert and consists of chapters written by the leaders in a particular field. They are richly illustrated and contain comprehensive bibliographies. All the chapters provide substantial background material relevant to the particular subject. We hope that as the volumes become available the effort put in by us, the publisher, the book editors, and individual authors will contribute to the further development of brain research. The extent that we achieve this goal will be determined by the utility of these books.

Sidney A. Simon, PhD
Miguel A.L. Nicolelis, MD, PhD

Foreword

Fat—a word that strikes emotions in all of us. Being initially associated with healthy and nutritious food attributes after World War II the connotation changed toward a more negative one over the past few decades. It goes without saying that fat—used as an adjective—for example to describe bodily appearance is close to being politically incorrect.

At the same time impressive insights accumulated on the role of fat in health and nutrition. We understand the metabolic breakdown of fat in our bodies as well as the synthesis and its storage in adipocytes. The role of triglycerides in diabetes and cardiovascular disease is undisputed and obviously the global obesity epidemic is firmly linked to food intake and fat.

With the above in mind it is surprising to realize that one aspect of fat never has been addressed in a comprehensive way—I am talking about the perception, taste, and smell of dietary fat.

In the present book *Fat Detection: Taste, Texture, and Post Ingestive Effects,* edited by Jean-Pierre Montmayeur and Johannes le Coutre, a superb collection of contributions has been compiled by the pre-eminent authors in the field to address this topic.

I leave it up to the readers to discover the exciting evolutionary role of fat intake and to learn how the detection of fat can be monitored from the tongue to the brain and subsequently how we develop a clear preference for fat—and sometimes also an aversion.

The important contribution of this book is the demystification of fat from being the culprit in today's nutrition toward a positioning of "good fat" as an essential building block of food to deliver enhanced development and performance as well as a desirable impact on the prevention of disease.

For the educated amateur scientist with an interest in the principles of sensory physiology and nutrition the book offers a comprehensible introduction to the field. For the established expert it provides a balanced overview of the latest developments in the field.

I am convinced this book will make its contribution toward a more educated but also toward a more relaxed way of interacting with fat so that we can go to a good restaurant and consume our fried eggplants, risotto, fish, and dessert knowing that enjoyable and good food does have a positive impact on our health.

Günter Blobel
John D. Rockefeller, Jr. Professor
Investigator, HHMI Laboratory of Cell Biology
The Rockefeller University, New York

Preface

The idea to put together a book compiling the current knowledge on dietary fat taste, perception and intake came about somewhere between a slice of foie gras in Prenois and a portion of Vacherin cheese in Lausanne during Sid Simon's visit to Europe in the spring of 2007. We realized then that the exquisite taste of these foods and hard-to-resist second helpings might have been related to their fat content.

Why is fat so tasty, why do we crave it, and what is the impact of dietary fat on health and disease? These are the fundamental questions this book aims to answer through 22 chapters contributed by leading scientists in the field who deserve all the credit and all our gratitude for making this project not only possible but most importantly a very pleasant experience.

The undeniable sensory appeal of fat may have deep roots; some might even argue that the ability to select energy-dense foods is crucial for survival. Part I of this book lays down the evolutionary reasons for a fat detection system. In Chapter 1, William Leonard and colleagues convey archeological arguments linking the availability of docosahexaenoic acid and diets rich in arachidonic acid with the evolution of larger brain size in mammals, and in Chapter 2 Frits Muskiet provides an in-depth review of the importance of dietary fats in development, including geographical and sociocultural arguments guiding nutritional habits.

Over the last two decades, numerous studies investigating the gustatory mechanisms and sensory factors underlying the innate preference for dietary fat have accumulated. Part II of this book summarizes essential findings emanating from this significant body of work through five chapters. In Chapter 3, Timothy Gilbertson and colleagues review the candidate receptor mechanisms underlying oral fat detection and provide a clear image of the state of the art in fat taste transduction through a new model piecing together the current players. In Chapter 4, David Pittman highlights important findings on the modulation of fatty acids responsiveness by gender and strain. Next, in Chapter 5 Jennifer Stratford and Robert Contreras lay out behavioral studies that contributed to the mapping of the circuits linking preference and peripheral detection mechanisms. Then, in Chapter 6 James Smith documents how carefully designed behavioral paradigms helped establish that gustatory, textural, and olfactory information all contribute to fat detection. Finally, in a comprehensive review, in Chapter 7 Richard Mattes reflects on 15 years of psychophysical, behavioral, electrophysiological, and molecular studies, making the case for an oral fat detection system.

In Part III, Edmund Rolls (Chapter 8) provides a detailed account of the brain regions processing the signals elicited by a fat stimulus including flavor, aroma, and texture; their modulation by hunger; and their impact on the pleasantness of food. In Chapter 9, Ulrike Toepel and colleagues contribute a stimulating chapter on the cortical regions implicated in reward assessment and decision-making, demonstrating that visual food stimuli initiate a rapid processing of the food's energetic content.

There are many explanations for the appeal of fat. Part IV explores some of them through the contribution of Yasuko Manabe and colleagues (Chapter 10), who review pioneering behavioral experiments in rodents, and together represent the most compelling evidence that fat is detected in the mouth. In Chapter 11, Adam Drewnowski and Eva Almiron-Roig accomplish a remarkable feat by covering the many factors responsible for the sensory appeal of foods rich in fat.

Several of the factors contributing to fat preference, including fat content, palatability, satiety, pleasure, and reward, are discussed in great detail in Part V. First, Karen Ackroff and Anthony Sclafani (Chapter 12) skillfully summarize key experiments exploring the impact of postoral factors on dietary fat appetite. In Chapter 13, Brian Baldo and colleagues provide a logical and detailed analysis of their findings on the brain mechanisms associated with appetitive behaviors and the hedonic experience connected with food consumption. Next, Charlotte Erlanson-Albertsson (Chapter 14) presents an overview of the effect of fatty acids intake on the release of appetite regulating peptides and makes the case for enterostatin as a potential therapeutic target to control fat intake. Finally, Rania Abou Samra (Chapter 15) discusses the effects of various fats and fatty acids on satiety and appetite as well as those of other dietary components.

Genetic predisposition and physiological status can influence the intake and metabolism of fats. In Part VI, Danielle Reed (Chapter 16) addresses the genetic components of human fat preference with an in-depth look at heritability through family and twin studies. In Chapter 17, José Lopez-Miranda and Carmen Marin focus on the impact of lifestyle and the fatty acid composition of meals on postrandial lipemia, pointing out a possible link with atherosclerosis.

It has become apparent that there is a link between obesity and the rise in the consumption of energy-dense diets such as those rich in dietary fats. The first section of Part VII gathers clinical observations on the link between essential fatty acids and neurological disorders through the contribution of Kiran Panickar and Sam Bhathena (Chapter 18) who point out studies suggesting that diets enriched in ω-3 fatty acids seem to help improve the conditions of Huntington's disease, stroke, and multiple sclerosis patients. In addition, Michel Bégin and colleagues (Chapter 19) discuss the protective value of a seafood diet on cognitive impairment and dementia based on the results of epidemiological studies. In the remaining chapters of Part VII, Madhu Chari and colleagues (Chapter 20) present a very thorough account of the current knowledge on the hypothalamic fatty acid sensing mechanisms as they relate to energy homeostasis. In Chapter 21, Marc Brown and colleagues review a vast array of investigations on the contribution of dietary fat and carbohydrates to the development of diseases tied to the metabolic syndrome, including detailed molecular mechanisms underpinning the protective effect of ω-3 polyunsaturated fatty acids on insulin resistance and obesity. Finally in Chapter 22, Jennifer Smilowitz and colleagues investigate the connection between fat metabolism, the fat content of foods, and obesity, making a loud statement in favor of personalized studies contributing insights on the impact of diet on the metabolic syndrome.

By gathering information from the many different fields covered, we hope that this book will not only spark new interests toward this very important topic, but also

provide a general reference for students, scientists, physicians, and professionals in the field of food science.

We would like to thank the following people for their help and support throughout this project: Maia Kokoeva, John Langone, Ronit le Coutre, Anna Montmayeur, James Stellar, and Gary Strichartz. Most of all we are grateful to Sidney Simon for extending the invitation to get involved in this series as well as to Jill Jurgensen and Barbara Norwitz at Taylor & Francis for their patience and efficiency.

A final note: over the course of this project the neuroscience community lost two distinguished members. Doctors Ann E. Kelley and Sam J. Bhathena are being remembered with special dedications in Chapters 13 and 18, respectively. We are indebted to Brian Baldo and Kiran Panickar for kindly accepting to undertake these chapters on their behalf despite the circumstances.

Jean-Pierre Montmayeur
Johannes le Coutre

Editors

Jean-Pierre Montmayeur was introduced to G protein-coupled receptors and G protein signaling as a graduate student in the laboratory of Emiliana Borrelli before receiving his PhD in molecular biology from the University of Strasbourg in 1993. Dr. Montmayeur then did a short postdoctoral term in the laboratory of Andrius Kazlauskas at the National Jewish Center in Denver before starting a second, longer postdoctoral fellowship with Linda Buck at Harvard Medical School. It is during this time that he developed a taste for the sensory systems and decided to pursue his investigations in this field. In 2001, he joined the Centre National de la Recherche Scientifique to work in Dijon, France.

Dr. Montmayeur's research focuses on the receptors responsible for the detection of sapid molecules by the tongue and in particular the signaling mechanisms downstream of these receptors. His recent work includes the identification of variants of the T1R1–T1R3 taste receptor in human fungiform papillae as well as investigations of the contribution of gustatory nerves in the perception elicited by an oral linoleic acid stimulus.

Johannes le Coutre obtained his MSc in biology from the University of Regensburg. In 1995, with the support of a Boehringer Ingelheim Fellowship, he obtained his PhD in biophysics at the Max Planck Institute for Molecular Physiology in Dortmund for discoveries related to the transfer of protons in bacteriorhodopsin, a photosynthetic protein of *H. salinarium*. In 1996, funded by a Human Frontiers Science Program award, Dr. le Coutre went to the University of California, Los Angeles to establish a research project on structure–function relationships in the *E. coli* lactose permease and various other membrane proteins using predominantly Fourier transform infrared spectroscopy as well as mass spectrometry.

In 2000, he joined the Nestlé Research Center in Lausanne, Switzerland to work on various aspects related to perception physiology and, in particular, on taste signal transduction. Most recently, Dr. le Coutre has been contributing to understanding the role of transient receptor potential (TRP) channels in the perception of metallic and spicy tastes and recently he started to investigate the brain integration and representation of gustatory signals in humans.

Contributors

Karen Ackroff
Department of Psychology
Brooklyn College and the Graduate School
City University of New York
Brooklyn, New York

Eva Almiron-Roig
Department of Biological Sciences
University of Chester
Chester, United Kingdom

Brian A. Baldo
Department of Psychiatry
University of Wisconsin Medical School
Madison, Wisconsin

Michel E. Bégin
Research Centre on Aging
Health and Social Services Centre
Sherbrooke Geriatrics University Institute
Sherbrooke, Québec, Canada

Sam J. Bhathena
Diet, Genomics, and Immunology Laboratory
Beltsville Human Nutrition Research Center
Agricultural Research Service
U.S. Department of Agriculture
Beltsville, Maryland

Ian L. Brown
School of Health Sciences
University of Wollongong
Wollongong, New South Wales, Australia

and

Center for Human Nutrition
University of Colorado
Aurora, Colorado

Marc A. Brown
School of Health Sciences
University of Wollongong
Wollongong, New South Wales, Australia

Madhu Chari
Department of Physiology
University of Toronto
and
Toronto General Hospital Research Institute
University Health Network
Toronto, Ontario, Canada

Robert J. Contreras
Department of Psychology and Program in Neuroscience
Florida State University
Tallahassee, Florida

Johannes le Coutre
Nestlé Research Center
Lausanne, Switzerland

Stephen C. Cunnane
Research Centre on Aging
Health and Social Services Centre
Sherbrooke Geriatrics University Institute
Sherbrooke, Québec, Canada

Adam Drewnowski
Nutritional Sciences Program and Center for Obesity Research
University of Washington
Seattle, Washington

Paul L. Else
School of Health Sciences
University of Wollongong
Wollongong, New South Wales,
Australia

Charlotte Erlanson-Albertsson
Department of Experimental Medical
Science
University of Lund
Lund, Sweden

Tohru Fushiki
Laboratory of Nutrition Chemistry
Division of Food Science and
Biotechnology
Graduate School of Agriculture
Kyoto University
Kyoto City, Japan

J. Bruce German
Nestlé Research Center
Lausanne, Switzerland

and

Department of Food Science
and Technology
University of California
Davis, California

Timothy A. Gilbertson
Department of Biology
and
The Center for Advanced Nutrition
Utah State University
Logan, Utah

Janine A. Higgins
Center for Human Nutrition
University of Colorado
Aurora, Colorado

Xu-Feng Huang
School of Health Sciences
University of Wollongong
Wollongong, New South Wales,
Australia

Julie Hudry
Nestlé Research Center
Lausanne, Switzerland

Ann E. Kelley
Department of Psychiatry
University of Wisconsin Medical
School
Madison, Wisconsin

Jean-François Knebel
Neuropsychology and
Neurorehabilitation Service
Centre Hospitalier Universitaire
Vaudois
University of Lausanne
Lausanne, Switzerland

Carol K.L. Lam
Department of Physiology
University of Toronto
and
Toronto General Hospital Research
Institute
University Health Network
Toronto, Ontario, Canada

Tony K.T. Lam
Departments of Physiology and
Medicine
University of Toronto
and
Toronto General Hospital Research
Institute
University Health Network
Toronto, Ontario, Canada

William R. Leonard
Department of Anthropology
Northwestern University
Evanston, Illinois

José Lopez-Miranda
Department of Medicine
Reina Sofía University Hospital
University of Córdoba
Córdoba, Spain

and

CIBER Physiopathology of Obesity and Nutrition
Instituto de Salud Carlos III
Madrid, Spain

Yasuko Manabe
Laboratory of Nutrition Chemistry
Division of Food Science and Biotechnology
Graduate School of Agriculture
Kyoto University
Kyoto City, Japan

Carmen Marin
CIBER Physiopathology of Obesity and Nutrition
Instituto de Salud Carlos III
Madrid, Spain

Shigenobu Matsumura
Laboratory of Nutrition Chemistry
Division of Food Science and Biotechnology
Graduate School of Agriculture
Kyoto University
Kyoto City, Japan

Richard D. Mattes
Department of Foods and Nutrition
Purdue University
West Lafayette, Indiana

Micah M. Murray
Neuropsychology and Neurorehabilitation Service
Radiology Service
EEG Brain Mapping Core
Center for Biomedical Imaging
Centre Hospitalier Universitaire Vaudois
and
University of Lausanne
Lausanne, Switzerland

Frits A.J. Muskiet
Laboratory Medicine
University Medical Center
Groningen, the Netherlands

Kiran S. Panickar
Diet, Genomics and Immunology Laboratory
Beltsville Human Nutrition Research Center
Agricultural Research Service
U.S. Department of Agriculture
Beltsville, Maryland

Fabien Pifferi
Research Centre on Aging
Health and Social Services Centre
Sherbrooke Geriatrics University Institute
Sherbrooke, Québec, Canada

David W. Pittman
Department of Psychology
Wofford College
Spartanburg, South Carolina

Mélanie Plourde
Research Centre on Aging
Health and Social Services Centre
Sherbrooke Geriatrics University Institute
Sherbrooke, Québec, Canada

Wayne E. Pratt
Department of Psychology
Wake Forest University
Winston-Salem, North Carolina

Danielle R. Reed
Monell Chemical Senses Center
Philadelphia, Pennsylvania

Marcia L. Robertson
Department of Anthropology
Northwestern University
Evanston, Illinois

Edmund T. Rolls
Oxford Centre for Computational Neuroscience
Oxford, United Kingdom

Rania Abou Samra
Nestlé Research Center
Lausanne, Switzerland

Anthony Sclafani
Department of Psychology
Brooklyn College and the Graduate School
City University of New York
Brooklyn, New York

Bhavik P. Shah
Department of Biology
and
The Center for Advanced Nutrition
Utah State University
Logan, Utah

Jennifer T. Smilowitz
Department of Food Science and Technology
University of California
Davis, California

James C. Smith
Department of Psychology and Program in Neuroscience
Florida State University
Tallahassee, Florida

J. Josh Snodgrass
Department of Anthropology
University of Oregon
Eugene, Oregon

Len H. Storlien
Institute of Obesity, Nutrition and Exercise
University of Sydney
Sydney, New South Wales, Australia

Jennifer M. Stratford
Department of Psychology and Program in Neuroscience
Florida State University
Tallahassee, Florida

and

Department of Cell and Developmental Biology
University of Colorado
Aurora, Colorado

Linda C. Tapsell
School of Health Sciences
University of Wollongong
Wollongong, New South Wales, Australia

Ulrike Toepel
Neuropsychology and Neurorehabilitation Service and Radiology Service
Centre Hospitalier Universitaire Vaudois
University of Lausanne
Lausanne, Switzerland

Tian Yu
Department of Biology
and
The Center for Advanced Nutrition
Utah State University
Logan, Utah

Angela M. Zivkovic
Department of Food Science
and Technology
University of California
Davis, California

Part I

Importance of Dietary Fat

1 Evolutionary Perspectives on Fat Ingestion and Metabolism in Humans

William R. Leonard, J. Josh Snodgrass, and Marcia L. Robertson

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1.1 INTRODUCTION

Increasingly, biomedical researchers are coming to recognize the importance of an evolutionary perspective for understanding the origin and nature of modern human health problems. This is particularly true when examining "nutritional/metabolic" disorders such as obesity and cardiovascular disease. Research in human evolutionary biology over the last 20 years has shown that many of the key features that distinguish humans from other primates (e.g., our bipedal form of locomotion and large brain sizes) have important implications for our distinctive nutritional needs (Aiello and Wheeler, 1995; Leonard and Robertson, 1997; Leonard, 2002). The most important of these features is our high levels of encephalization (large brain:body mass). The energy demands (kcal/g/min) of brain and other neural tissues are extremely high—approximately 16 times that of skeletal muscle (Kety, 1957; Holliday, 1986). Consequently, the evolution of large brain size in the human lineage came at a very high metabolic cost.

Compared to other primates and mammals of our size, humans allocate a much larger share of their daily energy budget to "feed their brains." The disproportionately large allocation of our energy budget to brain metabolism has important implications for our dietary needs. To accommodate the high energy demands of our large brains, humans consume diets that are of much higher quality (i.e., more dense in

energy and fat) than those of our primate kin (Leonard and Robertson, 1992, 1994). On average, we consume higher levels of dietary fat than other primates (Popovich et al., 1997), and much higher levels of key long-chain polyunsaturated fatty acids (LC-PUFAs) that are critical to brain development (Crawford et al., 1999; Cordain et al., 2001). Moreover, humans also appear to be distinctive in their developmental changes in body composition. We have higher levels of body fatness than other primate species, and these differences are particularly evident early in life.

The need for an energy-rich diet also appears to have shaped our ability to detect and metabolize high-fat foods. Humans show strong preferences for lipid-rich foods. Recent work in neuroscience has shown that these preferences are based on the smell, texture, and taste of fatty foods (Sclafani, 2001; Gaillard et al., 2008; Le Coutre and Schmitt, 2008), and that our brains have the ability to assess the energy content of foods with remarkable speed and accuracy (Toepel et al., 2009). Additionally, compared to large-bodied apes, humans have an enhanced capacity to digest and metabolize higher fat diets. Our gastrointestinal (GI) tract, with its expanded small intestine and reduced colon, is quite different from those of chimpanzees and gorillas and is consistent with the consumption of a high-quality diet with large amounts of animal food (Milton, 1987, 2003). Finch and Stanford (2004) have recently shown that the evolution of key "meat-adaptive" genes in hominid evolution were critical to promoting enhanced lipid metabolism necessary for subsisting on diets with greater levels of animal material.

This chapter draws on both analyses of living primate species and the human fossil record to explore the evolutionary importance of fat in the nutritional biology of our species. We begin by examining comparative dietary data for modern human groups and other primate species to evaluate the influence that variation in relative brain size has on dietary patterns among modern primates. We then turn to an examination of the human fossil record to consider when and under what conditions in our evolutionary past key changes in brain size and diet likely took place. Finally, we explore how the evolution of large human brains was likely accommodated by distinctive aspects of human growth and development that promote increased levels of body fatness from early in life.

1.2 COMPARATIVE PERSPECTIVES ON PRIMATE DIETARY QUALITY

The high energy costs of large human brains are evident in Figure 1.1 which shows the scaling relationship between brain weight (grams) and resting metabolic rate (RMR) (kcal/day) for humans, 35 other primate species, and 22 nonprimate mammalian species. The solid line denotes the best-fit regression for nonhuman primate species, and the dashed line denotes the best-fit regression for the nonprimate mammals. The data point for humans is denoted with a star.

As a group, primates have brains that are approximately three times the size of other mammals (relative to body size). Human brain sizes, in turn, are some 2.5–3 times those of other primates (Martin, 1989). In caloric terms, this means that brain metabolism accounts for ~20%–25% of RMR in an adult human body, as compared to about 8%–10% in other primate species, and roughly 3%–5% for nonprimate mammals (Leonard et al., 2003).

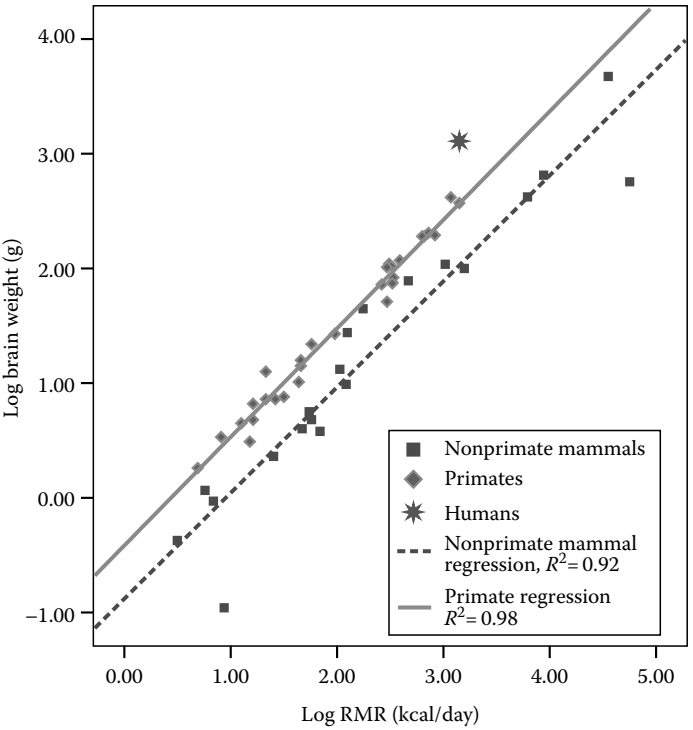

FIGURE 1.1 Log–log plot of brain weight (BW, g) versus RMR (kcal/day) for humans, 35 other primate species, and 22 nonprimate mammalian species. The primate regression line is systematically and significantly elevated above the nonprimate mammal regression. For a given RMR, primates have brain sizes that are three times those of other mammals, and humans have brains that are three times those of other primates.

To accommodate the metabolic demands of our large brains, humans consume diets that are denser in energy and nutrients than other primates of similar size. Figure 1.2 shows the association between dietary quality and body weight in living primates, including modern human foragers. The diet quality (DQ) index is derived from the work of Sailer et al. (1985), and reflects the relative proportions (percentage by volume) of (a) structural plant parts (***s***; e.g., leaves, stems, bark), (b) reproductive plant parts (***r***; e.g., fruits, flowers), and (c) animal foods (***a***; including invertebrates):

$$\text{DQ index} = \boldsymbol{s} + 2(\boldsymbol{r}) + 3.5(\boldsymbol{a})$$

The index ranges from a minimum of 100 (a diet of all leaves and/or structural plant parts) to 350 (a diet of all animal material).

There is a strong inverse relationship between DQ and body mass across primates; however, note that the diets of modern human foragers fall substantially above the regression line in Figure 1.2. Indeed, the staple foods for all human societies are

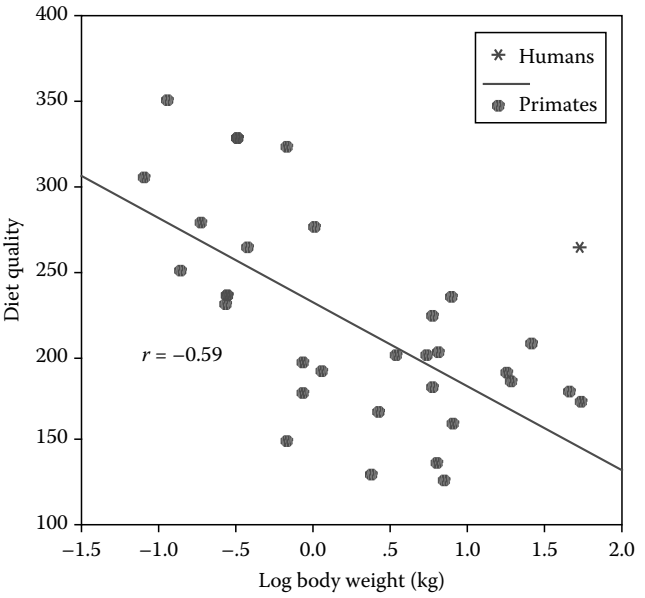

FIGURE 1.2 Plot of DQ versus log body mass for 33 primate species. DQ is inversely related to body mass ($r = -0.59$ [total sample]; -0.68 [nonhuman primates only]; $P < 0.001$), indicating that smaller primates consume relatively higher quality diets. Humans have systematically higher quality diets than predicted for their size. (Adapted from Leonard, W.R. et al., *Comp. Biochem. Physiol., Part A*, 136, 5, 2003.)

much more nutritionally dense than those of other large-bodied primates. Although there is considerable variation in the diets of modern human foraging groups, recent studies have shown that modern human foragers typically derive over half of their dietary energy intake from animal foods (Cordain et al., 2000). In comparison, modern great apes obtain much of their diet from low-quality plant foods. Gorillas derive over 80% of their diet from fibrous foods such as leaves and bark (Richard, 1985). Even among common chimpanzees (*Pan troglodytes*), only about 5%–10% of their calories are derived from animal foods (Teleki, 1981; Stanford, 1996). This "higher quality" diet means that we need to eat less volume of food to get the energy and nutrients we require.

Table 1.1 presents comparative data on macronutrient intakes of selected human groups, compared to those of chimpanzees and gorillas living in the wild. The dietary information for human populations was derived from the U.S. NHANES data (Briefel and Johnson, 2004) and from a recent review of the diets of contemporary hunter-gatherers (foragers) by Cordain et al. (2000). Data for chimpanzees and gorillas were obtained from foraging studies in the wild (Richards, 1985; Tutin and Fernandez, 1992, 1993; Popovich et al., 1997) and compositional analysis of commonly consumed food items (Dufour, 1987; Popovich et al., 1997). Contemporary foraging societies derive between 28% and 58% of their daily energy intake from dietary fat. Those groups living in more northern climes (e.g., the Inuit) derive a

TABLE 1.1
Percent (%) of Dietary Energy Intake Derived from Fat, Protein, and Carbohydrates (CHO) in Selected Human Populations, Chimpanzees (*Pan troglodytes*), and Gorillas (*Gorilla gorilla*)

Species/Group	Fat	Protein	CHO	References
Humans (*Homo sapiens*)				
United States (2000)	33	14	53	Briefel and Johnson (2004)
Modern foragers	28–58	19–35	22–40	Cordain et al. (2000)
Chimpanzees (*P. troglodytes*)	6	21	73[a]	Richard (1985)
				Tutin and Fernandez (1992, 1993)
				Popovich et al. (1997)
Gorilla (*G. gorilla*)	3	24	73[a]	Popovich et al. (1997)

[a] Includes estimated energy derived from fermentation of dietary fiber.

larger share of their diet from animal foods, and thus have higher daily fat intakes. Conversely, tropical foraging populations generally have lower fat intakes because they obtain more of their diet from plant foods. In comparison, Americans, and other populations of the industrialized world fall within the range seen for hunter-gatherers, deriving about a third of their daily energy intake from fat (Millstone and Lang, 2003; Briefel and Johnson, 2004).

In contrast to the levels seen in human populations, the great apes obtain only a small share of calories from dietary fat. Popovich et al. (1997) estimated that Western lowland gorillas derive approximately 3% of their energy from dietary fats. Chimpanzees appear to have higher fat intakes than gorillas (about 6% of dietary energy), but they are still well below the low end of the modern forager range. Thus, the higher consumption of meat and other animal foods among human hunter-gatherers is associated with diets that are higher in fat and denser in energy.

The link between brain size and dietary quality is evident in Figure 1.3, which shows relative brain size versus relative dietary quality for the 33 different primate species for which we have metabolic, brain size, and dietary data. Relative brain size for each species is measured as the standardized residual (z-score) from the primate brain versus body mass regression, and relative DQ is measured as the residual from the DQ versus body mass regression. There is a strong positive relationship ($r = 0.63$; $P < 0.001$) between the amount of energy allocated to the brain and the caloric density of the diet. Across all primates, larger brains require higher quality diets. Humans fall at the positive extremes for both parameters, having the largest relative brain size and the highest quality diet.

Thus, the high costs of the large, metabolically expensive human brain is partially offset by the consumption of diet that is more dense in energy and fat than those of other primates of similar size. This relationship implies that the evolution of larger hominid brains would have necessitated the adoption of a sufficiently high-quality diet

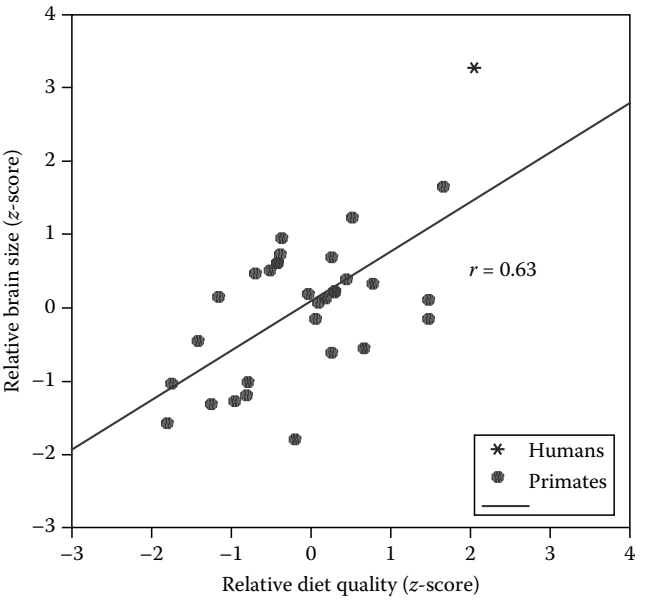

FIGURE 1.3 Plot of relative brain size versus relative DQ for 31 primate species (including humans). Primates with higher quality diets for their size have relatively larger brain size ($r = 0.63$; $P < 0.001$). Humans represent the positive extremes for both measures, having large brain:body size and a substantially higher quality diet than expected for their size. (Adapted from Leonard, W.R. et al., *Comp. Biochem. Physiol., Part A*, 136, 5, 2003.)

(including meat and energy-rich fruits) to support the increased metabolic demands of greater encephalization.

Relative to other large-bodied apes, humans show important differences in the size and morphology of their GI tracts that are tied to the consumption of a more energy-rich diet. Compared to chimpanzees and gorillas, humans have small total gut volumes, reduced colons, and expanded small intestines (Milton, 1987, 2003). In many respects, the human gut is more similar to that of a carnivore and reflects an adaptation to an easily digestible diet that is higher in energy and fat.

In addition, recent work in human evolutionary genetics suggests that the selection for key "meat-adaptive" genes were critical for allowing our hominid ancestors to more effectively exploit diets with higher levels of animal fat. Finch and Stanford (2004) argued that the evolution of the unique E3 allele in *Homo* at the apolipoprotein E (apoE) locus was important for allowing our ancestors to exploit diets with greater animal material. ApoE plays a critical role in regulating the uptake of cholesterol and lipids throughout the body (Davignon et al., 1988). The E3 allele is evident in humans, but not in chimpanzees and gorilla, and is associated with reduced metabolic and cardiovascular risks with the consumption of higher fat diets (Finch and Stanford, 2004).

In light of these important morphological and genetic adaptations to enhanced DQ, it is not surprising that humans also show preferences for foods that are rich in

fat and energy. Until recently, it was thought that human preference for "fatty foods" was based largely on smell and texture (Sclafani, 2001); however, we now know that taste plays a critical role (Gaillard et al., 2008). Neuroimaging studies also suggest that the human brain has a remarkable ability to assess the energy content of potential food items with speed and accuracy (Toepel et al., 2009).

Across human populations, variation in the degree of preference for both sweet and fatty foods has been well documented (e.g., Messer, 1986; Johns, 1996; Salbe et al., 2004). Recent work by Lussana et al. (2008) has shown that nutritional status during development may play an important role in shaping taste preferences. Drawing on analyses from the Dutch Famine Birth Cohort, these authors show that prenatal exposure to famine conditions is associated with greater preference for fatty foods and increased risk of poor serum lipid profiles in adulthood.

1.3 EVOLUTIONARY TRENDS IN DIET, BRAIN SIZE, AND BODY SIZE

When we look at the human fossil record, we find that the first major burst of evolutionary change in hominid brain size occurred at about 2.0–1.7 million years ago (mya), associated with the emergence and evolution of early members of the genus *Homo* (see Table 1.2). Prior to this, our earlier hominid ancestors, the

TABLE 1.2
Geological Ages (mya), Brain Size (cm^3), Estimated Male and Female Body Weights (kg), and Postcanine Tooth Surface Areas (mm^2) for Selected Fossil Hominid Species

Species	Geological Age (mya)	Brain Size (cm^3)	Body Weight		Postcanine Tooth Surface Area (mm^2)
			Male (kg)	Female (kg)	
A. afarensis	3.9–3.0	438	45	29	460
A. africanus	3.0–2.4	452	41	30	516
A. boisei	2.3–1.4	521	49	34	756
A. robustus	1.9–1.4	530	40	32	588
H. habilis (sensu strictu)	1.9–1.6	612	37	32	478
H. erectus (early)	1.8–1.5	863	66	54	377
H. erectus (late)	0.5–0.3	980	60	55	390
H. sapiens	0.4–0.0	1,350	58	49	334

All data from McHenry and Coffing (2000), except for *H. erectus*. Early *H. erectus* brain size is the average of African specimens as presented in McHenry (1994b), Indonesian specimens from Antón and Swisher (2001) and Georgian specimens from Gabunia et al. (2000, 2001). Data for late *H. erectus* are from McHenry (1994a).

australopithecines, showed only modest brain size evolution from an average of 400–510 cm^3 over a span of 2 million years from 4 to 2 mya. With the evolution of the genus *Homo* there is rapid change, with brain sizes of, on average, ~600 cm^3 in *Homo habilis* (at 2.4–1.6 mya) and 800–900 cm^3 in early members of *Homo erectus* (at 1.8–1.5 mya). Furthermore, while the relative brain size of *H. erectus* has not yet reached the size of modern humans, it is outside of the range seen among other living primate species.

The evolution of *H. erectus* in Africa is widely viewed as a "major adaptive shift" in human evolution (Wolpoff, 1999; Antón et al., 2002; Antón, 2003). Indeed, what is remarkable about the emergence of *H. erectus* in East Africa at 1.8 mya is that we find (a) marked increases in both brain and body size, (b) the evolution of human-like body proportions, and (c) major reductions of posterior tooth size and craniofacial robusticity (McHenry, 1992, 1994a,b; Ruff et al., 1997; McHenry and Coffing, 2000). These trends clearly suggest major energetic and dietary shifts: (a) the large body sizes necessitating greater daily energy needs; (b) bigger brains suggesting the need for a higher quality diet; and (c) the craniofacial changes suggesting that they were consuming a different mix of foods than their australopithecine ancestors.

The ultimate driving factors responsible for the rapid evolution of brain size, body size, and cranio-dental anatomy at this stage of human evolution appear to have been major environmental changes that promoted shifts in diet and foraging behavior. The environment in East Africa at the Plio-Pleistocene boundary (2.0–1.8 mya) was becoming much drier, resulting in declines in forested areas and an expansion of open woodlands and grasslands (Vrba, 1995; Reed, 1997; Bobe and Behrensmeyer, 2002; deMenocal, 2004; Wynn, 2004). Such changes in the African landscape likely made animal foods an increasingly attractive resource for our hominid ancestors (Harris and Capaldo, 1993; Behrensmeyer et al., 1997; Plummer, 2004).

This can be seen by looking at the differences in ecological productivity between modern-day woodland and savanna ecosystems of the tropics. Despite the fact that tropical savanna environments produce only about half as much plant energy per year as tropical woodlands (4050 versus 7200 kcal/m^2/year), the abundance of herbivores (secondary productivity) is almost three times greater than in the savanna (10.1 versus 3.6 kcal/m^2/year) (Leonard and Robertson, 1997). Consequently, the expansion of the savanna in Plio-Pleistocene Africa would have limited the amount and variety of edible plant foods (to things like tubers, etc.) for hominids, but also resulted in an increase in the relative abundance of grazing mammals such as antelope and gazelle. These changes in the relative abundance of different food resources offered an opportunity for hominids with sufficient capability to exploit the animal resources.

The archeological record provides evidence that this occurred with *H. erectus*, as this species is associated with stone tools and the development of the first rudimentary hunting and gathering economy. Meat does appear to have been more common in the diet of *H. erectus* than it was in the australopithecines, with mammalian carcasses likely being acquired through both hunting and confrontational scavenging (Plummer, 2004; Bunn, 2006). In addition, the archaeological evidence indicates that butchered animals were transported back to a central location (home base) where the resources were shared within foraging groups (Potts, 1988a,b; Harris and Capaldo,

1993; Bunn, 2006). Increasingly sophisticated stone tools (i.e., the Acheulean industry) emerged around 1.6–1.4 mya, improving the ability of these hominids to process animal and plant materials (Asfaw et al., 1992). These changes in diet and foraging behavior would not have turned our hominid ancestors into carnivores; however, the addition of even modest amounts of meat to the diet (10%–20% of dietary energy) combined with the sharing of resources that is typical of hunter-gatherer groups would have significantly increased the quality and stability of the diet of *H. erectus*.

In addition to the energetic benefits associated with greater meat consumption, it appears that such a dietary shift would have also provided increased levels of key fatty acids necessary for supporting the rapid hominid brain evolution (Cordain et al., 2001). Mammalian brain growth is dependent upon sufficient amounts of two LC-PUFAs: docosahexaenoic acid (DHA), and arachidonic acid (AA) (Crawford et al., 1999; Cordain et al., 2001). Because the composition of all mammalian brain tissue is similar with respect to these two fatty acids, species with higher levels of encephalization have greater requirements for DHA and AA (Crawford et al., 1999). It also appears that mammals have a limited capacity to synthesize these fatty acids from dietary precursors. Consequently, dietary sources of DHA and AA were likely limiting nutrients that constrained the evolution of larger brain size in many mammalian lineages (Crawford, 1992; Crawford et al., 1999).

Cordain et al. (2001) have demonstrated that wild plant foods available on the African savanna (e.g., tubers, nuts) contain only tiny amounts of AA and DHA, whereas muscle tissue and organ meat of wild African ruminants provide moderate to high levels of these key fatty acids. As shown in Table 1.3, brain tissue is a rich source of both AA and DHA, whereas liver and muscle tissues are good sources of AA and moderate sources of DHA. Other good sources of AA and DHA are freshwater fish and shellfish (Broadhurst et al., 1998; Crawford et al., 1999). Cunnane and Crawford (2003) have suggested that the major increases in hominid encephalization were associated with systematic use of aquatic (marine, riverine, or lacustrian) resources. However, there is little archeological evidence for the systematic use of aquatic resources until much later in human evolution (see Klein, 1999).

TABLE 1.3
Energy, Fat, Protein, AA, and DHA Contents of African Ruminant, Fish, and Wild Plant Foods per 100 g

Food Item	Energy (kcal)	Fat (g)	Protein (g)	AA (mg)	DHA (mg)
African ruminant (brain)	126	9.3	9.8	533	861
African ruminant (liver)	159	7.1	22.6	192	41
African ruminant (muscle)	113	2.1	22.7	152	10
African ruminant (fat)	745	82.3	1.0	20–180	Trace
African fish	119	4.5	18.8	270	549
Wild tuber/roots	96	0.5	2.0	0	0
Mixed wild plants	129	2.8	4.1	0	0

Source: Data derived from Cordain, L. et al., *World Rev. Nutr. Diet*. 90, 144, 2001.

Overall, the available evidence seems to best support a mixed dietary strategy in early *Homo* that involved the consumption of larger amounts of animal foods than with the australopithecines. Greater consumption of animal foods would have increased total dietary fat consumption in early *Homo*, and markedly increased the levels of key fatty acids (AA and DHA) necessary for brain development. Together the nutritional stability provided a critical foundation for fueling the energy demands of larger brain sizes.

1.4 BRAIN METABOLISM AND HUMAN BODY COMPOSITION: THE IMPORTANCE OF FAT

In addition to improvements in dietary quality and greater fat intakes, the increased metabolic cost of larger brain size in human evolution also appears to have been supported by developmental changes in body composition. During the human life course, the metabolic demands of our large brains are most dramatic in infancy and early childhood, when brain:body weight ratios are largest and when brain growth is most rapid. Whereas brain metabolism accounts for 20%–25% of resting needs in adults, in an infant of under 10 kg, it uses upwards of 60% (Holliday, 1986)! Table 1.4 shows changes in the percent of RMR allocated to the brain over the course of human growth and development.

To accommodate the extraordinary energy demands of the developing infant brain, human infants are born with an ample supply of body fat (Kuzawa, 1998; Leonard et al., 2003). At ~15%–16% body fat, human infants have the highest body fat levels of any mammalian species (cf., Dewey et al., 1993; Kuzawa, 1998). Further, human infants continue to gain body fat during their early postnatal life. During the first year, healthy infants typically increase in fatness from about 16% to about 25% (see Table 1.4). Thus, the very high levels of adiposity seen in early human growth and development coincide with the periods of greatest metabolic demand of the brain.

TABLE 1.4
Body Weight (kg), Brain Weight (g), Percent Body Fat (%), Resting Metabolic Rate (RMR; kcal/day), and Percent of RMR Allocated to Brain Metabolism (BrMet, %) for Humans from Birth to Adulthood

Age	Body Weight (kg)	Brain Weight (g)	Body Fat (%)	RMR (kcal/day)	BrMet (%)
New born	3.5	475	16	161	87
3 months	5.5	650	22	300	64
18 months	11.0	1045	25	590	53
5 years	19.0	1235	15	830	44
10 years	31.0	1350	15	1160	34
Adult male	70.0	1400	11	1800	23
Adult female	50.0	1360	20	1480	27

All data are from Holliday (1986), except for percent body fat data for children 18 months and younger, which are from Dewey et al. (1993).

Human infants and toddlers also appear to show metabolic adaptations to preserve body fatness in face of nutritional and disease stressors. Research on children of the developing world suggests that chronic, mild to moderate undernutrition has a relatively small impact on a child's fatness. Instead of taking away the fat reserves, nutritional needs appear to be downregulated by substantially reducing rates of growth in height/length—producing the common problem of infant/childhood growth stunting or growth failure that is ubiquitous among impoverished populations of the developing world (Martorell and Habicht, 1986).

Figure 1.4 shows an example of this process based on growth data collected from the Tsimane' farmers and foragers of lowland Bolivia (from Foster et al., 2005). Note that stature early in life closely approximates the U.S. median, but by age 3–4 years it has dropped below the 5th percentile, where it will track for the rest of life. In contrast, body fatness (as measured by the sum of the triceps and subscapular skinfolds) compares more favorably to U.S. norms, tracking between the 15th and 50th U.S. percentiles. The problem of early childhood growth failure is the product of both increased infectious disease loads and reduced dietary quality.

Recent work among impoverished children in Brazil provides insights into the physiological mechanisms associated with the preservation of body fatness under conditions of growth stunting. In a study of children (8–11 years) living in the shantytowns of São Paulo, Hoffman et al. (2000) found that children who were growth stunted had significantly lower rates of fat oxidation than those of their "nonstunted" group. The observed difference in fat oxidation levels under fasting conditions suggested that the stunted children derived about 25% of the resting energy needs from fat, as compared to 34% in the nonstunted group. It appears that the reductions in insulin-like growth factor I (IGF-I) commonly observed with early childhood growth stunting may promote impaired fat oxidation and increased fat storage (Sawaya et al., 1998, 2004; Hoffman et al., 2000). Indeed, because IGF-I has been shown to increase

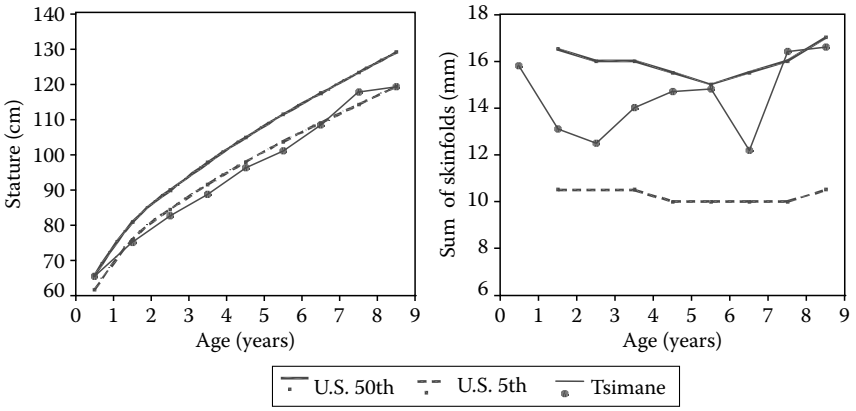

FIGURE 1.4 Patterns of physical growth in stature (cm) and body fatness (as sum of triceps and subscapular skinfolds, mm) in Tsimane' girls of lowland Bolivia. Growth of Tsimane' girls is characterized by marked linear growth stunting, whereas body fatness compares more favorably to U.S. norms. (Data from Foster, Z. et al., *Am. J. Phys. Anthropol*., 126, 343, 2005.)

lipolysis (Hussain et al., 1994), significant reductions in IGF-I during growth can be expected to result in decreased fat oxidation.

Overall, key aspects of human growth and development of body composition are shaped by the very high metabolic demands of brain metabolism early in life. Human infants are born altricially (relatively underdeveloped for their age), and unlike other primates, continue rapid brain growth into early postnatal life (Martin, 1989; Rosenberg, 1992). To provide energy reserves for the high metabolic demands of large, rapidly growing brains, human infants are born with high body fat levels, and continue to gain fat during the first year of postnatal life. Further, under conditions of chronic nutritional stress, human infants show the capacity to preserve brain metabolism by (a) "downregulating" linear growth, (b) reducing fat oxidation, and (c) increasing fat storage. These adaptive responses are evidenced in the preservation of body fatness among "growth stunted" children, and in the tendency of stunted children to gain weight and body fatness later in life (see Frisancho, 2003; Grillo et al., 2005; Hoffman et al., 2007).

1.5 CONCLUSIONS

The evolution of large human brain size has had important implications for the nutritional biology of our species. Humans expend a much larger share of their resting energy budget on brain metabolism than other primates or nonprimate mammals. Comparative analyses of primate dietary patterns indicate that the high costs of large human brains are supported, in part, by diets that are rich in energy and fat. Relative to other large-bodied apes, modern humans derive a much larger share of their dietary energy from fat. Among living primates, the relative proportion of metabolic energy allocated to the brain is positively correlated with dietary quality. Humans fall at the positive end of this relationship, having both a very high-quality diet and a large brain.

Greater encephalization also appears to have consequences for human body composition, particularly in early life. Human infants have higher levels of adiposity than the infants of other mammals. These greater levels of body fatness allow human infants to accommodate the growth of their large brains by having a ready supply of stored energy. Under conditions of nutritional stress, human infants and toddlers preserve body fat reserves for brain metabolism by reducing rates of linear growth. This process of "linear growth stunting" is also associated with reduced rates of fat oxidation and increased rates of fat storage. Thus, humans appear to show important adaptations in fat metabolism to accommodate the high energy demands of the brain early in life.

The human fossil record indicates that major changes in both brain size and diet occurred in association with the emergence of early members of the genus *Homo* between 2.0 and 1.7 mya in Africa. With the evolution of early *H. erectus* at 1.8 mya, we find evidence of an important adaptive shift—the evolution of the first hunting and gathering economy, characterized by greater consumption of animal foods, transport of food resources to "home bases," and sharing of food within social groups. *H. erectus* was human-like in body size and proportions, and had a brain size beyond that seen in nonhuman primates, approaching the range of modern humans. In addition, the

reduced size of the face and grinding teeth of *H. erectus*, coupled with its more sophisticated tool technology suggest that these hominids were consuming a higher quality and more stable diet that would have helped to fuel the increases in brain size. Consequently, while dietary change was not the prime force responsible for the evolution of large human brain size, improvements in dietary quality and increased consumption of dietary fat appear to have been a necessary condition for promoting encephalization in the human lineage.

Associated with the evolution of our high-quality diet, humans developed distinct molecular pathways for detecting and metabolizing high-fat foods. We show preferences for foods that are rich in fat and energy. Key genetic mutations during later hominid evolution were critical to promoting the enhanced lipid metabolism necessary for subsisting on diets with greater levels of animal material. Moreover, accumulating evidence highlights the remarkable capacity of the human brain and sensory system for accurately assessing the energy content of potential food items. In sum, the ability to effectively detect, metabolize, and store fats likely provided tremendous selective advantages to our hominid ancestors, allowing them to expand into diverse ecosystems around the world. Further research is needed to better understand the nature of the dietary changes that took place with the emergence of early human ancestors and how they are associated with distinctive aspects of our own nutritional biology.

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2 Pathophysiology and Evolutionary Aspects of Dietary Fats and Long-Chain Polyunsaturated Fatty Acids across the Life Cycle

Frits A.J. Muskiet

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Dietary fat is our second most important energy-producing macronutrient. It also contains fatty acids and vitamins essential for growth, development, and maintenance of good health. Dietary fat quantity and quality have been subject to tremendous change over the past 10,000 years. This has, together with other man-made changes in our environment, caused a conflict with our slowly adapting genome that is implicated in "typically Western" diseases. Rather than reducing our life expectancy, these diseases notably diminish our number of years in health. Important changes in dietary fat quality are the increased intakes of certain saturated fatty acids (SAFA) and linoleic acid (LA), introduction of industrially produced *trans* fatty acids, and reduced intakes of ω3 fatty acids, notably alpha-linolenic acid (ALA) from vegetable sources and eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) from fish. The pathophysiological effects of these changes are diverse, but are increasingly ascribed to induction of a proinflammatory state that progresses easily to chronic low-grade inflammation. The latter might affect virtually all organs and systems, possibly beginning at conception, and possibly even prior to gametogenesis through epigenetic alterations. Low-grade inflammation might be a common denominator of the metabolic syndrome and its sequelae (e.g., coronary artery disease (CAD), diabetes mellitus type 2, some types of cancer, and pregnancy complications), some psychiatric diseases (e.g., major and postpartum depression, schizophrenia, and autism), and neurodegenerative diseases (e.g., Alzheimer's disease, Parkinson's disease). The long-chain polyunsaturated fatty acids (LCPUFA) arachidonic acid (AA), EPA, and DHA are intimately related to the initiation and resolution of inflammatory responses. The current balance between AA and EPA + DHA is however disturbed by the dominance of AA, which originates from the diet or synthesis from LA. LCPUFA are together with their highly potent

metabolites (prostaglandins, thromboxanes, leukotrienes, resolvins, and (neuro)protectins) involved in the functioning of membrane-bound receptors, transporters, ion channels, and enzymes, and also in signal transduction and gene expression. Among their many targets are nuclear receptors which, upon ligation with LCPUFA and their metabolites, function as transcription factors of a variety of genes functioning in many pathways. For instance, the targeted peroxisome proliferators-activated receptors (PPARs) are strategic intermediates in the coordinated expression of proteins with functions in, for example, lipid and glucose homeostasis and inflammatory reactions. Many interventions have been conducted with LCPUFA, especially EPA and DHA, aiming at primary and secondary CAD preventions, improvement of fetal and newborn (brain) development by supplementation during pregnancy or early postnatal life, and in psychiatric diseases. Consensus has been reached that those in CAD and depression are positive, although more large-scale trials are needed. Many recommendations for the intakes of saturated fat, *trans* fat and EPA + DHA have been issued, notably for CAD prevention, and also for EPA + DHA intakes by pregnant women and for AA, EPA, and DHA intakes by newborns. The ultimate goal might, however, be to return to the fat quality of our ancient diet on which our genes have evolved during the past million years of evolution, while this actually applies for our entire dietary composition and lifestyle, as translated to the culture of the current society.

2.1 INTRODUCTION

Dietary fats and especially their caloric content and individual constituents, such as essential fatty acids (EFA) and fat-soluble vitamins, are indispensable for growth, development, and maintenance of good health. The nutritional value of dietary fats and their fatty acid composition cannot be considered in isolation since they are part of a diet that in its optimal form is balanced to fulfill the nutritional requirements of adults, and, in infants, children, and adolescents, should also be appropriate for growth and development. The corresponding nutrient needs, as defined by the Dietary Reference Intakes (DRIs) for both sexes and the various life stage groups, have been established by authoritative organizations such as the Food and Nutrition Board of the Institute of Medicine (DRI USA, 2008).

The influence of maternal diet prior to conception, during pregnancy and in early life on child development, and adult onset of many diseases is increasingly recognized (Gluckman et al., 2005). Many Nutritional Boards, for example that of The American Heart Association, recommend exclusive breastfeeding for 4–6 months, and, if possible, its continuation to 12 months, because of its beneficial effects on the prevention of obesity and its possible impact on lower blood pressure in later childhood, and on lower blood cholesterol at adult age. Some of these effects may be on the account of better self-regulation of energy intake and taste preference at later ages (Gidding et al., 2005). The period from weaning to a mature diet, usually from 4 to 6 months to 2 years, is characterized by the introduction of complementary foods* and should be guided by the physiologic and developmental readiness of the infant,

* Any energy-containing foods that displace breastfeeding and reduce the intake of breast milk.

nutrient requirements for growth and development, and other health considerations (Butte et al., 2004). Special emphasis is laid on the moderation of the consumption of "kid foods," which tend to be high in fat and sugar, including excess juice, juice-based sweetened beverages, French fries, and nutrient-poor snacks, and may easily cause the exceeding of energy requirements. For those aged 2 years and older the emphasis is on a diet that primarily relies on fruits and vegetables, whole grains, low-fat and nonfat dairy products, beans, fish, and lean meat, which translates to low intakes of saturated fat, *trans* fat, cholesterol, and added sugar and salt, adequate intake of micronutrients, and an energy intake and physical activity appropriate to maintain a normal weight for height (Gidding et al., 2005).

After an introduction on the evolutionary aspects of our changing diet during the past 10,000 years and its relation with the development of "typically Western disease," and stressing the importance of intrauterine and early postnatal diet, this chapter focuses on the influence of LCPUFA* on development, health, and disease.

2.2 OUR CHANGING DIET FROM AN EVOLUTIONARY PERSPECTIVE

Humans share a common ancestor with the chimpanzee and bonobo that probably lived in East Africa some 6 million years ago. Hominins have since then experienced a tremendous brain growth and assumed an upright position, which coincided with a change from a vegetarian to a hunting-gathering omnivore–carnivore. The oldest *Homo sapiens* found to date originates from the current Ethiopia and is about 160,000 years old (White et al., 2003). Since about 100,000 years ago humans have started to spread across the whole world to become the only *Homo* species currently inhabiting this planet. The "Out-of-Africa" Diaspora necessitated adaptation to new conditions of existence. Changes in physical characteristics gave rise to the concept of "race," which is however better described by "geographical location" of origination as shown by studies of human genetic variation (Rosenberg et al., 2002). The number of new alleles that has been added to *H. sapiens*' gene pool since the "Out-of Africa" Diaspora is small compared with the genetic variation that was already present in its founder population. If the total genetic variance is set at 100%, it becomes apparent that 93%–95% of the variance can be ascribed to differences between individuals belonging to a single population within a single race and that the differences between populations within a single race account for only 2%, so that the five races do not differ by more than 3%–5% (Rosenberg et al., 2002).

The vast majority of alleles that have been added to the already existing variance results from the selection pressure that was experienced by the changing environment since 160,000 years ago. They include alleles for protection against sunlight (skin (de)pigmentation, Jablonski and Chaplin, 2000; Harris and Meyer, 2006) and infectious diseases such as malaria (Cserti and Dzik, 2007), small pox, yellow fever, typhus, and cholera (Balter, 2005), and also for new diets such as milk consumption at adult age (lactase persistence, Harris and Meyer, 2006), gluten (grains, Cordain,

* Long-chain polyunsaturated fatty acids are straight-chain fatty acids with ≥20 carbon atoms and ≥3 double bonds in the methylene-interrupted *cis* configuration.

1999), and starch-rich diets (amylase copy number variation, Perry et al., 2007). The molecular clock hypothesis estimates that our nuclear DNA changes with an average rate of about 0.5% per million years (1.7×10^{-9} substitutions/site, year; Ingman et al., 2000). The genuine rate of genomic change is obviously dependent on the actual mutation rate, the magnitude of selection pressure, and other driving forces in population genetics, such as genetic drift (i.e., loss of genes in small populations). There is evidence that the rapidity became increased since the diminishing influence of famine and infection and the concomitant growth of the human population from some 40,000 years ago (Hawks et al., 2007), but basically we remained almost the same as the first *H. sapiens*, some 160,000 years ago. In other words, genetically, we are for the greater part still adapted to the East African ecosystem on which our genome evolved, with some adaptations since the Out-of-Africa Diaspora. It is clear that any change of environment is capable of introducing new selection pressure, with outcomes ranging from no effect to extinction, but also to an intermediate outcome that confers a loss of number of years in health without major influence on the chance to reach reproductive age. This basic principle of evolution was already noticed by Darwin in 1859.

2.2.1 Conflict between Current Diet and Our Ancient Genome

Since the agricultural revolution (i.e., some 10,000 years ago) we have gradually changed our diet and accelerated these changes from the beginning of the industrial revolution (100–200 years ago). The seven major dietary changes as recognized by Cordain et al. (2005) are summarized in Figure 2.1. Briefly, we have shifted our

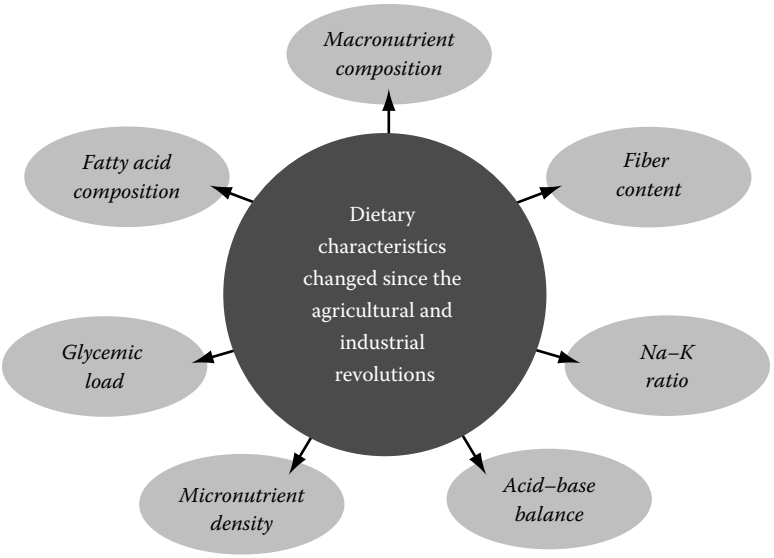

FIGURE 2.1 The seven crucial nutritional factors that have been changed since the agricultural and industrial revolutions. (Data derived from Muskiet, F.A. et al., *Prostag. Leukot. Essent. Fatty Acids*, 75, 135, 2006. With permission.)

dietary macronutrient composition toward carbohydrates at the expense of protein, increased the intakes of ω6 fatty acids (notably LA from refined seed oils), SAFA and industrially produced *trans* fatty acids, decreased our ω3 fatty acid intake (both ALA and those from fish oil), shifted to a carbohydrate-rich diet that contains a high percentage refined carbohydrates with high glycemic indices (e.g., highly processed grains, sucrose, fructose), decreased the intake of certain micronutrients (e.g., folate, vitamin D, magnesium, zinc), shifted toward acid-producing foodstuffs (like meat, grains) at the expense of base-producing counterparts (fruits, vegetables), increased our sodium (salt) intake and reduced our potassium intake, and decreased the intake of fiber.

Table 2.1 compares the EFA intakes from the reconstructed Paleolithic diet with that of a typically Western diet and with current recommendations. Estimates of EFA intakes were calculated for a savanna-like diet (models 1 and 2, Eaton et al., 1998; Eaton and Eaton, 2000; Cordain et al., 2000) with different plant–animal subsistence ratios. In contrast to the savanna diet, the unpublished data of Kuipers et al. (models 3 and 4) assume a shore-based diet in which meat was mixed with fish harboring fatty acid compositions of those living in current Tanzanian lakes and the ocean (Kuipers et al., 2005, 2007). The median intakes (model 3) and their ranges (model 4), indicate that the mixing of fish into the savanna-like Paleolithic diet greatly increases the intakes of the sum of the fish oil fatty acids (EPA + DHA) to a median of 12.05 (range: 3.98–28.7 g/day), while the intakes of ALA and AA in all models are high, and that of LA is low. The calculated high LCPUFAω3 intakes compare well with those of the Eskimo's who have lifetime high consumption of marine foods (see Section 2.5.1.2). The models were constrained at a maximum intake of 35 en% from protein (Cordain et al., 2000), an intake of more than 1.7 en% LA to prevent biochemical signs of EFA deficiency (Barr et al., 1981) and an (EPA + DHA)/AA ratio above about 2.0. Low values for the latter ratio are related to CAD and neuroinflammatory diseases (Sections 2.5.1.6, 2.5.3.2, 2.5.4.2, and 2.5.4.4). The (EPA + DHA)/AA constraint was calculated from the current recommended intake of 450 mg EPA + DHA/day, while the upper limit of the mean dietary intake of AA from typical Western diets is about 220 mg/day (Section 2.5.1.2, Astorg et al., 2004).

The EFA and other changes in our diet together with an energy intake that does not match with our current sedentary lifestyle has caused a conflict with our genome that is likely to be at the basis of "typically Western" diseases. The resulting human phenotype centers around the metabolic syndrome,* which is a risk factor for associated diseases such as cardiovascular disease (CAD), diabetes mellitus type 2, osteoporosis, and certain types of cancer, notably those of the breast, prostate, and colon (Reaven, 2005; WCR/AICR, 2007). Many, if not all, of the polymorphisms† that have been implicated in Western disease up to now give rise to

* The metabolic syndrome, also referred to as the insulin resistance syndrome, is a combination of four frequently occurring symptoms, i.e., high blood pressure, disturbed glucose homeostasis, dyslipidemia, and overweight. The syndrome confers high risk of diabetes mellitus type 2, cardiovascular disease, and many other typically Western diseases. Microalbuminuria is a symptom that is also considered to be part of the metabolic syndrome by some.

† Polymorphism refers to differences in DNA sequences that occur with ≥1% frequency in the population. A mutation has a frequency <1%.

TABLE 2.1
Estimated Intake of EFA from a Paleolithic Diet, as Compared with Intakes from a Current Typically Western Diet, RDAs, AIs, and AMDRs

Nutrient	Unit	Paleolithic Diet, Model 1, Savanna	Paleolithic Diet, Model 2, Savanna	Paleolithic Diet, Model 3, Meat/Fish Mixed	Paleolithic Diet, Model 4, Meat/Fish Mixed	2003 NL Diet (19–30 Years)	U.S. Recommendations RDA, AI of AMDR
Animal/plant ratio							
Animal{meat/fish}/plant	en%{en%/en%}/en%	35{100/0}/65	55{100/0}/45	35{30/70}/65	70–30{0–40/60–100}/30–70		
Macronutrients							
Energy	kcal/day	3000	3000	3000	3000	M 2760 and F 1921	M 2842 and F 2477[a]
Protein	en%	37	35	30	22–36	14.3	AMDR 10–35
Carbohydrate	en%	41	28	39	19–48	48.2	AMDR 45–65
Fat	en%	22	37	31	20–46	34.4	AMDR 20–35
EFA							
ALA (18:3ω3)	g/day	12.61	15.4	16.3	7.9–21.6		AI M 1.6 and F 1.1
EPA (20:5ω3)	g/day	0.39	0.63	2.98	1.14–6.67		
DPA (22:5ω3	g/day	0.42	0.91	2.09	0.78–4.71		
DHA (22:6ω3)	g/day	0.27	0.35	9.07	2.84–22.0		
EPA + DHA	g/day	0.66	0.98	12.05	3.98–28.7	M 0.103 and F 0.84	United States, UK, NL 0.45
LCPω3	g/day	1.08	1.89	14.14	4.65–32.5		

(*continued*)

TABLE 2.1 (continued)
Estimated Intake of EFA from a Paleolithic Diet, as Compared with Intakes from a Current Typically Western Diet, RDAs, AIs, and AMDRs

Nutrient	Unit	Paleolithic Diet, Model 1, Savanna	Paleolithic Diet, Model 2, Savanna	Paleolithic Diet, Model 3, Meat/Fish Mixed	Paleolithic Diet, Model 4, Meat/Fish Mixed	2003 NL Diet (19–30 Years)	U.S. Recommendations RDA, AI of AMDR
ω3	g/day	13.7	17.2	30.4	19.3–40.5		
LA (18:2ω6)	g/day	8.84	12.6	8.0	5.2–10.6		AI M 17 and F 12
AA (20:4ω6)	g/day	1.81	2.24	4.85	1.91–10.7	M 0.195 and F 0.160	
LCPω6	g/day	7.28	3.40	8.80	3.73–18.7		
ω6	g/day	16.1	16.0	16.8	10.5–24.7		
LCP	g/day	8.36	5.30	22.9	8.49–52.0		
LA/ALA	g/g	0.70	0.83	0.50	0.39–0.89		
EPA + DHA/AA	g/g	0.36	0.44	2.45	2.09–2.69		
LCPω3/LCPω6	g/g	0.15	0.56	1.61	0.71–1.76		
ω3/ω6	g/g	0.85	1.08	1.81	0.93–2.11		
ALA (18:3ω3)	en%	4.0	4.4	5.1	2.49–6.80	M 0.63 F 0.59	AMDR 0.6–1.2
LCPω3	en%	0.3	0.6	4.4	1.46–10.23		AMDR 0.06–0.12
LA (18:2ω6)	en%	2.78	3.97	2.52	1.63–3.32	M 5.8 F 5.5	AMDR 5–10
LCPω6	en%	2.29	1.07	2.77	1.17–5.88		AMDR 0.5–1.0
Other fats and fatty acids							
SAFA	g/day	21.2	30.3	27.4	13.0–49.2	33.4	As low as possible
MUFA	g/day	25.7	33.5	24.2	15.5–34.2		

PUFA	g/day	29.8	33.3	47.2	31.7–68.3		
Trans fatty acids	g/day					2.8	As low as possible
P/S ratio	g/g	1.40	1.10	1.83	1.16–2.72		
Cholesterol	mg/day	480	830	502	321–764		As low as possible
SAFA	en%	6.66	9.52	8.61	4.09–15.48	M 12.9 and F 13.1	As low as possible; NL < 10
MUFA	en%	8.08	10.56	7.61	4.88–10.76	M 11.4 and F 11.2	
PUFA	en%	9.38	10.47	14.86	9.97–21.49	M 6.8 and F 6.5	NL 12
ω3	en%	4.31	5.43	9.58	6.07–12.74	M 0.7 and F 0.66	
ω6	en%	5.07	5.04	5.29	3.30–7.77	M 5.85 and F 5.52	
Trans fatty acids	en%					1.1	As low as possible; NL < 1

Data for model 1 are derived from Eaton et al. (1997, 1998), who used an animal{meat/fish}/plant composition of 35{100/0 en%}/65 en%. The content of an individual fatty acid per 100 g meat was calculated by organ or tissue as % total edible *x* % total fatty acids in organ or tissue *x* that individual fatty acid as % of total fatty acids in organ or tissue. The fat percentage of the total edible meat was 4.89%. Model 2 is based on data from Cordain et al. (2000) using a 55[100/0]/45 composition, 5% plant fat and 5% meat fat. The plant fatty acid composition was as previously described (Guil et al., 1996; Eaton et al., 1998). Model 3 is the median outcome of all models by Kuipers et al. (unpublished) in which animal/plant ratios and meat/fish ratios were varied. The meat fat contents varied from 5% to 10%, the plant fat content from 2.5% to 5% and the fish fat content from 2.5% to 10%. The fish fatty acid composition of Tanzanian lakes and ocean was derived from Kuipers et al. (2005). Rejected were models that did not comply with the following constraints: protein intake below 35 en%, LA intake above 1.7 en% and (EPA + DHA)/AA above 2.0 g/g. Model 4 shows the ranges of intakes, as derived from all models by Kuipers et al. (unpublished) complying with the above constraints. Data from the Western diet are derived from the 2003 Dutch National Food Survey (Ocke et al., 2004; Kruizinga et al., 2007) and the U.S. Food and Nutrition Board 1989 (Eaton et al., 1997), AA intake is taken from Astorg et al. (2004), recommendations (RDA, AI, AMDR, UL) are from the U.S. DRI (DRI USA, 2008) and EPA + DHA intake recommendations are from Psota et al. (2006) and Gezondheidsraad (2006). LCPω6 is overestimated since it was calculated as "the gap" between PUFA and all ω3 and ω6 fatty acids. For fatty acid abbreviations see list of abbreviations; NL, the Netherlands; UK, United Kingdom; M, male; F, female; RDA, recommended dietary allowance; AI, adequate intake; AMDR, acceptable macronutrient distribution range.

[a] 30 years, length: 1.65 m, body mass index: 24.99 kg/m2, active (physical activity level, PAL: 1.6–1.9).

perfectly normal genes that do not cause disease by themselves and are likely to have been with us since the first *H. sapiens*. With a generation time of 20–25 years, the nucleotide sequence of our genome could simply not have become adapted to these new conditions of existence. Polymorphisms giving rise to variant proteins with different sensitivities to dietary factors, such as vitamins (Ames et al., 2002), are widespread, and may cause a higher need in the homozygous and occasionally heterozygous states. If the prevalence of these genotypes exceeds 2.5% they are at least partially included into the recommended dietary allowance (RDA) for that dietary factor, which by definition equals the recommended intake for a certain nutrient that is sufficient for 97.5% of the population (Yates et al., 1998). Illustrative are homozygotes for the methylenetetrahydrofolate reductase 677 C → T (Ala222 → Val) allele and protein (MTHFR TT), which compared to MTHFR CT and CC counterparts, require higher folate status for optimal functioning of the variant enzyme. MTHFR TT has a prevalence of about 10%–20% in the white population, but is also widespread among the other races. Such polymorphic genes might better be referred to as "genes sensitive to faulty environment" (i.e., low folate), rather than "disease susceptibility genes."

The domination of famine during human evolution has shaped our genome to what has been named the "thrifty genotype"* (Chakravarthy and Booth, 2004; Prentice et al., 2005) by Neel as early as 1962 (Neel, 1999). It is conceivable that basically, we are all carriers of the "thrifty genotype." This hypothesis seems increasingly proven by genetic analyses, since the alleles with demonstrated risk of obesity in meta-analysis exhibit high frequency and confer low relative risk (Van den Berg, 2008), which might have been predicted from the evolutionary comprehensible phenomenon of high frequency–low penetrance and low frequency–high penetrance (Willett, 2002; The Wellcome Trust Case Control Consortium, 2007). It seems that our genetically determined "survival strategy" turns against us now that we can eat whatever we want and whenever we want, and need little physical activity for food procurement. This, so-called obesogenic environment has never existed in the past, was consequently not part of selection pressure, and genetic adaptations might therefore also not be expected. Consequently, obesity is not a genetic disease, apart from some rare mutations (Farooqi and O'Rahilly, 2006). The conclusion that it is "caused by an interaction between genes and environment" distracts from its causation by our current "faulty" environment and therefore does not carry useful information from a public health perspective. The identification of the underlying genes is nevertheless important from the point of view of health care, since it may help us target treatments in those who have developed disease from underexposure or overexposure to the underlying environmental factor(s).

* Thrifty genotype hypothesis: limiting food recourses have in the past favored alleles that promote efficient bodily storage of energy reserves. In conditions of plentiful nutrition, this genotype predisposes to disease. Thrifty phenotype hypothesis: limiting food resources cause physical and metabolic adaptations of the fetus that, when mismatched predisposes to disease in later life.

2.2.2 Number of Years in Health

Fortunately, the current conflict between environment and our genome has not affected our Darwinian fitness* to an appreciable extent, since at present the majority of Western diseases occurs typically after reproductive age. We have on the contrary witnessed a tremendous increase of survival to reproductive age and of life expectancy, with a concomitant explosion of the world population. This achievement is however largely on account of the elimination of the aforementioned unfavorable conditions of existence, that is, notably through elimination of famine and infections (Eaton et al., 2002). Elimination of the meanwhile voluntarily introduced unfavorable environmental conditions and return to the dietary balance, and lifestyle in general, on which our genome has evolved, might restore "homeostasis,"† increase the number of years in health, reduce the costs in healthcare, but not so much add to an increase of life expectancy (Eaton et al., 2002).

2.3 IMPORTANCE OF EARLY DIET: THE THRIFTY PHENOTYPE

Intrauterine undernourished or malnourished, so-called programmed, newborns are at risk of some typically Western diseases at adult age, notably when they become exposed to the nutrient-rich postnatal environment and sedentary lifestyle that is characteristic for affluent societies and increasingly so for rapidly developing countries, that is, the "societies in transition" (Prentice and Moore, 2005). This "fetal origins" hypothesis of the early 1990s by Barker (1995, 2007) was initially based on the epidemiological relation between low birth weight and the development of CAD at adult age, but is now known to be associated with many diseases and their risk factors in affluent societies, such as obesity, diabetes mellitus type 2, stroke, osteoporosis, polycystic ovary syndrome, abnormal vascular compliance, endothelial dysfunction, insulin resistance, compromised hypothalamic–pituitary–adrenal (HPA) axis, and schizophrenia (Godfrey and Barker, 2000). More precisely, the hypothesis states that "limiting food resources at a vulnerable time during intrauterine growth and development may cause physical and metabolic adaptations of the fetus that predispose to disease in later life." The hypothesis is also referred to as the "thrifty phenotype" and the "developmental origins of health and disease" (DOHaD) (Hales, 1997; Wells, 2003; Stocker et al., 2004; Prentice and Moore, 2005). It should, however, be noted that "low birth weight" is a proxy for intrauterine nutrient restriction, since "normal birth weight" does not preclude underdevelopment of particular organs or systems. Normal birth weight refers to quantity, not quality, and can, for example, still be attained if maternal nutrition is adequate in late gestation (Buckley et al., 2005). In addition, the hypothesis is nowadays not limited to the effects of undernourishment, since it has become clear that fetal overnutrition leading to "high birth weight" may exert similar

* The extent to which an organism is adapted to or able to produce offspring in a particular environment.

† Homeostasis is the "integration of, and the balance between, physiological functions." It refers to optimal interaction between environment and our genome: "our nature in balance with nurture."

effects (Cottrell and Ozanne, 2008). Such conditions are likely to become more prevalent, since "high birth weight" is clearly associated with disturbed maternal glucose homeostasis, such as occurring in diabetes mellitus type 2 and gestational diabetes.

2.3.1 Evolution and the Thrifty Phenotype

Low birth weight is usually derived from maternal malnutrition, undernutrition, placental dysfunction, or abnormal fetal handling (Godfrey and Barker, 2000). An overview of biological factors influencing prenatal growth and development is shown in Figure 2.2 (Ceelen et al., 2008). Dependent on type, timing, and duration, these insults may compromise growth of those organs developing in the affected time window or preclude the reach of the organism's genetically determined maximum growth. Mechanistically, the ensuing growth restriction, and notably its hierarchy in terms of which organ becomes affected first, is likely to have an epigenetic* background aiming at adjusted development of the various organs and systems. Analogous

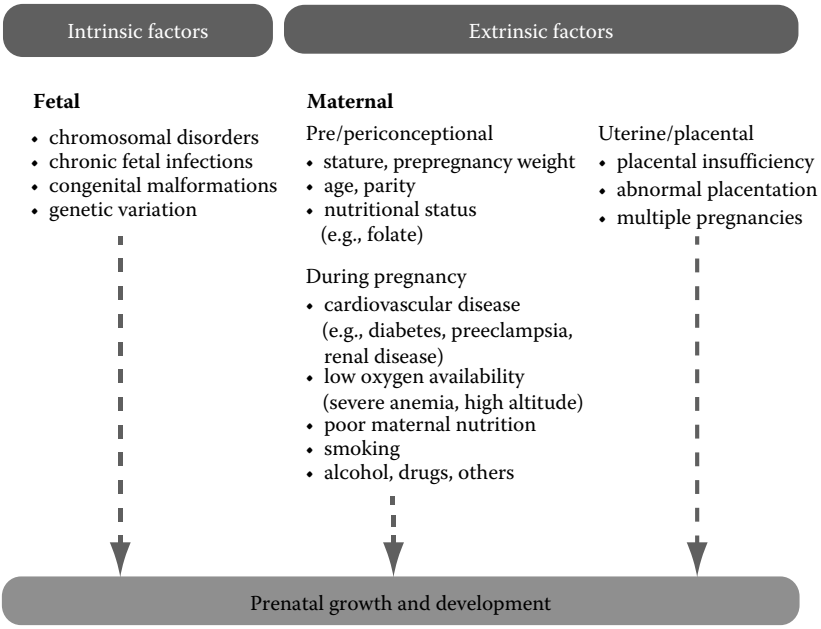

FIGURE 2.2 Biological factors influencing prenatal growth and development. Effect sizes depend on the developmental stage (e.g., organogenesis, fetal period) of the conceptus. (Adapted from Ceelen, M. et al., *Fertil. Steril*., 90, 1662, 2008. With permission.)

* Epigenetics is the study of the changes in gene expression that occur without changes in DNA sequence. These changes are inherently unstable, but stable during mitosis (by which a liver cell remains a liver cell) and occasionally during meiosis (in which case phenotype is transmitted to the next generation). Epigenetics is at the basis of the phenotypic differences between a liver cell and neuron carrying the same genome. The environment transmits instructions to the genome through epigenetics to keep our phenotype perfectly adapted at both short and middle-long term.

to the "thrifty genotype," the "thrifty phenotype" is also likely to find its origin in evolution. Dependent on the magnitude of the insult, these adaptations may either be of immediate value to survival (the so-called immediate adaptive response) or improve Darwinian fitness at a later stage of development (the so-called predicted adaptive response; PAR). They are to be distinguished from insults that cause gross pathology or even intrauterine death, such as the central nervous system pathology caused by severe iron, iodine, or folic acid deficiencies, of which the latter is known for its causal relation with neural tube defects. The immediate adaptive response comes with long-term costs in the sense of higher chance of disease development. The PAR is advantageous from an evolutionary point of view, because it allows for better adaptation to the predicted environment and thereby confers a higher chance of survival and reproductive success. Disease may, however, ensue if the predicted environment mismatches with the actual environment. The combination of the thrifty phenotype and a postnatal nutrition-rich environment, might constitute a not-readily observed mismatch between intrauterine and postnatal conditions in humans, that explains much of the epidemiology of Western disease (Bateson et al., 2004; Gluckman et al., 2005, 2007a; Gluckman and Hanson, 2006; Godfrey et al., 2007; Waterland and Michels, 2007; Hanson and Gluckman, 2008; Malina and Little, 2008).

2.3.2 The Thin Fat Baby

Many valuable data with regard to the thrifty phenotype have come from India. Comparison of the body compositions of a 2700 g newborn in India with a 3500 g newborn in the United Kingdom, revealed that the Indian baby has preserved brain volume, but has developed a relatively large adipose tissue compartment at the expense of muscle and visceral organs such as liver, pancreas, and kidneys (Figure 2.3) (Yajnik,

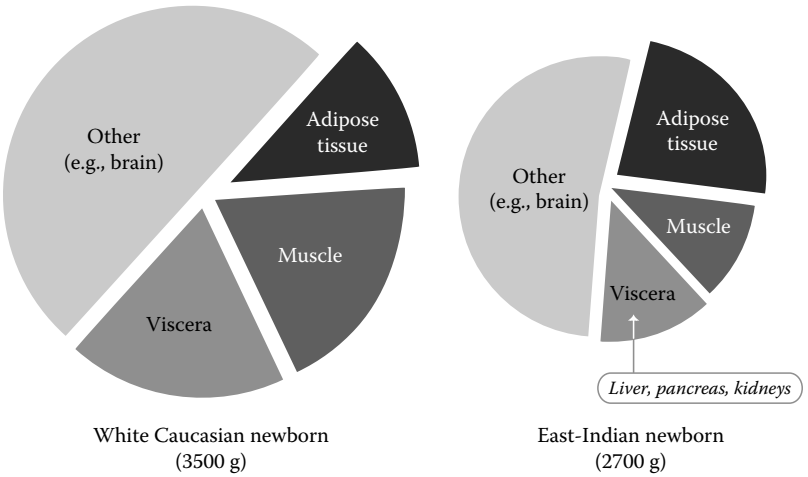

FIGURE 2.3 Body compositions of white Caucasian United Kingdom and East-Indian newborns. The growth retarded Indian baby, also referred to as the "thin fat baby," has preserved its brain volume and adipose tissue compartment at the expense of muscle and abdominal viscera. (Adapted from Yajnik, C.S., *Proc. Nutr. Soc.*, 63, 387, 2004. With permission.)

2004). The cord blood insulin and glucose levels of these "thin fat babies" are higher than those of counterparts born in the United Kingdom, while similar signs of insulin resistance are noticeable at the age of 4 and 8 years, especially when they grow fast (Yajnik, 2000). Interestingly, these differences in body composition seem to persist to adult age, since at similar body mass index, East Indians have higher percentage body fat, compared with Caucasians (Deurenberg et al., 1998; Yajnik and Yudkin, 2004). This fat mass is notably located in the abdominal cavity which is a feature of the metabolic syndrome in which low-grade inflammation, insulin resistance, and compensatory hyperinsulinemia are central (Reaven, 2005).

2.3.3 Animal Studies

Although the "fetal origins" hypothesis will remain unproven from randomized controlled trials (RCTs), it is well supported by animal studies (Buckley et al., 2005). Various intervention modes have been employed, including maternal nutrient restriction (e.g., protein, energy, iron), uterine artery ligation, and hormonal insults, such as glucocorticoid overexposure (Stocker et al., 2005). For instance, it was shown that the low birth weight offspring of undernourished pregnant mice develop obesity on a high-fat postnatal diet (Yura et al., 2005). Dietary protein restriction experiments in pregnant rats increases the susceptibility to insulin resistance and diabetes of their offspring if they receive a high-fat postnatal diet (Stocker et al., 2005). On a molecular basis, such experiments caused upregulated expression of genes for the glucocorticoid receptor (GR) and the PPAR-alpha in the offspring liver. These receptors are important to growth and development and have been implicated in the "fetal origins" hypothesis. Diminished methylation of their gene promoter regions was demonstrated to be at least one of the underlying mechanisms (Lillycrop et al., 2005). The state of hypomethylation of the PPAR-alpha gene promoter became conserved up to adult age (Lillycrop et al., 2008), but proved correctable by fortifying the protein-restricted diet with folic acid (Lillycrop et al., 2005). Importantly, these studies were the first to suggest a background of the fetal hypothesis in epigenetics. They demonstrate that the acquired epigenetic marks *in utero* may persist to adult age, while it becomes increasingly clear that these marks may also become transmitted to the next generation, giving rise to a seemingly genetic origin of the associated traits (Gluckman et al., 2007b).

Few studies have as yet been conducted to link the fetal origins hypothesis with dietary fat in pregnancy. One study in rats showed that a low fat intake in pregnancy retarded pulmonary maturation (Nelson et al., 1982). High fat intakes, especially those rich in SAFA and ω6 fatty acids, by pregnant animals caused similar effects on their offspring, when compared with administration after weaning. High SAFA intakes during pregnancy caused features of the metabolic syndrome in the offspring, including increased body fat, increased liver weight and triglyceride content, elevated circulating glucose and triglycerides, vascular dysfunction, permanent alterations in structure and function of the pancreas with faster and greater insulin responses upon a glucose challenge, severe endothelial dysfunction, hypertension, insulin resistance and secretory deficiency, and mitochondrial abnormalities that predispose to metabolic disease. Diets high in ω6 fatty acids caused disturbed glucose homeostasis and insulin

responsiveness suggestive of insulin resistance, elevated body fat and abdominal fat at normal body weight, hepatic triglyceride accumulation, and downregulated expression of key-proteins in the insulin-signaling cascade (Buckley et al., 2005).

2.3.4 Decreasing Influence with Age

Consistent with the closure of the "window of opportunity" for the development of most organs, the influence of nutrition on reaching optimal organ cell numbers and subsequent development becomes less apparent and mechanistically less attributable to the thrifty phenotype *per se* with advancing age. The brain is a clear exception because of its growth up to about 2 years of age, with the time of delivery showing the highest growth rate. This rapid growth is also referred to as the "brain growth spurt" and in humans extends from the third trimester up to 18 months after birth (Innis, 1991). The chances of acquired mutations in the germ line and somatic cells obviously increase with advancing age, but the influence of environment also exerts cumulating effects on our epigenome, as elegantly demonstrated in a study of twins: it was shown that monozygous twins exhibit more epigenetic differences at older age, compared with infancy. This suggests that, dependent on environmental factors, our phenotype may progress in different directions with time and thereby explain part of the disease discordance in genetically identical twins (Fraga et al., 2005).

The identification of factors causing postnatal amplification and reversal of the metabolic adaptations of the thrifty phenotype is of crucial importance. Rapid postnatal growth is a well-studied risk factor, and it becomes increasingly clear that the stimulus of this growth and the ensuing obesity might largely be programmed *in utero* by altered leptin signaling (Cottrell and Ozanne, 2008). Importantly, it was recently shown in rats that some of the adverse effects associated with, dexamethasone-induced, low birth weight were offset by a postnatal diet rich in the fish oil fatty acids EPA and DHA. This treatment completely blocked the associated hyperleptinemia and hypertension, suggesting that fish oil fatty acids might prevent or at least reduce some of the adverse effects associated with low birth weight (Wyrwoll et al., 2006).

2.4 POSTNATAL NUTRITION WITH SPECIAL REFERENCE TO FAT

The influence of the postnatal diet on development of typically Western diseases has been studied intensively. Although fat and cholesterol consumption and high serum cholesterol have for long been blamed as the principal causes of the CAD epidemic, we now know that this so-called lipid–heart hypothesis" is at least incomplete and that dietary fat and cholesterol quantities hold questionable relations with CAD. There is no solid evidence that a high intake of fat *per se* is harmful in terms of CAD, cancer, or obesity in adults and also the influence of dietary cholesterol has been, and is still, exaggerated. For instance, consumption of one cholesterol-rich egg (about 200 mg/egg) daily up to six times per week does not increase risk of CAD or heart failure (Djousse and Gaziano, 2008a,b). The focus is nowadays mostly on fat quality (Lichtenstein, 2003) (see Section 2.4.2), while there is increasing evidence that carbohydrates, especially those with high glycemic indices, and food products with high glycemic loads play important roles in the etiology of obesity,

insulin resistance, and CAD, and that a high protein intake reduces obesity risk (Last and Wilson, 2006). Food products with high glycemic loads, especially when consumed in isolation, cause transient hyperinsulinemia (which is associated with CAD) and postprandial hypoglycemia (which is associated with the stimulation of appetite) (Last and Wilson, 2006). Compared with three other diets the low-carbohydrate Atkins diet proved superior for weight loss within a 1 year RCT with overweight premenopausal women (Gardner et al., 2007). It was also shown that isoenergetic diets with high protein (25 en%) or monounsaturated fatty acids (MUFA; 21 en%) have more favorable effects on systolic blood pressure, serum lipid profile and CAD risk, when compared with a diet with 58 en% carbohydrates (Appel et al., 2005). Low-carbohydrate/high-protein diets have by now proven their favorable influence on weight loss (Last and Wilson, 2006), which however does not imply that all of these are healthy or easily maintained (Alhassan et al., 2008). It nevertheless gains acceptance that the for long propagated replacement of SAFA and *trans* fatty acids with an isoenergetic percentage carbohydrates has unfavorable effects on CAD risk. They might for this purpose better be replaced by unsaturated fatty acids (UFA), protein, or both. Also the messages that vegetable oils are "good" and animal fat is "bad" proved incorrect and so is the notion that all SAFA are "wrong" and that MUFA and polyunsaturated fatty acids (PUFA) are "right." Categorizing fats into "tropical fats" and "nontropical fats" does not seem to make sense either. There are no "good" and "wrong" naturally occurring fats that have been part of our diet since the first *H. sapiens*, because nutrition is about the "balance" on which our genome has become to what it currently is.

2.4.1 Lipoproteins as Risk Factors

A high low-density lipoprotein (LDL)-cholesterol and low high-density lipoprotein (HDL)-cholesterol belong to the classical CAD risk factors that are nowadays, together with age, gender, systolic blood pressure, and smoking used for CAD risk assessment. The total-cholesterol/HDL-cholesterol ratio is the CAD risk factor most often applied in algorithms for CAD risk assessment, such as the SCORE, PROCAM, and Framingham algorithms (Graham, 2006). For instance, both the Pathobiological Determinants of Atherosclerosis in Youth (PDAY) research group (PDAY Research Group, 1990) and the Bogalusa Heart Study (Berenson et al., 1998) indicated a positive relation of atherosclerotic lesions in postmortem aortas and coronary arteries of children, young adolescents, and young adults with serum total-cholesterol and LDL-cholesterol on the one hand and a negative relation with serum HDL-cholesterol on the other hand. Other classical CAD risk factors identified in these studies, and in which (dietary) fats may be involved, were obesity and blood pressure. The observation that a composite of PDAY risk factors, including HDL- and non-HDL-cholesterol, body mass index (BMI), and blood pressure, in 12–24 years children and adolescents predicted carotid artery intima-media thickness at ages 27–39 years (Graham, 2006) added to the notion that dietary fats may exert effects on classical CAD risk factors such as dyslipidemia and hypertension.

LDL-cholesterol lowering by statins has indeed proven to lower CAD risk in both primary and secondary prevention trials. These studies have shown that the lowest

LDL-cholesterol confers lowest risk, and have set the stage for current treatment goals. Interestingly, the current LDL-treatment goals proved similar to LDL-levels encountered in traditionally living hunter-gatherer societies (O'Keefe and Cordain, 2004; O'Keefe et al., 2004). Statins however have pleiotrophic effects (Liao and Laufs, 2005) and their LDL-cholesterol lowering relates to a concomitant reduction of C-reactive protein (O'Keefe et al., 2006). Moreover, the pastoral living Maasai eating a saturated fat and cholesterol-rich diet (about 600 mg/day) from milk and meat have very low serum total-cholesterol, but extensive atherosclerosis with lipid infiltration and fibrous changes of their aortas, together with intimal thickening of their coronary arteries. They are however virtually free from signs of CAD, have smaller hearts, their blood pressure shows only a slight tendency to increase with age and they are remarkably fit (Mann et al., 1964, 1965, 1972). In addition, Central African Pygmies and Kalahari bushmen showed "prolonged hypoglycemia" after exogenous insulin and exhibited "low insulin responses" upon a glucose tolerance test (Joffe et al., 1971; Merimee et al., 1972). Taken together this suggests that the insulin sensitivity that comes with fitness and low BMI might be of crucial importance. With regard to lipids it might notably be of importance to prevent the production of small dense LDL- and HDL-particles that are associated with insulin resistance and part of the so-called atherogenic lipid triad, that is, elevated triglycerides, low HDL-cholesterol and small dense LDL particles (Krauss, 2004; Reaven, 2005).

2.4.2 Fat Quality as Risk Factor

It is widely accepted that atherosclerosis is associated with the consumption of SAFA and *trans* fatty acids by their ability to increase serum total- and LDL-cholesterol with no effect or decrease of HDL-cholesterol (Gidding et al., 2005). A detailed review on the effects of dietary fatty acids on serum cholesterol showed that isoenergetic replacement of 1 en% carbohydrates with SAFA, such as lauric acid (12:0*), myristic acid (14:0), and palmitic acid (16:0) increases LDL-cholesterol, but also HDL-cholesterol (Mensink et al., 2003). Stearic acid (18:0) does not influence LDL- and HDL-cholesterol to an appreciable extent. However, lauric acid induces a significant decrease of the total-cholesterol/HDL-cholesterol ratio, which implies that replacement of carbohydrate with the *saturated* 12:0 lowers CAD risk from at least a theoretical point of view. Replacement of 1 en% carbohydrates with SAFA, *cis*-MUFA, PUFA, or *trans*-MUFA does not induce much change in the total-cholesterol/HDL-cholesterol ratio for SAFA, causes a steep decrease for *cis*-MUFA and PUFA, but produces an increase for *trans*-MUFA. Replacement of 10 en% of the typical U.S. diet with carbohydrates or a variety of naturally occurring fats or oils would theoretically cause the steepest increase of the total-cholesterol/HDL-cholesterol ratio for carbohydrates and the steepest decrease of this ratio for rapeseed oil, soybean oil, and olive oil (Figure 2.4; Mensink et al., 2003). Taken together, these data suggest favorable effects of reduced carbohydrate consumption and are illustrative for the

* Fatty acids are denoted as a:*b*ω*c*, in which a is the (usually even) number of straight chain carbon atoms, *b* the number of double bonds in the methylene-interrupted *cis* configuration, and *c* the number of atoms from the methyl end to the first double bond.

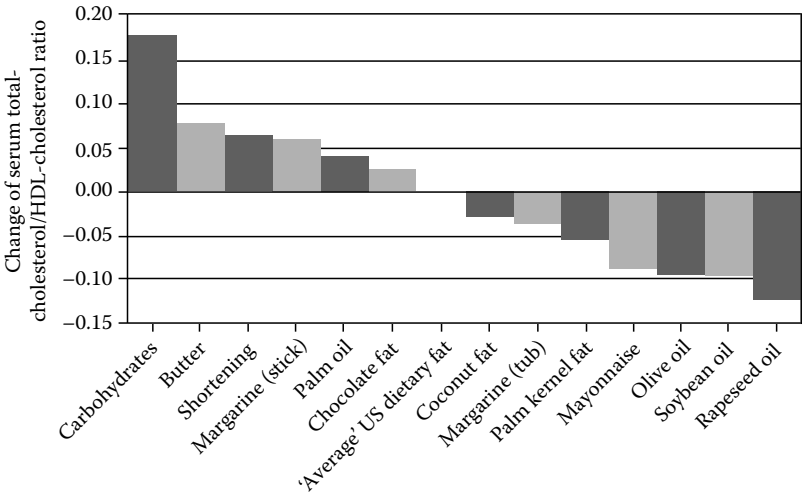

FIGURE 2.4 Predicted changes in serum total-cholesterol/HDL-cholesterol ratio if a mixed fat constituting 10% of energy in the "average" U.S. diet is isoenergetically replaced by carbohydrates or various fats and oils. (Adapted from Mensink, R.P. et al., *Am. J. Clin. Nutr.*, 77, 1146, 2003. With permission.)

nuance with regard to the influence of naturally occurring fatty acids, fats, and oils on our serum lipid risk profile. In addition, EPA and DHA have little effects on serum cholesterol, apart from a modest increase of LDL-cholesterol. Their favorable effects on CAD risk are attributed to their antiarrhythmic, antithrombotic, antiatherosclerotic, and anti-inflammatory effects, while they improve endothelial function, and lower both blood pressure and serum triglycerides (Din et al., 2004; Lee et al., 2008; Mozaffarian, 2008).

It has become clear that the dietary fatty acid composition may not only adversely affect serum cholesterol, but also influences coagulation, endothelial function, inflammation, abdominal obesity, insulin sensitivity, development of type 2 diabetes mellitus, and arrhythmias (Erkkila et al., 2008). Such adverse conditions are likely to have been introduced by the increasing intake of SAFA, *trans* fatty acids and ω6 fatty acids (notably LA) in the Western diet during the past century and the concomitant decrease of the ω3 fatty acids intake from vegetable oils and fish (Figure 2.5; Simopoulos, 1999, 2006). A diet-induced proinflammatory and prothrombotic state may be part of the chronic low-grade systemic inflammation that is associated with the insulin resistance and compensatory hyperinsulinemia of the metabolic syndrome (Reaven, 2005). The current dominance of the ω6 fatty acid series (LA and AA) over those from the ω3 series (ALA, EPA, and DHA) may at least partially be at the basis of this proinflammatory and prothrombotic state (Innis, 2007b; Siddiqui et al., 2008). Low-grade inflammation is emerging as a common feature of the metabolic syndrome (Tilg et al., 1994) and its sequelae, but also of neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, multiple sclerosis (Skaper, 2008), and also major depression (Leonard, 2007) (see Section 2.5.4.4). The relation with disbalanced ω6/ω3 ratio might find

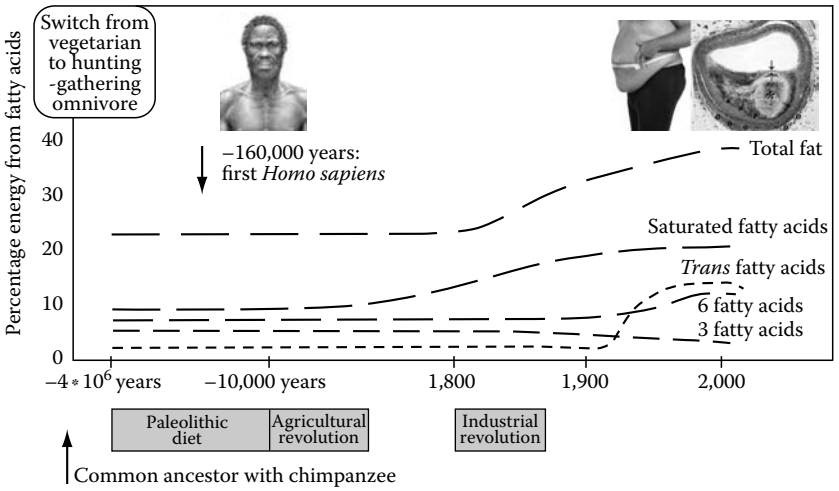

FIGURE 2.5 Fat intake of hominins and *H. sapiens* since our common ancestory with the current chimpanzee. Inlays: Reconstruction of *H. sapiens Idaltu* dated about 160,000 years ago (White et al., 2003), central obesity as a characteristic of the metabolic syndrome, and an instable coronary artery plaque characterized by a thin fibrous cap and a large lipid core (arrow indicates activity of inflammatory cells). (Modified from Simopoulos, A.P., *Am. J. Clin. Nutr.*, 70, 560S, 1999. With permission.)

its origin in ω6-mediated aggravation of inflammatory responses in conjunction with insufficient ω3-mediated compensatory anti-inflammatory responses. Recent research indicates that the inflammation initiation/resolving balance is orchestrated by the proinflammatory actions of prostaglandins and leukotrienes from AA, and the inflammation-resolving actions of the lipoxins from AA, and the resolvins and (neuro)protectins from EPA and DHA (Calder, 2006; Mayer and Seeger, 2008; Serhan et al., 2008). Evidence from both observational and experimental studies indicates that industrially produced *trans* fatty acids, notably those from LA, may also be proinflammatory by as yet are poorly understood mechanisms (Mozaffarian and Willett, 2007). Other adverse effects of *trans* fatty acids include a decrease of incorporation of other fatty acids in cell membranes, decrease of HDL-cholesterol and increase of LDL-cholesterol, fatty acid desaturase 2 (FADS2) inhibition, decrease of testosterone, lowering of birth weight, increases of platelet aggregation, lipoprotein (a), body weight, and cholesterol ester-transfer protein, and abnormal morphology of sperm (Simopoulos, 2008).

2.4.3 Prospective Studies, Trials, and Recommendations

The risk of the type of fat has been reasonably well established, notably for CAD development. There are obviously no population-based RCTs for primary prevention with hard endpoints, which makes it difficult to establish the causality of the relation of dietary fat quality with diseases like CAD. A recent review on prospective cohort studies and trials with hard endpoints concluded that SAFA and industrially

produced *trans* fatty acids increased CAD risk in several studies, while both ω6 (notably LA) and ω3-PUFA are associated with lower CAD risk. From the ω3 fatty acids, both EPA and DHA are associated with decreased risk for especially fatal CAD outcomes, while the role of ALA is less clear (Erkkila et al., 2008; Stark et al., 2008). There are few studies with children and all of those available carry soft endpoints. Reduction of elevated LDL-cholesterol in 8–10 years old children was accomplished by the consumption of a low-fat diet with reduced SAFA and cholesterol (Obarzanek et al., 2001). Repeated dietary counseling to lower SAFA and cholesterol intake from 7 months, as monitored up to 14 years of age, has recently been found effective in lowering total- and LDL-cholesterol, notably in boys, without affecting growth, BMI, pubertal development, and age of menarche (Niinikoski et al., 2007). A high intake of fish oil fatty acids is epidemiologically associated with reduced CAD risk, while a low intake has been associated with depression. Favorable effects of fish oil on hard CAD endpoints have clearly been demonstrated in secondary prevention studies (Lee et al., 2008). Favorable effects of EPA on depression have been shown in RCTs, although more data are needed (Ross, 2007) (see Section 2.5.4).

The current (2005) acceptable macronutrient distribution range (AMDR) issued by the U.S. Institute of Medicine for fat intake by adults (>18 years) amounts to 20–35 en% and, dependent on age group, amounts to 14–17 g/day ω6-PUFA (LA) for men and 11–12 g/day for women (5–10 en%), and to 1.6 g/day ω3-PUFA (ALA) for men and 1.1 g/day for women (0.6–1.2 en%). About 10% of total PUFA may come from LCPω3 or LCPω6. Since it is contended that there is an increase in plasma total- and LDL-cholesterol with increased intake of SAFA or *trans* fatty acids or with cholesterol at even very low levels in the diet, it is recommended that the intakes of each should be minimized while consuming a nutritionally adequate diet (DRI USA, 2008). The current (2006) adequate intakes (AIs) in the Netherlands are: 20–40 en% fat (tolerable upper intake level; UL 40 en%), as low as possible SAFA (UL < 10 en%) and *trans*-MUFA (UL < 1 en%), PUFA (UL = 12 en%), MUFA + PUFA 8–38 en%, LA 2 en% for prevention of EFA deficiency, ALA 1 en% and EPA + DHA 450 mg/day (Health Council of the Netherlands, 2006). Recommendations for fish oil fatty acid intake for primary prevention have been issued by authoritative Nutritional Boards ranging from "choose fish as food item more often" to two to three servings of fish per week (Psota et al., 2006). For secondary prevention the American Heart Association recommends 1 g EPA + DHA daily, preferably from fatty fish or otherwise from fish oil supplements, while 2–4 g daily is recommended for serum triglyceride lowering (Kris-Etherton et al., 2002). The currently advised intake of about 450 mg LCPω3/day confers virtually maximum antiarrhythmic effects, but is below the estimated dosage that confers maximum benefits for other CAD risk factors responding favorably to fish oil (Mozaffarian, 2008). A recent study from Belgium showed that at the recommended consumption of 0.3 en% EPA + DHA (i.e., about two times fatty fish/week) the intake of methyl mercury remains well within the tolerable daily intake, but that the dioxin-like compounds approach the current limit at more than two times fatty fish per week (Sioen et al., 2007). The benefits of two seafood servings per week exceed the potential risk, notably when a variety of fish is chosen and the consumption of species with high contaminant levels is avoided (Mozaffarian and Rimm, 2006).

2.5 LCPUFA IN HEALTH AND DISEASE

There is good evidence to show that the evolution to *H. sapiens* took place on an ω3-rich diet from East-African ecosystems that were notably located in places where the land meets freshwater. The sites at which the fossil remains of our ancestors have been discovered are in support of this notion (Gibbons, 2002b), while the Out-of-Africa Diaspora has largely taken place via the coastal lines (Stringer, 2000) also after the crossing of the Bering Strait (Wang et al., 2007). Compared with hunting in the savanna, food from the land–water ecosystems is relatively easy to obtain and rich in iodine, vitamins A and D, and ω3 fatty acids from both vegetables and fish. Each of these nutrients has important functions in brain development and growth. Exploitation of this ecosystem, and its abundant "brain food," might be at the basis of our remarkable brain growth during the past 6 million years of evolution since our common ancestry with the present chimpanzee. This dietary composition seems somewhat abandoned since the Out-of-Africa Diaspora, since deficiencies of many of these particular nutrients are among the most widely encountered in the current world population (Broadhurst et al., 1998, 2002; Crawford et al., 2001; Holick and Chen, 2008). Iodine is added to table salt in many countries, and margarines and milk have become popular food products for fortification with vitamins A and D. The dietary composition of our ancestors has also become clear from our current (patho)physiology: epidemiological data demonstrated a negative association of fish consumption with CAD (see Section 2.4) and (postpartum) depression (Hibbeln, 1998, 2002), while landmark trials with ALA (de Lorgeril et al., 1999) and fish oil (GISSI-Prevenzione trial, 1999; Lee et al., 2008) in CAD, and with EPA in depression and schizophrenia (Peet and Stokes, 2005) supported the causality of these relations. It has become clear that the intake of the parent EFA and their chain elongation/desaturation metabolites, the so-called LCPUFA (≥20 straight-chain carbon atoms and ≥3 methylene-interrupted *cis*-double bonds) is important to our health across the entire life cycle, but that their intakes have been subject to tremendous change. This section deals with their (patho)biochemistry and (patho) physiology and specifically concentrates on their role in pregnancy, neonatal nutrition, and psychiatric disease.

2.5.1 (Patho)biochemistry and Physiology of LCPUFA

2.5.1.1 LCPUFA Synthesis, Regulation, and ω3/ω6 Balance

LA (18:2ω6, precursor of the ω6-series fatty acids) and ALA (18:3ω3, precursor of the ω3-series) are the parent EFA for humans (Innis, 1991, 2003). "Essential" implies that the nutrient in question cannot be synthesized to sufficient amounts and therefore needs to be obtained from the diet to prevent development of disease. Most of the dietary LA and ALA is used for energy generation, converted to other compounds or used for structural purposes. Especially LA can become stored to reach high levels in adipose tissue. The parent EFA are also converted to LCPUFA by microsomal desaturation (delta-6 and delta-5 desaturases; also referred to as FADS2 and fatty acid desaturase 1 (FADS1), respectively), microsomal chain elongation, and peroxisomal chain shortening (Figure 2.6). Humans do not possess a delta-4 desaturase,

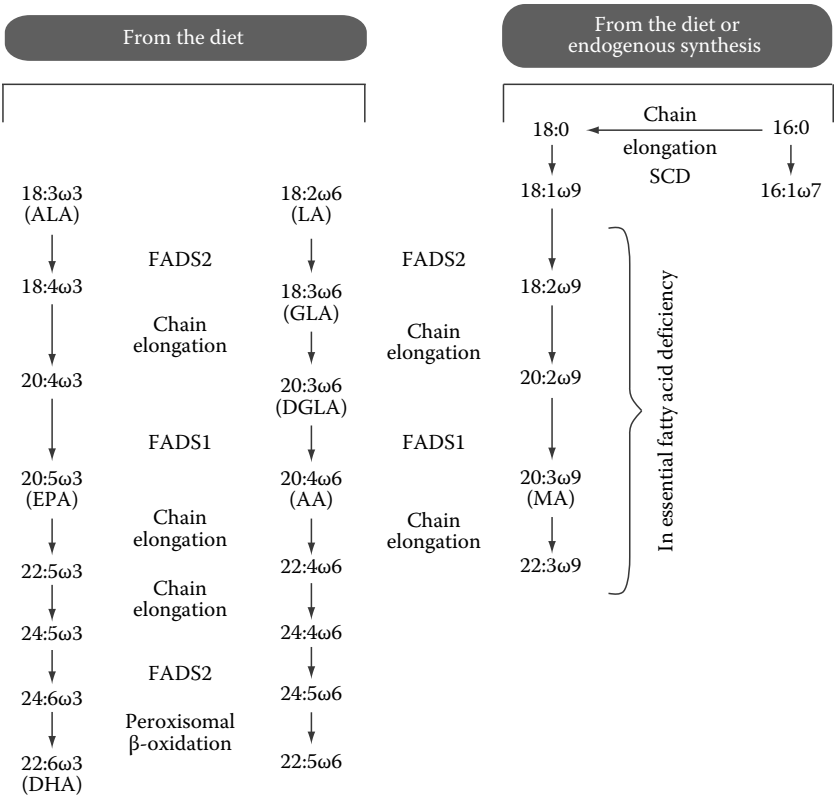

FIGURE 2.6 Chain elongation and desaturation pathways of the parent essential fatty acids ALA and LA, and of stearic and palmitic acids. *Abbreviations*: ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; GLA, gamma-linolenic acid; DGLA, dihomo-gamma-linolenic acid; AA, arachidonic acid; MA, Mead acid; FADS1, fatty acid desaturase 1 (delta-5 desaturase); FADS2, fatty acid desaturase 2 (delta-6 desaturase); SCD, steroyl-CoA desaturase (delta-9 desaturase).

which precludes the direct synthesis of DHA from its precursor 22:5ω3. Direct desaturation is circumvented by initial elongation of 22:5ω3 to 24:5ω3, followed by delta-6 desaturation by FADS2 and a single cycle of beta-oxidation in peroxisomes to yield DHA. The output of this pathway is, however, limited (Muskiet et al., 2004; Burdge, 2006; Williams and Burdge, 2006) as may, for example, be derived from the lower DHA status of vegans compared with omnivores (Fokkema et al., 2000a), and the inability to augment DHA status by supplementation with ALA (Fokkema et al., 2000b) or very high EPA (Horrobin et al., 2003). Supplemental ALA does however increase EPA and 22:5ω3 status (Stark et al., 2008).

The sharing of the elongating and desaturating enzymes by ω6 and ω3 fatty acids causes them to compete for conversion into the various LCPUFA. It must however be appreciated that the ω3 and ω6 fatty acids also compete in many other pathways,

such as beta-oxidation, incorporation into lipids, release from lipids, conversion into highly active metabolites, and binding to receptors. Circulating AA levels are relatively constant at LA intakes ranging from 3 to 10 en% (Innis, 2007b). Consumption of a high LA diet by healthy adults increases plasma phospholipid LA and decreases EPA with no effect on AA (Liou et al., 2007), suggesting that our current high LA intake contributes to our low EPA/AA ratio. Generally, high EPA and DHA intakes lower AA status in some tissues, erythrocytes (RBC), and circulating lipids (Innis, 2003), but this does not seem to occur to the same extent in all compartments, notably not in the brain. For instance at variable DHA status, DHA and 22:5ω6 exhibit inverse relationships in rat frontal cortex, with little effect on AA (McNamara and Carlson, 2006). AA in the postnatal baboon central nervous system (CNS) seems tightly controlled and much less sensitive to dietary manipulation than DHA, possibly because the pathway for AA synthesis from the abundant LA (by three endoplasmic steps) might be more efficient than the pathway of DHA from the less abundant ALA (by six endoplasmic and one peroxisomal step). Postnatal feeding with formulae without DHA causes a DHA drop in most CNS structures of the baboon infant, which can be prevented by alternative feeding with a formula with DHA (Diau et al., 2005; Brenna and Diau, 2007). Brain LCPUFA profiles exhibit remarkable similarity among different mammals (Tassoni et al., 2008) and the data on the DHA *vs.* AA relation in animal brain are in line with the limited cross-sectional data from the brains of young infants. These show lower DHA and somewhat higher (Farquharson et al., 1992) or equal (Makrides et al., 1994) AA in children who received infant formula without LCPUFA, compared with counterparts receiving breastmilk (which contains LCPUFA). Nevertheless, the complex interrelationships between the two EFA series puts emphasis on the need of dietary "ω6/ω3 balance" to reach "homeostasis." The dietary composition producing this "balance" is as yet unknown, but it may be postulated that it is part of our ancient diet, because it is that diet on which our genes evolved.

The regulation of LCPUFA synthesis is complex and notably targets FADS1 and FADS2. Both enzymes are widely expressed in human tissues, notably in liver (Nakamura and Nara, 2004), but also in the brain, placenta, and the mammary gland (Innis, 2005, 2007a,c). The liver enzymes seem major origins of the LCPUFA synthesized from LA and ALA. The contributions of the brain, placenta, and mammary gland enzymes to brain, fetal, and newborn LCPUFA status are uncertain but probably small. For instance, DHA synthesis in the adult rat liver is sufficient to keep brain DHA constant while the brain itself is unable to do so (Rapoport et al., 2007). There are no differences in the capacities to convert ALA to DHA between prematures, term babies, and adults (Innis, 2007a). Women have higher capacity to synthesize DHA, which might be mediated by estrogens (Williams and Burdge, 2006). Compared with LCPUFA synthesized from their parent precursors, there is a clear preference for preformed AA and DHA from the diet for incorporation into brain and other tissues (Lin and Su, 2007; DeMar, Jr. et al., 2008) and for preformed DHA originating from transplacental transfer and milk for fetal and newborn tissue accretion (Innis, 2005). Dietary LCPUFA downregulate LCPUFA synthesis by negative feedback inhibition (Nakamura and Nara, 2004). Insulin stimulates the expression of both enzymes, while it also stimulates expression of delta-9 desaturase (steroyl-CoA

desaturase) (Brenner, 2003; Jump, 2004). The latter is the third important desaturase that is notably related to *de novo* fatty acid synthesis and is, for example, induced in adipose tissue during insulin resistance (Sjogren et al., 2008). Other factors affecting LCPUFA synthesis are various hormones, cofactors like vitamin B_6 and zinc, and inflammatory stimuli (Nakamura and Nara, 2003; Jump, 2004). Downregulation of LCPUFA synthesis by dietary LCPUFA, the presumed high LCPUFA intakes from our ancient diet (Table 2.1), the occurrence of FADS1 and FADS2 polymorphisms with lower activities (see Section 2.5.1.7), and our difficulty to synthesize DHA suggest that the LCPUFA synthesis machinery has only been used during long periods with low LCPUFA intakes, such as during famine or limited availability of animal foods. The low AA level and higher ratio between dihomo-gamma-linolenic acid and AA (DGLA/AA) in plasma phospholipids of Greenland and Danish Eskimos, compared with Danes, has led to the suggestion that Eskimos may have low FADS1 activity. This would render them obligate carnivores who, similar to cats, are in need of a dietary LCPUFA source (Gibson and Sinclair, 1981).

The FADS1 and FADS2 genes are located in a head-to-head orientation on chromosome 11. The proximity of their promoters suggests that they might be coordinately controlled by common regulatory sequences but this has not been demonstrated as yet. The FADS2 promoter contains binding sites for the (ligated) PPAR-alpha (see Section 2.5.1.5) and the active form of the sterol regulatory-binding protein-1c (SREBP1c). Binding of the ligated PPAR-alpha and SREBP-1c proved necessary for FADS2 transcription and this was found to occur notably during LCPUFA demand, such as in EFA deficiency. Consequently, PPAR-alpha and SREBP-1c may collectively be considered as our "LCPUFA sensors" (Nakamura and Nara, 2004). The strong feedback control on LCPUFA synthesis by LCPUFA is mediated by reduced FADS2 transcription secondary to the reduction of the active form of SREBP-1c. This mode of regulation implies that excess of dietary LCPUFA from either the ω3 or ω6 series may shut off synthesis of both LCPUFA series, which is a strong argument for the need of dietary LCPω3/LCPω6 balance, and may play a role in the AA lowering effect of DHA supplementation (Nakamura and Nara, 2004). Transcription of the opposite strand of FADS1, named reverse-FADS1, gives rise to an antisense transcript that is able to bind to complementary sequences of the FADS1 transcript and thereby lowers or even switches off FADS1 translation. Fasting and refeeding with a high glucose fat-free diet and administration of fish oil were found to be triggers for the induction of the antisense regulator of FADS1, which probably exerts its action by accelerating FADS1 mRNA degradation or interference with its translation (Dreesen et al., 2006). One-carbon metabolism is linked to LCPUFA metabolism and status by the methylation of phosphatidylethanolamine (PE) to phosphatidylcholine (PC) via the phosphatidylethanolamine-*N*-methyltransferase (PEMT) pathway (Watkins et al., 2003; Umhau et al., 2006). PC may also become synthesized via the CDP-choline pathway, but the resulting species carry less AA and DHA than those deriving from the PEMT pathway. In addition, hyperhomocysteinemia was recently shown to influence LCPUFA synthesis by the silencing of FADS2 in mouse liver. The mechanism was by hypermethylation of the FADS2 promoter and was accompanied by higher PE, and lower AA and DHA in liver phospholipids (Devlin et al., 2007).

2.5.1.2 Dietary LCPUFA Sources and Intakes

Both LA and ALA are predominantly derived from vegetable oils. The various edible oils differ widely in LA and ALA contents and their ratio. Sunflower oil and corn oil are, for example, high in LA, and soy bean oil and especially linseed oil have (much) higher ALA/LA ratio. Meat, eggs, and poultry are rich sources of AA, while EPA and DHA are derived notably from fish, meat, and eggs. The EPA and DHA contents and ratios in fish are dependent on their position in the food chain and the composition of the phytoplankton from which these LCPω3 ultimately originate. Freshwater fish may synthesize their own EPA and DHA from the appropriate precursors (Sargent, 1997). There are little differences in reported intakes of LA in Western countries, which are typically in the 11–18 g/day range. Also AA intake is rather constant, amounting to 160–230 mg/day for men and 120–200 mg/day for women. This may, together with the constancy of AA across a wide range of LA intakes (3–10 en%), explain the constancy of AA status in Western countries (Liou et al., 2007; Innis, 2007b). In contrast, there are wide differences in ω3 fatty acid intakes and the resulting ω3-status. ALA intake in Western countries is typically 1.2–1.8 g/day, but is up to 1.7–2.2 g/day in Japan. The LA/ALA ratio amounts to 4–8 in Japan, 6–10 in most Western countries, and >13 in South-European countries (Astorg et al., 2004), but has also been estimated at 15–16 for the typical Western diet (Simopoulos, 2001). Notably LCPω3 intake is highly variable among countries. EPA + DHA intakes in Japan are 1.0–1.5 g/day for men and 0.7–1.1 g/day for women. They amount to 0.7–1.0 g/day in Norway and 0.7 g/day in Spain, while relatively low intakes have been reported for the United States (210–240 mg/day), Germany (215–315 mg/day), and Australia (175 mg/day) (Astorg et al., 2004). Vegetarians consume 30 mg DHA/day (Haggarty, 2004). Lifetime LCPω3 intakes by Eskimos has been estimated at 14 g/day, while the intake in Denmark was 3 g/day (Feskens and Kromhout, 1993). The latter figures compare favorably with our estimates for an East-African freshwater-based diet of our ancestors (see Table 2.1). Higher LCPω3 intakes than the 450 mg/day as currently advised would not only provide maximal effects on arrhythmia risk, but also on the lowering of serum triglycerides, heart rate and blood pressure, and on thrombosis risk (Mozaffarian, 2008). Docosapentaenoic acid (22:5ω3) intake seems to occur notably from meat, with total daily intakes of 85–105 mg in Japan, 60–80 mg in Sweden and France, and 71 mg in Australia (Astorg et al., 2004; Howe et al., 2006). Gamma-linolenic acid (GLA) is abundant in borage, black current, and evening primrose oils (Kapoor and Huang, 2006).

2.5.1.3 EFA and LCPUFA Function

The parent EFA (notably LA) and LCPUFA are building blocks of the membrane phospholipids of all cells. They contribute to the membrane's physicochemical properties and thereby the functions of membrane-bound receptors, transporters, ion channels, enzymes, and other membrane-bound biochemical processes, such as those involved in signaling pathways. LA has a clear structural function of its own. As building block of ceramides in our skin, LA contributes to the skin's barrier function to limit transepidermal water loss. In contrast to LA, ALA gets stored in our body to only a limited extent. ALA is largely degraded to acetyl-CoA for the generation

of energy or *de novo* synthesis of SAFA, MUFA, and cholesterol, for example, in the brains of the fetus and newborn ("recycling," Cunnane et al., 2006). LA and LCPUFA are also stored in adipose tissue, but especially AA exhibits preference for phospholipids (Nelson et al., 1997) perhaps to control AA release and subsequent conversion to its highly potent eicosanoids. EFA make up 20% of brain dry weight, including about 6% for AA and 8% for DHA. Brain levels of LA and ALA are low. DHA and AA are notably located in the synaptosomes, where AA is of special importance as a second messenger in (synaptic) signal transduction and DHA provides the needed fluidity for appropriate neurotransmitter receptor activity. DHA is the major fatty acid in the structural phospholipids of the retinal photoreceptor outer segment membrane, where its fluidity is essential to accommodate the extremely rapid conformational changes of rhodopsin (Kurlak and Stephenson, 1999). Part of the retinal phospholipids is composed of PE, phosphatidylserine (PS), and PC that carry two DHA acyl moieties. Brain AA and DHA reach highest levels in gray matter and especially those areas involved in motor activities (Diau et al., 2005). DHA's role in neurodevelopment has been grouped into effects on gene expression, monoaminergic neurotransmission, protection against apoptotic cell death, and neurite outgrowth from the cell body (Innis, 2007a). DHA also has an important function in spermatozoa, where DHA's unique structure contributes to motility (Tavilani et al., 2006). Both AA and DHA are important to maintain a healthy endothelium of our cardiovascular system (Calder, 2004). The importance of AA/DHA balance in myocardial phospholipids to preserve a low arrhythmogenic eicosanoid profile has been emphasized (McLennan and Abeywardena, 2005). LCPUFA synthesis from parent precursors may also be subject to "programming" (see Section 2.3). A diet high in SAFA given to pregnant rats caused reduced AA and DHA and increased LA and ALA in the aorta of their offspring, suggesting poor conversion of parent EFA to LCPUFA. These abnormalities coincided with vascular dysfunction and persisted to adulthood (Ghosh et al., 2001).

2.5.1.4 Eicosanoids, Resolvins, Protectins, and Others

LCPUFA are precursors of highly potent metabolites that are involved in various signaling processes. After their release from membrane phospholipids by phospholipase A_2 (PLA_2) some of the C_{20} LCPUFA, that is, AA (20:4ω6), DGLA (20:3ω6), and EPA (20:5ω3) may become converted to eicosanoids (prostaglandins, thromboxanes, leukotrienes, hydroxyeicosatetraenoic acids, and epoxy-eicosatrienoic acid), which is a family of lipid mediators synthesized via the cyclooxygenase (COX1 and COX2), lipooxygenase (LOX), and the cytochrome P450 pathways. DGLA gives rise to prostaglandins, prostacyclins, and thromboxanes of the one-series (e.g., PGD_1, E_1, $F_{1\alpha}$), AA leads to the two-series (PGD_2, E_2, $F_{2\alpha}$, PGI_2, TXA_2) and EPA to the three-series (PGD_3, E_3, $F_{3\alpha}$, PGI_3, TXA_3). AA is also converted to the four-series leukotrienes (LTA_4, B_4, C_4, D_4), while EPA gives rise to the five-series leukotrienes (LTA_5, B_5, C_5, D_5). These mediators are involved in a variety of occasionally opposing functions such as smooth muscle contraction, fever induction, augmentation of vascular permeability, pain, vasodilation (PGE_2), vasoconstriction and platelet aggregation (TXA_2), vasodilation and platelet aggregation inhibition (PGI_2), contraction of smooth muscles in the respiratory tract, vessels, and intestine, leukocyte

chemotaxis, increased vascular permeability, enhanced local blood flow, and release of lysosomal enzymes and reactive oxygen species (LTB_4) (Calder, 2006).

The eicosanoids from EPA are believed to be less potent than those of AA. For instance, TXA_3 is a weak agonist of TXA_2 in inducing vasoconstriction and platelet aggregation, while PGI_2 and PGI_3 exert similar vasodilating and antiaggregating effects. Also LTB_5 is much less potent in eliciting neutrophil chemotaxis than LTB_4. Eicosanoids from AA are widely considered to be proinflammatory, whereas those from EPA are weakly inflammatory or anti-inflammatory. This must however be viewed upon as a generalization, since newly identified mediators of AA are not only involved in the initiation, but also in the resolution of inflammatory reactions. Prostaglandins and leukotrienes are intimately involved in the initiation of inflammation following its triggering by, for example, microbial infection, surgical trauma, hypoxia, and reperfusion injury. The outcome is progression to chronic inflammation, scarring and fibrosis, or complete resolution. Resolution is accompanied by a switch to the production of lipid mediators with anti-inflammatory and proresolving properties. These mediators include AA-derived lipoxins, which function as stop signals and are followed by the appearance of EPA-derived resolvins from the E-series and DHA-derived resolvins from the D-series and protectins (named neuroprotectins when generated in the neural tissue). Mediators from EPA and DHA stimulate resolution and the return to homeostasis (Serhan et al., 2008; Serhan and Chiang, 2008).

2.5.1.5 LCPUFA and Gene Expression

LCPUFA are not only important structural elements of membranes. Together with their eicosanoid products and other fatty acids, they are also firmly implicated in gene expression. For example, dietary LCPUFA are ligands of PPARs and suppress the expression of SREBPs, nuclear transcription factor kappa-B and others. These are nuclear transcription factors that can be considered as main switches in the coordinated expression and repression of a variety of (key) enzymes and proteins in intermediary metabolism, thermoregulation, energy partitioning, growth and differentiation, and inflammatory responses (Duplus and Forest, 2002; Clarke, 2004; Jump, 2004; Lapillonne et al., 2004). PPARs are among our bodily "lipid sensors" and, importantly, they constitute a link between lipid and glucose homeostasis, and inflammation (Figure 2.7). The serum triglyceride lowering effect of fish oil fatty acids is, for example, attributable to their interaction with PPAR-alpha. Recent data show that DHA and EPA play key regulatory roles in the coordinated expression of genes involved in glycolysis, *de novo* lipogenesis, fatty acid elongation, desaturation, and oxidation in the liver. They target many nuclear receptors that are part of at least three major transcriptional regulatory networks controlling hepatic carbohydrate and lipid metabolism, that is, by activation via the PPARs/retinoid X receptor (RXR) heterodimer and suppression of the nuclear abundances of SREBP-1 and the carbohydrate regulatory element-binding protein (ChREBP)/Max-like factor X (MLX) heterodimer. DHA weakly activates PPAR-alpha and strongly suppresses SREBP-1, while its hepatic retroconversion metabolite EPA is a strong PPAR-alpha activator. PUFA of both the ω3- and ω6-series suppress ChREBP/MLX abundance (Jump, 2008). Because of their strategic role in many metabolic and signaling routes

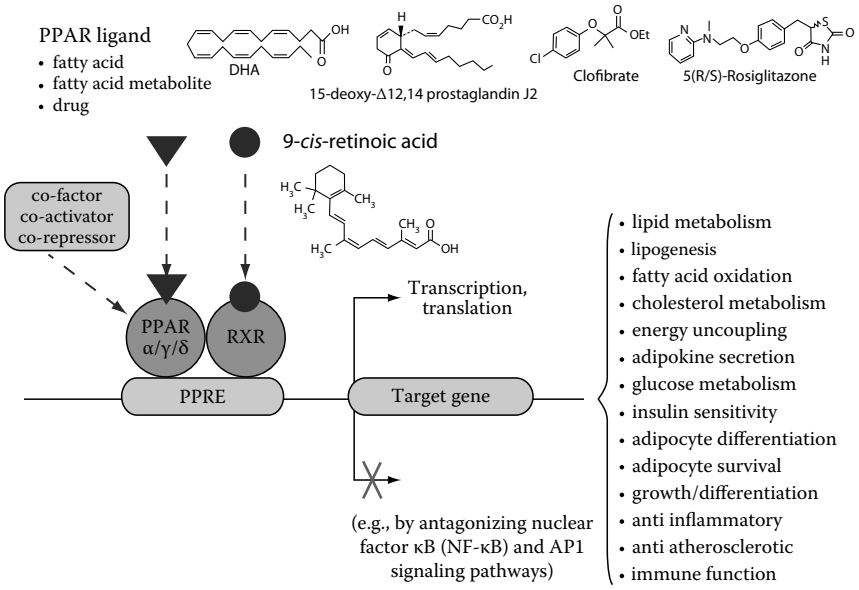

FIGURE 2.7 Role of PPARs in gene expression and repression. Ligated PPARs heterodimerize with the ligated RXR to interact with a PPAR response element (PPRE). This interaction leads to the coordinated expression of many proteins involved in a variety of pathways. Gene repression by PPARs occurs by antagonizing signaling cascades; an example is interaction with nuclear factor kappa-B (NFκB) causing suppressed transcription of genes involved in inflammatory reactions (Daynes and Jones, 2002). DHA and 15-deoxy-delta-12,14 prostaglandin J_2 are among the many natural ligands of the promiscuous PPARs. Clofibrate (triglyceride lowering) and rosiglitazone (insulin sensitivity enhancing) are examples of drugs with agonistic effects on PPAR-α and PPAR-γ, respectively. 9-*Cis*-retinoic acid is a natural RXR ligand.

PPARs are logical targets for drugs. For instance, the fibrates for serum triglyceride lowering are agonists of PPAR-alpha, while the insulin sensitivity increasing properties of the thiazolidinediones (glitazones) are based on their agonistic action toward PPAR-gamma. PPAR-gamma has been referred to as "the ultimate thrifty" gene, because of its ability to stimulate lipogenesis in the liver and adipose tissue (Auwerx, 1999). The unique 1300–1400 $Å^3$ Y-shaped cavity that constitutes part of the PPAR lipid-binding domain may be at the basis of their promiscuous ligand-binding characteristics, but recent data suggest that fish oil fatty acids and notably their derivatives are among the most powerful naturally occurring PPAR ligands (Deckelbaum et al., 2006; Bragt and Popeijus, 2008; Itoh and Yamamoto, 2008; Gani and Sylte, 2008; Gani, 2008).

2.5.1.6 EFA and LCPUFA Deficiency and Marginality

Clinical signs of ω6 fatty acid shortage include growth retardation, reproductive failure, fatty liver, and a dry (scaly) skin that is characterized by augmented transdermal water loss. Deficiency of ω3 fatty acids may cause dysfunctioning of the central

nervous system, including cognitive dysfunction and impaired vision. Biochemically, EFA deficiency (i.e., of both LA and ALA) gives rise to the accumulation of Mead acid (20:3ω9), because of the action of the chain elongation/desaturation system on the competing oleic acid (18:1ω9) (Figure 2.6). The order of substrate affinity of FADS2 is ALA>LA>oleic acid, which also explains the decrease of DHA with reciprocal increases of LCPω6, notably 22:5ω6 and to a lesser extent 22:4ω6 and AA, in isolated ALA deficiency. DHA may be conditionally essential (Muskiet et al., 2004), because of the difficulty by which it is synthesized and the relation of low DHA status with disease. "Conditionally essential" refers to a need of a dietary source during circumstances at which demand exceeds synthesis, such as during rapid growth, augmented nutrient loss, or declining synthesis, as for example, occurring in fetuses, young children, adolescents, pregnancy, lactation, and at advanced age. Age-dependent cutoff values for RBC 20:3ω9 (marker for EFA deficiency), RBC 22:5ω6/20:4ω6 (marker for ω3 deficiency), and RBC 22:5ω6/22:6ω3 (marker for ω3/DHA marginality and deficiency) have been proposed (Fokkema et al., 2002). Disbalances between AA, EPA, and DHA may change the ratio between EPA- and AA-derived eicosanoids with concomitant pathophysiological effects. For instance, there is a positive relation between the AA/EPA ratio in the platelets of different ethnic groups and the percentage of CAD deaths (Simopoulos, 2008). Competition between AA and EPA + DHA occurs to a large extent in compartments in which LCPUFA seem to have limited functionality apart from membrane flexibility or transport, such as RBC and plasma phospholipids. Consequently, measurements of LCPUFA in these compartments provide sensitive parameters for LCPUFA status assessment, of which the outcome is, however, as yet poorly defined in terms of pathophysiological consequences or disease risk. LCPUFA contents of RBC are considered a good reflection of brain LCPUFA (Makrides et al., 1994; van Goor et al., 2008a). The omega-3 index, that is, the sum of EPA and DHA in RBC, has been proposed as a risk factor for sudden cardiac death and may also serve as a fish oil treatment goal. An omega-3 index of >8 g/100 g RBC fatty acids (g%) is associated with 90% lower CAD risk, as compared with an index of <4% (von Schacky and Harris, 2007; von Schacky, 2008).

Biochemical and possibly clinical evidence of EFA deficiency is often present in protein-energy malnutrition (PEM). Typical symptoms in PEM, like skin changes, impaired resistance to infections, impaired growth rate, and disturbed mental functioning and development may be explained by coexisting EFA and LCPUFA deficiencies (Smit et al., 2002). Clinically apparent EFA deficiency in Western countries is rare. The sporadic cases are mostly based on heritable or acquired diseases that cause, or are accompanied by, poor gastrointestinal fat absorption.

2.5.1.7 Inborn Errors and Polymorphisms

Demonstrated genetic defects in LCPUFA synthesis comprise FADS2 deficiency and peroxisomal beta-oxidation defects such as those occurring in the Zellweger syndrome and adrenoleukodystrophy (Jump, 2004). The FADS2 deficiency was caused by an insertion in its transcription regulatory region, leading to symptoms consistent with EFA deficiency with low AA and DHA. The clinical symptoms, such as corneal ulceration, feeding intolerance, growth failure, photophobia, and

skin abnormalities, improved upon administration of AA and DHA (Williard et al., 2001; Nwankwo et al., 2003). Patients with generalized peroxisomal disorders such as present in Zellweger's syndrome, have profound brain DHA deficiency. DHA supplementation improves their vision, liver function, muscle tone, and social contact, but the treatment should be initiated as soon as possible after birth (Martinez et al., 2000; Martinez, 2001).

At least 18 polymorphisms have been demonstrated in the gene cluster of FADS2 and FADS1. These polymorphisms exhibit a high degree of linkage disequilibrium and are accompanied by diminished FADS1 and FADS2 activities. Carriers of the minor alleles had higher LA, 20:2ω6, 20:3ω6, and 18:3ω3, together with lower 18:3ω6, AA, 22:4ω6, EPA, and 22:5ω3 in their serum phospholipids. The underlying allele(s) explained 28% of the interindividual serum phospholipid AA variability (Schaeffer et al., 2006). Polymorphisms of FADS2 were also found to influence the association between breastfeeding and higher IQ. Children carrying the investigated major allele who received breast milk had higher IQ compared with counterparts receiving infant formula. Those carrying the major allele benefited more from breastmilk than children carrying the minor allele, and this observation did not seem related to maternal genotype (Caspi et al., 2007). Recently we showed that the minor allele of a FADS1 polymorphism is associated with lower RBC AA in pregnant women and that this genotype is sensitive to further AA lowering following DHA supplementation (Dijck-Brouwer et al., 2008). The polymorphisms of FADS1 and FADS2 are clear examples of the many "disease susceptibility genes," which do not cause disease by themselves, but only at unfavorable environmental conditions (here: low LCPUFA intakes). Their seemingly widespread occurrence might be taken as a testimony that the LCPUFA intakes from our ancient diet have been of sufficient magnitude to confer Darwinian fitness to their carriers.

2.5.2 LCPUFA in Pregnancy

2.5.2.1 Maternal and Fetal LCPUFA Metabolism and Transplacental Transport

Women of reproductive age and pregnant women have higher fractional conversion of ALA to EPA and DHA than men (Burdge, 2006; Williams and Burdge, 2006). This higher efficiency might be important, but is unlikely to compensate fully for the fetal and newborn needs. Sensitivity of the desaturation/elongation pathway for hormones is supported by the observation in rats that serum estrogens and progesterone exhibit positive correlations with LCPω3 status, while testosterone is negatively related (Childs et al., 2008). It has been shown that the fetal baboon is able to synthesize DHA from labeled ALA and that labeled DHA is transferred across the baboon placenta. The placenta secretes apolipoprotein B-containing particles and contains both FADS1 and FADS2 activities. This suggests that placental LCPUFA synthesis may contribute to fetal LCPUFA status, but the extent to which this occurs is unknown (Innis, 2005). During pregnancy DHA becomes notably enriched in plasma PC, which is likely to augment DHA bioavailability to the placenta and fetus (Burdge et al., 2006). Transplacental fatty acid transport is selective for LCPUFA, as concluded from the higher LCPUFA contents in the fetal circulation, compared with the maternal circulation, while the transfer of LA and ALA is nonselective

(van Beusekom et al., 1993; Duttaroy, 2004; Haggarty, 2004; Innis, 2005). The underlying mechanism of LCPUFA transplacental transfer, causing what has been named "biomagnification" (Crawford et al., 1976), is probably by a combination of many mechanisms, including selective hydrolysis of LCPUFA from maternal triglycerides, increasing maternal free fatty acids with advancing gestation in combination with higher fetal albumin, placental fatty acid-binding proteins (FABP) and fatty acid transfer proteins (FATP), a placenta-specific FABP with high DHA and AA affinity, and, importantly, the trapping of the transferred LCPUFA in the fetal circulation by albumin and alpha-fetoprotein or by esterification to lipids (Haggarty, 2004).

There is a nonsaturating linear relation between the phospholipid DHA/AA ratio in maternal and cord plasma for six populations with different fish intakes, including two with very high fish consumption (Otto et al., 1977; Jacobson et al., 2008). This relation coincided with rather uniform AA, but highly different DHA, in umbilical arteries and veins (Otto et al., 1997), suggesting that the current maternal DHA status does not cause saturation of transplacental DHA transport and that even higher infant DHA/AA ratios can be obtained at higher maternal DHA intakes. It has been suggested that AA does not become available for transport prior to the satisfaction of the high placental AA needs. The outcome of the various mechanisms is that fatty acids are transferred from mother to child with an order of preference of DHA > AA > ALA > LA (Haggarty, 2004). There is reasonable evidence to show that in Western countries the maternal body becomes somewhat depleted from LCPUFA, notably DHA, during pregnancy and subsequent breastfeeding. The following characteristics of LCPUFA physiology have been derived from the courses of the plasma phospholipids and RBC fatty acid compositions during pregnancy in Western countries: Maternal AA increases in early pregnancy and subsequently falls below prepregnancy levels, DHA increases in early pregnancy and remains above prepregnancy levels, the EFA/non-EFA ratio decreases while the DHA deficiency index (22:5ω6/22:4ω6) increases with gestational age, maternal and fetal LCPUFA (notably DHA) status are correlated, normalization of maternal DHA status after delivery is retarded by breastfeeding, and maternal plasma DHA is higher in primigravidae than multigravidae (Hornstra, 2000). Umbilical vessels at term (Muskiet et al., 2006) and RBC of newborns up to 0.2 years (Fokkema et al., 2002) contain relatively high amounts of Mead acid (20:3ω9, Figure 2.6; an index of EFA deficiency). Umbilical arteries contain higher 20:3ω9 than umbilical veins and each of these correlate inversely with corresponding AA and LA levels, and positively with 18:1ω9 levels. These data suggest that the intrauterine environment is characterized by low EFA status but may alternatively also be explained by the high fetal *de novo* fatty acid synthesis from glucose in the third trimester, causing increasingly successful competition of *the novo* synthesized 18:1ω9 for FADS2 and the occurrence of a state of "relative EFA/LCP deficiency," rather than an absolute deficiency (Muskiet et al., 2006).

2.5.2.2 Consequences of Low LCPUFA Status in Pregnancy

Mean EPA + DHA intakes by 19–30 years old Dutch females in 2003 has been estimated at 84 mg/day, while the current recommendation from 2006 is 450 mg/day (Kruizinga et al., 2007). An EFA deficiency initiated at conception in female mice caused biochemical signs of DHA depletion in maternal brain and severely impaired accretion of DHA and 22:4ω6 in the fetal brain. This implies that the growing fetal brain is more sensitive to low LCPUFA status development than maternal brain, at

least in mice (van Goor et al., 2008a). More extreme models with long-term ALA deficiency in female rats showed that maternal brain DHA is vulnerable to depletion, which becomes aggravated by pregnancy and lactation (Levant et al., 2006a) and affects specific brain regions differently (Levant et al., 2007). Thus, because of the high fetal demands, it seems possible that the marginal LCPUFA status in Western countries causes maternal brain to lose LCP, notably DHA, during pregnancy. Declining maternal DHA status was suggested to be involved in the compromised selective attention (a key component of cognition) during pregnancy (de Groot et al., 2003) and may also be related to postpartum depression (Hibbeln, 2002). However, both the former and the latter hypothesis remained unproven in RCTs with ALA (de Groot et al., 2004) and LCPω3 (Freeman et al., 2006a, 2008; Sinclair et al., 2007; Rees et al., 2008), respectively. A recent small trial did, however, show beneficial effects of LCPω3 supplementation on depression during pregnancy (Su et al., 2008).

Low LCPω3 status in pregnant animals may cause inadequacies in retinal, brain monoaminergic and behavioral function in the offspring, which could not all be restored by an ω3 adequate diet (Innis and Friesen, 2008). Intrauterine LCPUFA status or its relation with other nutrients has as yet not been implicated in "programming," but would be one of many plausible candidates, given the interaction of LCPUFA with various nuclear receptors (see Section 2.5.1.5). For example, PPARs are involved in both growth and development. They are also likely to constitute a link between dietary fatty acids and one-carbon metabolism and thereby epigenetics, since folic acid supplementation of dietary protein-restricted pregnant rats corrected the overexpression of the PPAR-alpha gene in the fetal liver (Lillycrop et al., 2005). Folic acid-stimulated methylation of CpG dinucleotides in the PPAR-alpha gene promoter was found to be the underlying mechanism. These methylation patterns persisted into adulthood (Lillycrop et al., 2008) and were passed to the next generation (Burdge et al., 2007). Modifiable PPAR-alpha expression by maternal dietary protein and folic acid might be expected to confer different sensitivities of their offspring to PPAR-alpha natural ligands (such as LCPUFA and its metabolites), but its causal relation to "programming" has not been delineated as yet.

AA and DHA status of premature and low birth weight infants correlate positively with anthropometrics and length of gestation. AA correlates most strongly with anthropometrics (Koletzko and Braun, 1991; Leaf et al., 1992) and DHA with length of gestation (Olsen et al., 2006, 2007). Positive, negative and insignificant correlations between newborn AA and DHA status and birth weight have been noted in term infants (Elias and Innis, 2001; Rump et al., 2001; Lucas et al., 2004). The discrepancy with prematures might be caused by the rapid accretion of *de novo* synthesized fat in fetal adipose tissue near term. This interindividually variable compartment in size may confound the relation, since LCPUFA status is rather related to lean body mass than birth weight. Maternal DHA is positively related to birth weight and head circumference, while AA is negatively related (Dirix et al., 2008; van Eijsden et al., 2008). A meta-analysis of supplementation studies with LCPω3 during pregnancy indicated a mean increase of 1.57 days of gestation and a 0.26 cm increase of head circumference, but no influence on percentage preterm deliveries, low-birth-weight rate, or rates of preeclampsia and eclampsia (Szajewska et al., 2006). LCPω3 supplementation of women with high-risk pregnancies reduced the risk of early preterm

delivery (i.e., <34 weeks), but there were no other effects on pregnancy outcomes such as recurrence of intrauterine growth retardation, and the rates of pregnancy-induced hypertension, preeclampsia, and caesarean section (Horvath et al., 2007). *Trans* fatty acids are negatively related to AA and DHA in umbilical vessels at birth and positively to Mead acid (Decsi et al., 2002). This relation probably indicates inhibition of LCPUFA synthesis by *trans* fatty acids and might be causal to their negative association with growth (Innis, 2006) and neurodevelopment (Bouwstra et al., 2006b), but other explanations are also possible (Innis, 2006).

A large observational study showed that 6 months to 8 years old children from mothers with seafood consumption below 340 g/week had lower verbal IQ, increased risk of suboptimal outcomes for prosocial behavior, fine motor skills, communication, and social development scores, compared with counterparts from mothers eating more than 340 g/week. The percentage of children with low verbal IQ was inversely related with the mother's LCPω3 intake from seafood (Hibbeln et al., 2007). Higher cord plasma DHA was associated with a more optimal visual development at 6 months and cognitive and motor developments at 11 months (Jacobson et al., 2008), and with lower internalizing problem behavior at 7 years (Krabbendam et al., 2007). Others showed that maternal plasma phospholipid DHA is related to a more mature sleep pattern of their neonates (Cheruku et al., 2002) and that infants of mothers with high RBC DHA performed better on psychophysiological measures (4, 6, and 8 months) and on free play attention and distractibility paradigms (12 and 18 months) (Colombo et al., 2004). In addition, maternal DHA intake during pregnancy was associated with better stereo acuity of their children at 3.5 years (Williams et al., 2001). Not only DHA, but also AA is associated with neurodevelopment. A positive association was found between umbilical vessel AA and the neurological optimality score at 2 weeks (Dijck-Brouwer et al., 2005b), general movements at 3 months (Bouwstra et al., 2006a), and the neurological optimality score at 18 months (Bouwstra et al., 2006b). Maternal AA intake during pregnancy was related to shorter brainstem auditory evoked potentials of their infants at 1 month (Parra-Cabrera et al., 2008). Another study revealed no relation between umbilical blood DHA or AA status with child cognition at 4 years (Ghys et al., 2002) and 7 years (Bakker et al., 2003).

Several trials have been conducted in which pregnant women were supplemented with LCPω3. These showed no lasting effects on child visual and cognitive developments during the first year, but a number of these aiming at evaluation at later age have been positive (Innis, 2007a). Some studies showing no effect reported associations similar to those observed in observational studies (Malcolm et al., 2003). The discrepancy may relate to a complex interplay between a ceiling effect in the dose–outcome relationship, benefits to those with suboptimal baseline status only, and interindividual differences in developmental potential (Innis and Friesen, 2008). Studies with supplemental DHA dosages ranging from 200 to 2200 mg/day have mostly shown benefits with high dosages (Decsi and Koletzko, 2005). Interventions with supplemental dosages above 500 mg LCPω3 daily usually give rise to noticeable augmentation of fetal LCPω3 status (Velzing-Aarts et al., 2001). Higher IQ at 4 years was demonstrated after 1183 mg DHA + 803 mg EPA (total 2494 mg LCPω3) per day from the 18th week till the 4th month postpartum (Helland et al., 2003). No effect on mental and psychomotor developments and behavior were seen at

10 months in infants of mothers receiving 4 g fish oil (1.2 g DHA + 1.8 g EPA) daily during the last trimester of pregnancy (Tofail et al., 2006). Infants of mothers who consumed 214 mg DHA/day via a functional food from 24 weeks to delivery had higher visual acuity scores at 4 months, but not at 6 months (Judge et al., 2007a), and better problem solving abilities, but not recognition memory at 9 months (Judge et al., 2007b). Positive effects on eye and hand coordination was noticed in 2.5 years old children whose mothers received 2.2 g DHA + 1.1 g EPA daily from the 20th week until delivery (Dunstan et al., 2008).

Both maternal and fetal LCPUFA status are compromised by gestational diabetes mellitus (GDM), types 1 and 2 diabetes mellitus, and preeclampsia. This might be of growing importance because affluent countries experience increasing prevalence of overweight and obesity in pregnancy, while high maternal BMI is a well-established risk factor for diabetes mellitus, preeclampsia, and fetal defects. Higher 16:0 and lower AA and DHA in RBC (Min et al., 2006) with similar abnormalities in plasma (Thomas et al., 2005) were observed in women with GDM and their offspring (Min et al., 2006). Overweight and obese women with GDM had lower RBC AA and DHA than lean counterparts (Min et al., 2004). Fetal RBC AA and DHA were inversely related to HbA_{1c} in healthy and GDM-complicated pregnancies (Wijendran et al., 2000), suggesting that fetal LCPUFA status is unfavorably affected by disturbed maternal glucose homeostasis. The placental phospholipids contained higher AA and DHA, whereas AA in placental triglycerides was lower (Thomas et al., 2005). Umbilical vessel walls of GDM and also type 1 diabetic pregnancies have lower EFA and LCPUFA status (Dijck-Brouwer et al., 2005a). Similarly, both AA and DHA status of pregnant women with types 1 and 2 diabetes and their newborns is lower than control (Ghebremeskel et al., 2004; Min et al., 2005). A number of studies have shown either higher AA, lower LCPω3, or lower PUFAω3 + ω6 in women with preeclampsia (Jensen, 2006). One case-control study showed higher potentially *de novo* synthesized fatty acids and lower LCPω3 and LCPω6 in umbilical vessels of preeclamptic births (Velzing-Aarts et al., 1999), which recently became confirmed in a population with high LCPω3 intakes from fish (Huiskes et al., 2009). The underlying mechanism of the lower fetal LCPUFA status in diabetes mellitus in pregnancy might be increased maternal free fatty acids and augmented fetal *de novo* fatty acid synthesis due to high transplacental glucose transport, causing dilution of LCPUFA (Dijck-Brouwer et al., 2005a; Muskiet et al., 2006). Compromised transplacental transport and higher *de novo* fatty acid synthesis in the maternal liver due to insulin resistance might be the cause of the lower LCPUFA status in the offspring of mothers with preeclampsia (Huiskes et al., 2009).

Taken together, both maternal and fetal DHA status might be at risk in current Western societies because of low maternal intake of fish and the increasing prevalence of maternal insulin resistance and compromised glucose homeostasis. The fetal preference of LCP, notably DHA, as compared with their parent EFA precursors is likely to point at the importance of fetal LCPUFA status. Positive effects of LCPω3 supplements on newborn neurodevelopment become especially noticeable at later age and following administration of high supplemental dosages from early pregnancy that are preferably continued during lactation.

2.5.3 LCPUFA in Neonatal Nutrition

2.5.3.1 Importance of LCPUFA in Neonatal Nutrition

Brain volume is about 400 mL at birth and increases to about 1100 mL at the age of 2 years. AA and DHA are among the brain's principal building blocks and it is clear that preformed DHA is more effective than ALA to cover the high prenatal (Greiner et al., 1997) and postnatal (Su et al., 1999; DeMar, Jr. et al., 2008) needs. Both preterm and term infants are able to convert LA to AA, and ALA to DHA (Innis, 2003), but the activity seems insufficient to keep postnatal LCPUFA status constant: numerous studies have shown a decline of AA and DHA in plasma and RBC of children receiving infant formulae without preformed LCPUFA, as compared to breastfed counterparts. A few autopsy studies on the corresponding brain LCPUFA levels have been conducted (Farquharson et al., 1992; Makrides et al., 1994; Martinez and Mougan, 1998; Jamieson et al., 1999). These revealed that infants receiving formula without DHA and AA had lower DHA in brain, but their AA was normal or somewhat higher. The brain DHA levels increased with age in breastfed infants, which was largely an effect of length of feeding, while the accretion of AA was dependent on age but not diet (Makrides et al., 1994). The lower DHA in frontal cortex PE, with concomitantly higher AA, 22:4ω6, and 22:5ω6, suggested low DHA status with compensatory chain elongation/desaturation of ω6 fatty acids (Innis, 2003). In other words, the occasionally higher AA and consistently lower DHA in the brain of formula-fed infants seem rather caused by DHA shortage with corresponding dominance of LCPω6 synthesis, notably of 22:5ω6, than by competition of DHA and AA for incorporation. The consequences in humans are as yet unclear, but partial replacement of DHA in the brain of rat pups with 22:5ω6, by either causing ω3 deficiency or postnatal feeding with 22:5ω6, led to loss of spatial task performance at adult age (Lim et al., 2005). Many functions of DHA in the brain are recognized to date, varying from membrane biogenesis, gene expression, neurite outgrowth, protection from oxidative stress and apoptosis, to modulation of neurotransmission (Innis, 2007a). Finally, low neonatal DHA status may be involved in "programming." An ALA-deficient diet administered to rats in the perinatal period increased blood pressure in later life, even if they were subsequently repleted with ω3 fatty acids (Weisinger et al., 2001).

2.5.3.2 Consequences of Low Perinatal LCPUFA Status

Many, but not all, RCTs using formulae with and without LCPUFA and measuring outcome parameters like retinal function, visual acuity, behavior, and cognitive and motor developments, have shown positive effects of LCPUFA, notably DHA, in both preterm and term infants (Makrides et al., 2005; McCann and Ames, 2005; Hadders-Algra et al., 2007; Simmer et al., 2008a,b). Prematures are likely to benefit most, but many of the effects seem transient. The observed effects are especially on account of DHA, but addition of AA might be important to preserve ω3/ω6 balance. This might especially be appropriate at high EPA + DHA dosages for their ability to shut off AA synthesis from LA. What the ω3/ω6 or AA/EPA + DHA balances should look like is, however, uncertain, but the principal concern in Western societies is not AA but correction of our very low DHA status. It is in this respect of importance to note

that differences in infant visual acuity have been found in the "normal" human milk DHA range, with high DHA corresponding to better visual acuity (Innis et al., 2001). Moreover, a recent study showed a relation between cord plasma DHA and better visual acuity and cognitive and motor developments in the offspring of Canadian Inuits, who have high intakes of marine foods (Jacobson et al., 2008).

A Cochrane meta-analysis based on 14 RCTs (11 of high quality) with relatively mature and healthy preterm infants showed (1) that most studies did not find differences in visual acuity over the first year, (2) that there was no effect on neurodevelopment at 12 or 18 months, and (3) that there were no effects on growth (weight, length, or head circumference) at 12 and 18 months. It was concluded that there are no clear long-term benefits for infants receiving formula supplemented with LCPUFA and that there is no evidence that formulas with LCPω3 and LCPω6 impair the growth of preterm infants (Simmer et al., 2008b). Based on 14 RCTs (11 of high quality) a Cochrane meta-analysis with term infants showed (1) that data for visual acuity up to 3 years are inconsistent, (2) that there are no benefits on mental or psychomotor developments through the first 2 years, and (3) that there are neither beneficial nor harmful effects on growth through the first 3 years of life. The final conclusion was that there are no beneficial effects of LCPUFA supplementation of formula milk on visual, physical, and neurodevelopmental outcomes for infants born at term, and that LCPUFA supplementation cannot be recommended on the basis of current evidence (Simmer et al., 2008a). These conclusions are consistent with "no effect on growth" in an independent meta-analysis (Makrides et al., 2005) and with "no robust benefits of LCPUFA on mental and psychomotor developments in prematures between 12 and 18 postnatal months" (Smithers et al., 2008). LCPUFA also do not alter the risk of diseases of prematurity, such as necrotizing enterocolitis and neonatal sepsis (Smithers et al., 2008).

In contrast to the inconsistency of human studies, animal experiments have shown consistent abnormalities in various cognitive and behavioral tests, while also the beneficial effects of DHA (e.g., in CAD) in other human life stages cannot be ignored. More pronounced brain DHA deficiency (typically 70% depletion) in animal studies than those in humans (20% difference between brain DHA of breastfed and formula-fed infants), insufficient sensitivity of currently available tests in humans, and brain plasticity are among the possible explanations for the discrepancy (McCann and Ames, 2005). Also of importance might be that in the majority of studies neurodevelopmental outcome was assessed at 6–24 months, which is an age of "latency" in the expression of neurological dysfunction (Hadders-Algra et al., 2007). Therefore, on the basis of combined human and animal studies and because of the positive relations between neonatal brain DHA and cognitive and behavioral performance, the difficulty to detect differences by RCTs using the currently available tools, and the inability to exclude that LCPUFA have long-lasting beneficial effects on neurodevelopmental outcomes at school age and beyond, it is concluded that addition of LCPUFA and notably DHA to infant formula should rather be recommended on the basis of common sense than on the currently available strength of scientific evidence (McCann and Ames, 2005).

2.5.3.3 Recommendations

Many recommendations for infant formulae have been issued by various nutritional boards and advisory committees. An example is AA ≥ 0.40 and DHA ≥ 0.35 g% for

prematures and AA ≥ 0.35 and DHA ≥ 0.20 g% for term infants (Koletzko et al., 2001). These recommendations are based on the study of milk of mothers living in Western countries. However, these have low intakes of ALA, high dietary LA/ALA ratio, and limited consumption of fish, which relates to CAD, postpartum depression, and suboptimal neurodevelopment as outlined above. Criticizing their diet is inherent to criticizing their milk contents of AA and DHA (and other fatty acids), since both milk AA (Smit et al., 2000; van Goor et al., 2009) and DHA are notably dependent on long-term dietary habits, with the majority of the LCPUFA deriving from maternal stores (Fidler et al., 2000; del Prado et al., 2001). The milk fatty acid composition is strongly dependent on the dietary fatty acid composition and the carbohydrate energy percentage (Francois et al., 1998; Innis, 2007c; Kuipers et al., 2007). Worldwide the highest biological variation is in milk DHA and EPA and the lowest in 16:0. Among the EFA and LCP, AA and 20:3ω6 exhibited the lowest variation (Smit et al., 2002). The low worldwide AA biological variation might however be caused by the aforementioned relatively constant LA and AA intakes by Western populations. It was recently shown that maternal AA supplementation does increase milk AA (van Goor et al., 2009) and there is evidence from Vancouver that both milk DHA and AA have declined about 50% from 1988 to 1998 (Innis, 2003), while in the United States milk LA content has increased 2.5-fold from 1940 to 1990, with little change in ALA (Ailhaud et al., 2006).

Comparison of current recommendations for infant formulae with the actual fatty acid composition of human milk in various developing countries gives rise to many discrepancies and these are notably on account of the medium-chain fatty acids (12:0 and 14:0), LA and ALA, and also AA and DHA (Smit et al., 2003). For instance, the milk of the women living in Doromoni (Lake Kitangiri, Tanzania) contains relatively high percentages AA, DHA, and EPA, together with a relatively low AA/DHA ratio. Their diet is composed of sunflower oil-fried local fish, maize, and local vegetables (Kuipers et al., 2005). Milk from the island of Chole (Indian Ocean, close to the Tanzania mainland) contains high levels of 12:0, 14:0, AA and DHA, but low levels of LA that compare favorably with those in the United States in 1940. The staple foods in Chole are coconut and preferably boiled marine fish, which are combined with a high intake of fruits. Even higher milk DHA levels have occasionally been encountered in fish eating Caribbean societies (Kuipers et al., 2007) and other populations with high intakes of marine foods. Our rapidly changing Western dietary habits, the derivation of *H. sapiens* from the East-African land–water ecosystem (Broadhurst et al., 1998, 2002; Crawford et al., 1999; Gibbons, 2002a) and the more traditional lifestyle of the investigated women in some developing countries add to the contention that their milk fatty acid composition reflects a diet that is much closer to the ancient diet on which the genes of *H. sapiens* have evolved. It raises the question of *who* should actually constitute the *H. sapiens* standard.

The recent guidelines for LCPUFA contents of infant formulas and baby foods, as endorsed by the World Association of Perinatal Medicine, the Early Nutrition Academy, and the Child Health Foundation recommend to add at least 0.2 g DHA/100 g fatty acids (0.2 g%) in formulae for term infants. DHA should not exceed 0.5 g% and the minimum amount of AA should be equivalent to DHA (Koletzko et al., 2008). The basis of this recommendation is that at least 0.2 g% DHA is

necessary to achieve benefits on functional endpoints, but that systematic evaluation of levels above 0.5 g% DHA was not published. The recommendations acknowledge that "breast is best," that pregnant and lactating women should aim at dietary intakes of at least 200 mg DHA/day and that higher intakes up to 1 g DHA and 2.7 g LCPω3 have been studied without significant adverse effects. This new advice seems inconsistent because women who adhere to current recommendations for the general public to consume 450 mg LCPω3/day (about 170 mg DHA) or to the recommendations of the International Society for the Study of Fatty Acids and Lipids (ISSFAL) to consume 300 mg DHA/day during pregnancy and lactation (ISSFAL, 2008) will have mature milk DHA contents ranging from 0.43 to 0.79 g% DHA, but likely above 0.5 g% (van Goor et al., 2008b).

Taken together it seems that recommendations based on human milk observational, mostly Western, studies are likely to create a vicious circle: Low LCPUFA intakes by Western mothers cause low LCPUFA contents in breastmilk and correspondingly low recommendations for infant formulae, while both the consumption of LCPUFA-poor breastmilk and formulae will cause maintenance of low infant LCPUFA status (Brenna et al., 2007; Muskiet et al., 2007). Given the difficulty to proof beneficial effects of LCPUFA in infants, it might at present be a preferable strategy to aim at breastmilk DHA and AA contents of mothers consuming diets with RCT-proven benefits for CAD prevention in adults. The ultimate goal might, however, be to return to the human milk LCPUFA contents to which our genes have become adapted during evolution. The superiority of such diets might be difficult to prove in RCTs, but insistence on solid scientific evidence basically ignores the existence of evolution during which genes become adapted to environment and not *vice versa*.

2.5.4 LCPUFA in Psychiatric Disease

2.5.4.1 LCPUFA and Brain

As outlined in the previous chapters, the brain has relatively high LCPUFA contents with functions in membrane-associated processes and eicosanoid production and also in gene expression. "Nutrigenomics" studies in rats revealed that LCPω3 modulate the expression and repression in brain of a sizeable number of genes that are involved in structure, energy metabolism, neurotransmission, signal transduction, and regulation (Kitajka et al., 2002, 2004). A study with newborn baboons showed that dietary DHA and AA dosages within the human milk intake range caused differential expression of many genes involved in lipid metabolism and transport, G-protein and signal transduction, development, visual perception, cytoskeleton, peptidases, stress response, transcription regulation, and others without defined function (Kothapalli et al., 2007). PPAR-gamma is abundant in mouse embryonic brain and both AA and DHA are ligands of RXR, which as a heterodimer with the retinoic acid receptor (RAR) regulates expression of many genes involved in development, such as those involved in neurogenesis, differentiation, and plasticity (Innis, 2007a). Dietary LCPUFA also influence neurotransmitter physiology and behavior. Experiments with young rats revealed that fish oil supplementation influences several neurochemical and behavioral features of monoaminergic function, causing an

increase of cerebral membrane PS, higher dopamine, reduction of monoamineoxidase-B activity and greater binding to dopamine D2 receptors in the frontal cortex, and also lower ambulatory activity (Chalon et al., 1998). Profound chronic dietary ALA deficiency decreased the brain dopamine pool in the frontal cortex, which relates to hypofunction of dopaminergic transmission in the frontal cortex, and to hyperfunction in the nucleus accumbens (Chalon et al., 2001). Variation in rat brain DHA content by dietary means was found to cause sex-specific alterations in locomotor activity, with males being most affected notably at postadolescent age. The observed DHA content–effect curve was bell-shaped, with both low and high brain DHA giving rise to lower locomotor activities compared with control and medium high DHA levels (Levant et al., 2006b). Not only the dopaminergic, but also the serotonergic and cholinergic systems are influenced and, importantly, not all of the abnormalities proved correctable by reversal diets. Their persistence throughout life suggests that disbalances of ω3 and ω6 fatty acids, notably low DHA, in early life might have long-term consequences in neurotransmission systems and thereby brain functioning, and that these may be related to neurological and psychiatric disorders (Chalon, 2006).

A recent review (McNamara and Carlson, 2006) concluded that studies in rodents have shown that DHA is important in the developing brain because of its neurotrophic properties in the promotion of neuronal arborization and synaptogenesis. The frontal cortex and hippocampus are the most sensitive to dietary DHA deficits and such deficits notably affect the developing brain as opposed to the fully matured brain. Low brain DHA is accompanied by a reciprocal increase of 22:5ω6 and a decrease of PS, and is associated with reduced extracellular levels of various neurotransmitters including serotonin, dopamine, and acetylcholine upon stimulation, along with increased serotonin receptor-binding density, and reduced dopamine receptor-binding density. Most of these abnormalities proved correctable if feeding with dietary ω3 fatty acids was initiated within the first two postnatal weeks, but not beyond, suggesting that there is a critical window of opportunity for normalization. Perinatal ω3 deficiency in rodents is related to abnormal spatial learning, working memory and olfactory discrimination learning, anxiety, aggression, depression, and deviant locomotor activity upon treatment with various drugs, including amphetamine and scopolamine. Experiments with nonhuman primates showed that prematurely born monkeys had lower brain DHA than term counterparts. Initiation of an ALA-low diet prior to conception caused low brain DHA in the offspring and reduced visual acuity. The DHA deficits proved slowly correctable by a postnatal high-ALA feeding regimen, with retinal DHA showing the slowest recovery. The ω3 deficiency in nonhuman primates is associated with reduced visual acuity, abnormal electroretinograms, polydipsia, and longer look duration to both familiar and novel stimuli.

2.5.4.2 LCPUFA and Behavior

Current research in psychiatric disease seems to fall short of the input of nutrition and may be somewhat overdosed with genetics and the traditional search for abnormal neurotransmitter metabolism *per se*. Low perinatal frontal cortex DHA accretion is associated with suboptimal development of the dopaminergic system and therefore a risk factor for attention-deficit/hyperactivity disorders (ADHD) and schizophrenia.

These anomalies may still be correctable within a certain perinatal time window but not beyond, which might explain the limited efficacy of supplementation studies at later age (McNamara and Carlson, 2006). It is possible that also the relation of LCPUFA with one-carbon metabolism is involved, since at least schizophrenia is strongly related with low intrauterine folate status (Muskiet and Kemperman, 2006). Low intake of the fish oil fatty acids EPA and DHA is implicated in the high incidence of depression in Western countries. This incidence has increased markedly in recent decades (Klerman and Weissman, 1989) and there is a strong inverse correlation between national dietary fish intakes and rates of major and postpartum depression (Hibbeln, 1998, 2002). Depressive symptoms are more likely to be encountered in infrequent fish consumers. Patients with depressive symptoms have low EPA and DHA status and their AA/EPA ratio is high (Freeman et al., 2006b), while plasma EPA in the elderly is inversely related to the severity of depressive symptoms (Feart et al., 2008). The close relationships between fish consumption and the incidence of CAD (see Section 2.4.2) and depression has fuelled the suggestion that depression should be included into the cluster of diseases that are associated with the metabolic syndrome (Peet, 2003).

Multiple hit scenarios have been proposed to explain the late onset of psychiatric diseases, such as schizophrenia. Initial hits may occur in the uterus, or even during gametogenesis, with subsequent insults occurring in adolescence or adulthood, causing accumulating epigenetic abnormalities ("epimutations," Petronis, 2004). There is indeed good evidence to show that birth weight and pregnancy complications are risk factors for the development of at least some psychiatric diseases, including schizophrenia (Godfrey and Barker, 2000; Wahlbeck et al., 2001). Very-low-birthweight babies are at risk for developing psychiatric symptoms and reduced social and academic skills at adolescent age, while term small-for-gestational age babies have higher risk of emotional, behavioral, and attention deficit symptoms (Indredavik et al., 2005). A study of perinatal risk factors for autism concluded that we might be dealing with risk factors for obstetric complications and that these may precipitate to autism by exposure to certain environmental stimuli (Glasson et al., 2004). Low birth weight was also noticed as a perinatal risk factor in another autism case-control study (Larsson et al., 2005). A meta-analysis of prospective population-based studies revealed that schizophrenia is associated with complications of pregnancy, abnormal fetal growth and development, and complications of delivery (Cannon et al., 2002). Dietary habits at young adult age may constitute the basis for a second hit. Data from the United Kingdom show that the peak age of schizophrenia onset (i.e., 19–24 years) coincides with the highest intake of burgers (i.e., saturated fat) and full-sugar carbonated drinks and the lowest intake of oily fish (Peet, 2004b). A meta-analysis of dietary patterns in various countries linked the intake of refined sugar and dairy products to a worse 2-year outcome of schizophrenia, while a high national prevalence of depression became predicted from low intake of fish and seafood (Peet, 2004a).

The outcome of RCTs have until now been rather disappointing, with some exceptions, notably in depression. A 2006 Cochrane meta-analysis concluded that the use of LCPω3 for schizophrenia treatment remains experimental and that there is a need for large well-designed, conducted, and reported studies (Joy et al., 2003). This outcome is in line with two other meta-analyses concluding that "LCPω3 failed to

improve schizophrenia symptoms" (Freeman et al., 2006b) and that the "available data are not supportive of the efficacy of LCPω3 in schizophrenia" (Ross et al., 2007). Two meta-analyses of RCTs showed beneficial effects of LCPω3 in patients with affective disorders (i.e., combined unipolar and bipolar depression) although the results were highly heterogeneous (Freeman et al., 2006b; Lin and Su, 2007). It was felt that more large-scale well-controlled trials were needed to identify favorable target subjects with mood disorders, therapeutic dosages, and LCPω3 compositions (Lin and Su, 2007). These conclusions are largely in agreement with two other recent reviews in which a dosage of 1–2 g LCPω3/day was considered to be effective (Ross et al., 2007) and in which it was noted that 7 out of 10 studies in adults with depression or bipolar disorders showed positive effects of either fish oil or ethyl EPA, while three were negative (Sinclair et al., 2007). A recent small RCT with LCPω3 supplementation in children with autistic disorders showed a trend for beneficial effects in hyperactivity and stereotypy (Berger et al., 2008). On the other hand, no beneficial effects of LCPω3 were noted in ADHD (Freeman et al., 2006b; Ross et al., 2007) and LCPω3 can therefore not be recommended as a primary treatment (Richardson, 2006). A combination of ω3 and ω6 supplements have shown benefits in three studies with ADHD patients (Freeman et al., 2006b) and a recent study revealed beneficial effects of an elimination diet (Pelsser et al., 2009). LCPω3 may also reduce aggression, impulsivity, and hostility in subjects with borderline personality disorder, the normal population, children with ADHD and reduce felony-level violence in prisoners, although the latter study also provided a multivitamin and mineral supplement (Freeman et al., 2006b).

Taken together, the current data suggest that low LCPω3 status is likely to be involved in the etiology of at least some psychiatric diseases, but also their presentation in terms of disease severity at later age. A fetal origin may prove difficult to correct, but this does not hold for the often poor nutritional status, including biochemical EFA deficiency and folate-sensitive hyperhomocysteinemia, that may be encountered in at least some psychiatric patients (Kemperman et al., 2006). The latter is of importance since contrary to popular belief the primary cause of death in schizophrenia is not suicide, but CAD, while they have high risk of diabetes, partially because of the use of the new generation antipsychotics. Basically, there seem to be little difference in the dietary risk factors for poor mental health, CAD, and some cancers, which adds to the notion that we are dealing with common insults that originate from our changed environment, produce adverse effects in different organs and systems at different life stages, affect the genetically most vulnerable first, but will with increasing dose and exposure time ultimately affect all of us.

2.5.4.3 Schizophrenia Phospholipid Hypothesis

There are (anecdotic) reports that (1) feverish illness in patients with schizophrenia ameliorates their psychiatric symptoms, (2) patients with schizophrenia rarely suffer from rheumatoid arthritis (suggesting a generalized reduced inflammatory response), (3) schizophrenic patients are less capable of producing the typical (prostaglandin-induced) cutaneous flush that follows nicotinic acid ingestion or topical application, and (4) schizophrenia in developing countries, with usually higher LCPUFA intakes, runs a less severe course (Christensen and Christensen, 1988; Hopper and

Wanderling, 2000; Horrobin, 2001; Peet, 2003). Horrobin (2001) linked these observations to the so-called phospholipid hypothesis stating that schizophrenia is a systemic disease with a central theme of insufficient AA release for the production of its eicosanoid metabolites to support adequate signal transduction (Horrobin, 1998). It was suggested that the disease might find its origin in a genetically determined generalized "abnormality" of phospholipid metabolism that is sensitive to prevention or may even become corrected in part by nutritional factors, including LCP. The postulated polymorphism(s) of patients with schizophrenia might in the past not have precipitated to disease since the LCP-rich diet of our ancestors enabled them to take full evolutionary advantage of the intelligence and creativity that is associated with schizophrenia (Horrobin, 2001).

2.5.4.4 LCPUFA and Low-Grade Neuroinflammation in Psychiatry: A Common Denominator?

Consistent with the increased LCPUFA losses postulated by the phospholipid hypothesis, both patients with schizophrenia (Peet, 2003; Ross, 2003) and autism (Bell et al., 2004) have increased activity of PLA_2, which releases AA from membrane phospholipids (a process vital to brain cell signaling), while their LCPUFA in RBC appear more sensitive to oxidative stress *in vitro* (Fox et al., 2003; Bell et al., 2004). Brain magnetic resonance spectroscopy studies in patients with schizophrenia showed signs of increased phospholipid turnover, electroretinograms of patients with schizophrenia are abnormal (suggesting low retinal DHA content), while incorporation of AA into phospholipids seems to occur with difficulty (Horrobin, 2001). Taken together, these data suggest local AA depletion and insufficient synthesis of certain AA-derived eicosanoids, which becomes, for example, noticeable by amelioration of psychiatric symptoms initiated by fever-associated eicosanoid release, pain resistance by eicosanoid shortage at basal conditions, and poor ability to exhibit an eicosanoid-induced flush upon nicotinic acid treatment. It was hypothesized that perinatal supplementation of LCP, especially EPA and DHA, may prevent schizophrenia in the adult. Schizophrenia has also been suggested to derive from low-grade systemic inflammatory disease with origins in the perinatal period, probably triggered by maternal infection in a genetically susceptible individual, leading to excess production of proinflammatory cytokines in both mother and fetus. The inflammation compromises LCPUFA status with devastating neurodevelopmental effects that should theoretically be favorably responsive to augmented LCPUFA status (Das, 2004).

It is nowadays widely recognized that insulin resistance and its sequelae associated with the metabolic syndrome (such as diabetes type 2, CAD, and certain cancer types) and neurodegenerative disorders (like Alzheimer's disease and Parkinson's disease) and depression may have a common origin in a state of low-grade inflammation that finds at least part of its origin in the currently high dietary intakes of SAFA, *trans* fatty acids and ω6 fatty acids, and the low intakes of ω3 fatty acids, notably those abundant in fish (Innis, 2007b). The resulting high ratio between AA and EPA + DHA might have driven us into a proinflammatory condition that may precipitate to a hyperinflammatory response upon a trigger (the "systemic inflammatory response syndrome;" SIRS) with collateral damage, scarring and fibrosis, and the subsequent development of immune paralysis ("compensatory anti-inflammatory response syndrome;" CARS) characterized by weakened host defense and susceptibility to

(secondary) infections (Mayer and Seeger, 2008). The putative normal immune response requires lipid mediators from both AA and EPA + DHA, in which those from AA are implicated in the initiation of the inflammatory reaction and also in the lipid mediator "class switch" to those of EPA and DHA, which are responsible for the termination and complete resolution of the immune reaction and return to homeostasis (see Sections 2.4.2 and 2.5.1.4) (Serhan et al., 2008). The chronic inflammation resulting from the unbalanced AA/EPA + DHA ratio might be central in the pathogenesis of the diseases of the metabolic syndrome and neurodegenerative disease, explain the relation between inflammation, depression, and dementia (Leonard, 2007), and explain the favorable effects of LCPω3 supplements, although these are not consistently observed. LCPω3 supplements might especially be effective in prevention. This is, for example, suggested by the outcomes of epidemiological studies on CAD and prospective studies on Alzheimer's disease (Bourre, 2005), and also from the favorable effects of LCPω3 in early disease stages. For instance, it was recently shown that LCPω3 supplements are effective in cognitive function in patients with mild cognitive impairment, but not in those with mild or moderate Alzheimer's disease (Chiu et al., 2008).

Mechanistically it has been shown in rats that about 5% of both brain AA and DHA per day is lost by metabolism and subsequently replaced (Rapoport, 2003). Brain DHA supply is dependent on dietary sources and its limited synthesis from ALA notably in the liver (Rapoport et al., 2007), while the efficiency of brain DHA synthesis during ALA deficiency does not become upregulated (Igarashi et al., 2007). Consequently, deprivation of dietary ω3 fatty acids lowers DHA in rat brain, and also lowers the activity of the DHA regulatory PLA_2 (i.e., calcium-independent $iPLA_2$) and COX1 in rat frontal cortex, and increases the activity of the AA selective calcium-dependent cytosolic PLA_2 ($cPLA_2$), secretory PLA_2 ($sPLA_2$), and COX2. The outcome is an increased half-life of brain DHA by downregulated $iPLA_2$, but augmented release of AA and production of its eicosanoid metabolites and other bioactive mediators involved in brain signaling and neuroinflammation. In other words, low brain DHA indirectly augments susceptibility to inflammation and other brain insults through an upregulated brain AA–COX2–prostaglandin cascade (Rao et al., 2007). An upregulated AA–COX2–prostaglandin cascade is present in acute neurological disorders, such as cerebral ischemia and head trauma, but also in chronic neurologic disorders, such as Alzheimer's disease and Parkinson's disease, while decreased DHA and its anti-inflammatory docosanoids have been demonstrated in postmortem brain samples from patients with Alzheimer's disease (Orr and Bazinet, 2008). Mood stabilizers like lithium, valproate, and carbamazepine inhibit the AA cascade by reducing AA turnover, but not DHA turnover, in rat brain phospholipids (Rao et al., 2008). These findings are to a large extent supportive for the phospholipids hypothesis of Horrobin.

2.6 CONCLUSIONS

H. sapiens has evolved on a diet that was high in ALA from vegetable sources, rich in AA, EPA, and DHA from a land–water ecosystem (including (lean) fish) and contained low LA, SAFA, and no *trans* fatty acids from industrial sources. We have, however, gradually changed this diet from about 10,000 years ago and accelerated

these changes from about 100–200 years ago. These, but also other, dietary changes are firmly implicated in the risk of typically Western diseases. Some of the relations, for example, the association of ALA and fish oil with CAD and LCPω3 with depression, have proven their causalities in intervention trials. Evolutionary medicine, and perhaps common sense, teaches us that we might have to return to the composition of the diet on which our genes have evolved. For this, we need rethinking of the very basics of "homeostasis" and avoidance of the vicious cycle that is initiated by taking observations from Western societies as a basis of dietary recommendations and lifestyle in general. Traditionally living societies may provide us with clues for absolute standards for human homeostasis, but unfortunately many of these have meanwhile become dependent on food programs with typically Western approaches (e.g., high carbohydrates), or adopted a (quasi) Western lifestyle in general. It may be questioned whether definite answers will come from the expensive RCT approaches of single nutrients, since these require large study numbers, long observation periods, elucidation of dose–response relationships, and investigation of numerous interactions with other nutrients (for example: one-carbon metabolism and LCPUFA). A combination of data from traditionally living societies, (patho)physiological insight, anthropometrics, archeology, genetics, nutrigenomics, and classical trials may lead to cleverly designed RCTs to answer the question on what diet our genes have evolved and what diet consequently promotes our health at best. The lessons from such studies might especially be relevant to early nutrition, because of its importance to development and the increasingly recognized relation between early development and the risk of disease at later life.

ABBREVIATIONS

AA	arachidonic acid
AI	adequate intakes
ALA	alpha-linolenic acid
AMDR	acceptable macronutrient distribution range
BMI	body mass index
CAD	coronary artery disease
ChREBP	carbohydrate regulatory element-binding protein
CNS	central nervous system
COX1 and COX2	cyclooxygenases 1 and 2
DGLA	dihomo-gamma-linolenic acid
DHA	docosahexaenoic acid
DOHaD	developmental origins of health and disease
DRIs	dietary reference intakes
EFA	essential fatty acids
En%	energy %
EPA	eicosapentaenoic acid
FABP	fatty acid-binding protein
FADS1	fatty acid desaturase 1 (delta-5 desaturase)
FADS2	fatty acid desaturase 2 (delta-6 desaturase)
FATP	fatty acid-transfer protein

GDM	gestational diabetes mellitus
GLA	gamma-linolenic acid
GR	glucocorticoid receptor
HDL	high-density lipoprotein
HPA	hypothalamic–pituitary–adrenal
LA	linoleic acid
LCP and LCPUFA	long-chain polyunsaturated fatty acids
LDL	low-density lipoprotein
LOX	lipoxygenase
MLX	Max-like factor X
MTHFR	methylenetetrahydrofolate reductase
MUFA	monounsaturated fatty acid
PAR	predicted adaptive response
PC	phosphatidylcholine
PDAT	pathobiological determinants of atherosclerosis in youth
PE	phosphatidylethanolamine
PEM	protein-energy malnutrition
PEMT	phosphatidylethanolamine-*N*-methyltransferase
PLA_2	phospholipase A_2
PPAR	peroxisomal proliferators-activated receptor
PS	phosphatidylserine
PUFA	polyunsaturated fatty acid
RAR	retinoic acid receptor
RBC	erythrocytes
RCT	randomized controlled trial
RDA	recommended dietary allowance
RXR	retinoid X receptor
SAFA	saturated fatty acid
SREBP	sterol regulatory element-binding protein
UFA	unsaturated fatty acid

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Part II

Taste of Fat: From Detection to Behavior

3 Gustatory Mechanisms for Fat Detection

Timothy A. Gilbertson, Tian Yu, and Bhavik P. Shah

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3.1 INTRODUCTION

The incidence of obesity continues to escalate, and with it, there has been a corresponding increase in as many as 30 diseases related to the obese state, including cardiovascular disease, diabetes, and end-stage renal disease. Recent estimates place the number of overweight and obese individuals in the United States at roughly one third and two thirds of the population, respectively (*CDC/DHHS,* Centers for Disease Control and Prevention). While obesity is clearly a disease that has multiple etiologies, there are compelling data indicating a link between

the recent surge in obesity and a corresponding increase in dietary fat intake (Bray and Popkin, 1998, 1999; Bray et al., 2004). Despite this link, there has been comparatively little attention paid to the sensory cues provided by dietary fats which might precipitate their intake over the short and long terms. Over the past decade, however, the idea that fats might provide salient cues to the gustatory system consistent with there being a "taste of fat" has gained credence. Research in this area holds great promise in understanding the role of the gustatory system in both the recognition of dietary fat and the eventual control of fat intake.

3.1.1 Taste: The Gateway to the Enteric Nervous System

The peripheral gustatory system has two primary functions. The first is the selective identification of essential nutrients the body requires to function. This appetitive property is generally reflected in the ability to respond to the primary tastes such as sweet (carbohydrates), salty (minerals), and umami (proteins/amino acids). In general, these stimuli drive ingestive behavior. The aversive role of the gustatory system, which is generally served by our bitter taste system, is meant to prevent the ingestion of potentially toxic or harmful compounds into the body and elicits an innate rejection reflex in most organisms. Interestingly, our primary taste for acidic compounds, sour, does not easily fit into one of these categories exclusively, and arguments have been made on both sides as to whether sour taste should be included in the appetitive or aversive category (Roper and Gilbertson, 1992). While there is no consensus over the direct role of taste in mediating ingestive behavior in terms of the control of food intake, it is clear that appetitive stimuli promote food intake and aversive stimuli do not.

The sense of taste is mediated by organs called taste buds situated throughout the oral cavity. The greatest density of taste buds is found in the tongue, where they reside within one of three taste papillae. In mammals, the fungiform papillae, which lie over the anterior two-thirds of the tongue, contain one, two, or four taste buds with a preponderance of single taste bud per papilla. In the posterior tongue, hundreds of taste buds lie along the sides of the deep crypts that make up the paired foliate papillae on the sides of the tongue or the circumvallate papillae just posterior to the intermolar imminence. While rodents have a single circumvallate papilla, humans may have a dozen or more. Significant numbers of taste buds are also found distributed in the soft palate, with a high density in the region between the hard and soft palate in a structure known as the geschmackstreifen ("taste stripe"). Other regions containing taste buds include the epiglottis, larynx, and nasopharynx. While these extra-lingual taste buds have been investigated to a limited degree (Gilbertson and Fontenot, 1998; Gilbertson et al., 2001; Gilbertson, 2002; Kataoka et al., 2006; Bezencon et al., 2007; Miura et al., 2007), the vast majority of taste transduction studies have been focused on signaling within the taste buds of the tongue. Although there are certainly regional differences within the taste buds of the tongue in terms of chemosensitivity, it is clear that the concept of a "tongue map" with specific areas of the tongue devoted to each modality is incorrect and that individual taste buds and, perhaps even individual cells (Gilbertson et al., 2001; Caicedo et al., 2002), may respond to multiple classes of taste primers.

An individual taste bud contains from 50 to 100 cells, and within this population, there exist multiple cell types such as cells that act as stem cells, sustentacular cells, receptor cells, and so-called output cells that release neurotransmitter onto the gustatory afferent nerves. It is generally accepted that taste buds contain at least three cells types, though the function of these cells has not been firmly established (Bartel et al., 2006; Miura et al., 2006, 2007). The most numerous cells within the taste bud, the Type I cells, have been proposed to play a supporting role in the taste bud, perhaps as a sustentacular cell that modulates the local environment of the taste bud. A recent report suggests, however, that Type I-like cells may be involved in salt taste perception (Vandenbeuch et al., 2008). Type II cells, the so-called receptor cells, are believed to represent the class of cells that contain receptors for sweet, bitter, and umami tastes as they express all the signaling components of the G protein–coupled receptors (GPCR)-mediated pathways. Type III cells have been suggested to be the output cells of the taste bud as they contain much of the synaptic machinery thought to be necessary to signal taste information to the afferent nerve. While this model is attractive, it cannot completely explain peripheral gustatory function, and the intercellular signaling within the taste bud remains somewhat controversial. The story of cell-to-cell communication within the taste bud is far from clear. A current empirically based model (DeFazio et al., 2006; Roper, 2006, 2007; Huang et al., 2007) favors the idea that Type II (receptor) cells communicate with Type III (output) cells via purinergic signaling mechanisms. Indeed, the loss of purinergic signaling results in rather nonspecific and widespread impairment in peripheral gustatory function (Finger et al., 2005).

3.1.2 Taste Transduction

The initial identification of chemical signals by the taste receptor cells (TRCs) has been shown to involve a series of steps culminating in the release of neurotransmitter from the TRCs onto the gustatory afferents. Initially, sapid chemicals must diffuse or be transported to interact with sites on the apical membranes of TRCs. Most taste stimuli are restricted to the apical regions due to the occlusion of tight junctions between taste cells. In most cases, taste stimuli interact either with receptors or directly with ion channels located on the apical membranes of taste cells. This interaction then leads to a conductance change and/or a release of intracellular Ca^{2+} within the taste cell. The conductance change activates voltage-dependent K^+ and Na^+ channels in the basolateral membrane leading to depolarization and generation of action potentials within the taste cell. Alternatively, release of intracellular Ca^{2+} may activate the nonspecific cation channel, transient receptor potential channel type M5 (TRPM5), and allow Na^+ influx during chemostimulation. Membrane depolarization, in turn, activates voltage-dependent Ca^{2+} channels, allowing Ca^{2+} influx and eventually transmitter release onto gustatory afferents. As discussed above, it is unclear if this pathway from taste stimulus binding through transmitter release occurs within a single cell in the taste bud or if there is a requirement for cell-to-cell communication (Type II to Type III) within the taste bud prior to afferent nerve activation for certain subsets of taste stimuli. For more detailed information, the reader is referred to a number of excellent reviews dealing with the specifics

of the taste transduction process (Herness and Gilbertson, 1999; Gilbertson et al., 2000; Gilbertson and Boughter, 2003; Chandrashekar et al., 2006; Simon et al., 2006; Sugita, 2006; Palmer, 2007; Roper, 2007).

3.2 FAT TASTE TRANSDUCTION

Given the role of the taste system in recognizing those compounds needed for survival, almost a decade ago, we began to test the idea that, as is the case with other nutrients, the gustatory system *should* be able to detect the essential fatty acids, i.e., those that are required in the diet. At this time, however, the prevailing idea was that fat had no taste, rather, its most (*only*) salient cue was its texture (Raats et al., 1993; Mela et al., 1994a,b). Clearly, the textural attributes of fat have been well documented (Rolls et al., 2003; Verhagen et al., 2003; Kadohisa et al., 2005) and contribute significantly to fat perception. The texture of fat is perceived largely through activation of the somatosensory system and specifically through activation of trigeminal nerve fibers that originate in the trigeminal ganglia and send projections into the oral cavity.

Our initial work in this area was focused on trying to determine if essential fatty acids, the *cis*-polyunsaturated fatty acids (PUFAs), could activate taste cells in a manner analogous to other taste stimuli. The utility of fatty acids as the prototypical taste stimulus was suggested by a number of observations at the time. First, there was an emerging literature that had demonstrated that free fatty acids could act as specific and potent extracellular messengers in a variety of systems including cardiac, smooth, and skeletal muscle (Ordway et al., 1991; Honore et al., 1994; Petrou et al., 1995). These pathways appeared to be independent of the ability of some PUFAs (i.e., arachidonic acid) to activate second messenger cascades (lipoxygenase, cyclooxygenase, and 20-hydroxyeicosatetraenoic acid) within cells. Second, while there are significant free fatty acid concentrations in most, if not all, fat-containing foods (Weiss, 1983), the oral cavity contains molecules predicted to play a critical role in the generation and transport of free fatty acids within this aqueous environment. These include lingual lipase, which is released from serous glands at the base of the circumvallate and foliate papillae and can break down triglycerides into the mono- and diglycerides, free fatty acids, and the von Ebner's gland proteins. The latter are structurally similar to the lipocalins, a family of proteins that play primary roles in the transport of lipophilic molecules (Akerstrom et al., 2000; Descalzi Cancedda et al., 2000; Flower et al., 2000; Grzyb et al., 2006). Interestingly, the von Ebner's gland proteins, which were originally hypothesized to transport lipophilic bitter molecules, bind no known tastants other than free fatty acids (Schmale et al., 1993; Kock et al., 1994; Creuzenet and Mangroo, 1998).

3.2.1 Delayed Rectifying K^+ Channels: The First Fatty Acid "Receptor"

Our original hypothesis that proposed a role for delayed rectifying K^+ (DRK) channels as important players in the initial transduction of fat taste stemmed from work in the early-to-mid-1990s that demonstrated that free fatty acids were potent primary messengers in a numbers of systems (Ordway et al., 1991; Petrou et al., 1995)

and their site of action was directly at the level of DRK channels (Grissmer et al., 1994; Honore et al., 1994; Poling et al., 1996). To test this idea, we began a series of electrophysiological assays to determine if free fatty acids could activate taste cells through inhibition of DRK channels. In rat TRCs, the original target in these early experiments, fatty acids could significantly inhibit current flow through DRK channels consistent with their action in other tissues. The predicted cellular consequence of this action would be the inhibition of the efflux of K^+ ions, resulting in a depolarization of the TRCs during chemostimulation with free fatty acids (Gilbertson et al., 1997). Block of K^+ channels, either directly or indirectly, has been shown to result in depolarization during the application of other tastants, such as sweet and sour stimuli (Kinnamon et al., 1988; Cummings and Kinnamon, 1992; Cummings et al., 1996; Herness et al., 1997). Interestingly, in the anterior tongue (i.e., fungiform taste buds), the only fatty acids that caused a significant reduction in DRK currents were the *cis*-PUFAs, also known as the essential fatty acids, which are required in the diet. This finding dovetailed with the role of the gustatory system in nutrient recognition (Gilbertson and Kinnamon, 1996; Herness and Gilbertson, 1999; Gilbertson et al., 2000). The effective concentration range for PUFA activation of taste cells via this mechanism was approximately 1.0 μM for each fatty acid and there was no significant effect of chain length or degree of unsaturation (Gilbertson et al., 1997). The mechanism of inhibition seemed to be a classical open channel block mechanism (Honore et al., 1994). The effective concentrations fell well within the range of free fatty acid concentrations that can be generated during fat feeding in rodents (Kawai and Fushiki, 2003) and found in most fat-containing foods (Weiss, 1983) regardless of lingual lipase activity. While the anterior tongue appeared specific for PUFAs, other areas in the oral cavity (taste buds from the foliate and circumvallate papillae and the soft palate) responded to a somewhat broader array of fatty acids, including the monounsaturated fatty acids, oleic acid, and palmitoleic acid, in a similar concentration range (Hansen et al., 2002, 2003).

3.2.2 KCNA5 Is the Major Fatty Acid-Sensitive DRK Channel in Taste Cells

Given the molecular diversity of DRK channels which exist in at least nine different forms within three different subfamilies, one of our initial goals was to identify the subtype(s) of DRK channels in the taste system that was playing the role of fatty acid "receptor." Pharmacological, electrophysiological, and molecular biological assays are consistent with TRCs expressing a rich array of DRK channels, including members from within the three major families of DRK channels, KCNA, KCNB, and KCNC (Liu et al., 2005). Of the DRK channels expressed, the four most highly expressed channels appear to be KCNA5, KCNC2, KCNC1, and KCNC2 (Kv1.5, Kv2.2, Kv3.1, and Kv3.2, respectively). This relative expression pattern is consistent across all lingual taste bud types and the enteroendocrine cell line, STC-1, which shares many of its chemosensory pathways with mammalian taste cells, may as such be a good model for (Type II) taste cells (Wu et al., 2002, 2005; Dyer et al., 2005; Masuho et al., 2005; Chen et al., 2006; Saitoh et al., 2007; Hao et al., 2008). Heterologous expression data demonstrate that members of the KCNA family of DRK channels are highly sensitive

to *cis*-PUFAs, while the KCNB and KCNC family members are moderately sensitive and insensitive, respectively (Shah et al., 2006). Based upon kinetics, pharmacology, and quantitative expression, the major DRK channel in taste cells appears to be the KCNA5 channel (Liu et al., 2005). Interestingly, KCNA5 (Kv1.5) is the major DRK channel expressed in the heart which exhibits similar fatty acid responsiveness as taste cells (Kim and Clapham, 1989; Kim and Duff, 1990; Wallert et al., 1991; Honore et al., 1994; Hu et al., 1998; Xu and Rozanski, 1998; Crumb et al., 1999; Doolan et al., 2002; Ogita et al., 2003; Guizy et al., 2008). KCNA5 appears to be fairly ubiquitous in its expression within the taste bud, since virtually all types of taste cells express this channel (Liu et al., 2005).

3.2.3 Implications of DRK Inhibition by Free Fatty Acids

The ability of free fatty acids to inhibit DRK channels, particularly KNCA5, in taste cells has two functional consequences. The first, already mentioned, suggests that this mechanism may act as a primary mechanism related to the transduction of fat by the peripheral gustatory system. Indeed, there is now a wealth of data from the molecular/cellular level through studies of behavior and human psychophysics to support the notion that free fatty acids are capable of being sensed by the taste system (see Chapters 4 and 7).

The other role of this mechanism has been suggested to be involved in the ability of free fatty acids to modulate the responses of taste cells to other taste stimuli (Gilbertson, 1993, 1998a,b, 1999; Gilbertson and Boughter, 2003; Mizushige et al., 2007). As mentioned above, KCNA5 shows rather broad expression patterns within the taste bud and, accordingly, might be expected to be found in cells that respond to a variety of taste primers. Because one role of DRK channels is to repolarize cells following activity, one would predict that tastant-induced activity would be altered in the presence of fatty acids. That is, a depolarization induced in a taste cell by another stimulus (sweet or salty) should be enhanced and last longer if these repolarizing DRK channels are inhibited by fatty acids, an effect we have shown at the cellular level in electrophysiological assays (Gilbertson et al., 1997). To provide support for this model at the behavioral level, we have performed 48 h preference tests in rodents, investigating the ability of free fatty acids to alter preference for a subthreshold concentration of a sweet stimulus, saccharin. In these assays, neither linoleic acid (5 and 20 μM) nor saccharin (0.5 mM) was preferred at these concentrations, but there was preference for the combination of these two stimuli (Gilbertson et al., 2005). However, this effect was limited to *cis*-PUFAs like linoleic acid as lauric acid at the same concentration did not affect preference for the subthreshold concentration of saccharin consistent with its inability to inhibit DRK channels. These findings were replicated in rodents using a short term behavioral paradigm (Pittman et al., 2006), which showed that linoleic and oleic acids could alter licking responses to sweet stimuli in a manner consistent with the ability of fatty acids to enhance responsiveness. However, this ability of fatty acids to enhance taste in humans appears equivocal. Some reports have suggested that linoleic acid is able to alter taste in humans (Kamphius, 2003; Kamphius et al., 2003), whereas others have reported that free fatty acids are not able to alter detection or thresholds for

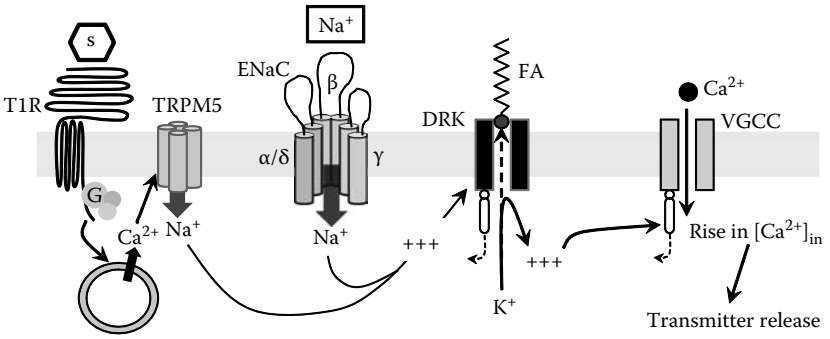

FIGURE 3.1 **(See color insert following page 166.)** The ability of fatty acids to inhibit DRK channels is consistent with roles as either a taste primer or a taste modulator. Fatty acids (FA), like linoleic acid, inhibit DRK channels by acting as an open channel blocker. This inhibition would prohibit the efflux of K^+ ions, causing depolarization of the taste cell and subsequent activation of downstream signaling elements such as voltage-gated Ca^{2+} channels (VGCC). Alternatively, the depolarization elicited by other taste stimuli like sweet working through T1R GPCRs/TRPM5 pathway or the permeation of Na^+ ions though epithelial sodium channels (ENaC) in the case of salty taste could be enhanced and prolonged in the presence of FAs. This is diagrammatic only and not meant to imply that all these elements are in the same individual cells. Our expression data would argue, however, that there is overlap between FA-sensitive DRK channels and these other taste modalities.

prototypical taste stimuli (Mattes, 2007). While the reason for these differences is unclear, the latter study used free fatty acid concentrations several orders of magnitude higher than those used in rodent studies and certain aspects of human taste performance show parabolic, not linear, relationships with concentration (Pangborn and Giovanni, 1984). Figure 3.1 summarizes the potential roles of fatty acids as a taste primer and taste modulator.

3.3 FAT TASTE, DRK CHANNELS, AND OBESITY

In the context of the emerging "epidemic of obesity," there has been significant interest in understanding the sensory cues for dietary fat due in part to the data suggesting a link between fat intake and obesity (Bray and Popkin, 1998, 1999; Bray et al., 2004). To this end, we compared fatty acid responsiveness in taste cells isolated from rats classified as either obesity-prone (fat-preferring) or obesity-resistant (Okada et al., 1992) to determine if the peripheral gustatory system responded differently to fatty acids. In these electrophysiological assays, DRK currents in TRCs from an obesity-prone strain (Osborne–Mendel; O–M) were significantly less responsive to *cis*-PUFAs than those from an obesity-resistant strain (S5B/Pl). That is, *cis*-PUFAs inhibited *less* of the total outward current through DRK channels in the O–M rats (Gilbertson et al., 1998) though the relative affinities of FAs were similar (e.g., EC_{50} in both strains were ~1 μM) (Gilbertson et al., 2005). From a cellular perspective, this would be predicted to exert a less robust signal from the TRC onto the gustatory afferent nerve (cf. Figure 3.1) in obesity-prone rats. Using

quantitative real-time PCR (qPCR) we determined that this disparity was due to a difference in expression of DRK channels (Gilbertson et al., 2005). These expression data coupled with our functional data showing the relative sensitivity to FAs in the three families of DRK channels (Shah et al., 2006) provided a framework for a model that suggested that the difference between the obesity-prone and -resistant rats was due, at least in part, to the fact that O–M rats expressed a lower ratio of FA-sensitive DRK channels:FA-insensitive (FA-s:FA-i) DRK channels. This ratio is approximately 1.0 in O–M rats and 5.7 in S5B/Pl rats (cf. Fig. 7 in Gilbertson et al., 2005). Based solely upon the data surrounding DRK expression and peripheral responsiveness to fatty acids in obesity-prone and -resistant rats, we developed a model that predicts an "inverse correlation" between peripheral responsiveness for fatty acids and dietary fat preference (Gilbertson et al., 1998, 1999, 2005). Basically, the reduced peripheral responsiveness to FAs in obesity-prone rats provides a weaker signal to the central nervous system which, in turn, uses this information to help coordinate patterns of food intake and dietary preference. Such inverse relationships between peripheral responsiveness and dietary intake have been shown for salt in rodent models (Curtis et al., 2001, 2008), consistent with this idea.

Our behavioral assay showing the ability of linoleic acid to enhance preference for a subthreshold saccharin concentration provided additional support for this model (Gilbertson et al., 2005). In this study, the linoleic acid was significantly more effective in enhancing the preference for saccharin in S5B/Pl (obesity-resistant) rats than in O–M rats, consistent with the electrophysiological data on fatty acid responsiveness in DRK channels described above. However, a direct comparison of taste thresholds for fatty acids in O–M and S5B/Pl rats conducted using the conditioned taste aversion paradigm produced contrary results (Pittman et al., 2008). Following formation of a conditioned taste aversion to linoleic acid, S5B/Pl and O–M rats were tested for their ability to avoid (i.e., detect) fatty acids. For all effective fatty acids, the obesity-prone rats were better able to detect and avoid fatty acids than their obesity-resistant counterparts. The conclusion from this series of experiments is that O–M rats have a lower threshold for fatty acid detection than S5B/Pl rats. Interestingly, female rats of both strains were significantly better at detecting and avoiding fatty acids than were males, suggesting that gender effects are significant in the chemoreception of dietary fat. This robust gender difference has not been explored at the cellular and molecular levels to date and appears warranted.

The explanation for this incongruity is not immediately clear. Based upon the cell-based assays and the ability of linoleic acid to enhance preference for sweet compounds, it was anticipated that the obesity-prone strain should be "less" responsive to the sensory cues for fatty acids. At the minimum, since the EC_{50} for FA-induced inhibition of DRK channels was identical across strains (Gilbertson et al., 2005), we would expect no difference between the two strains. Yet, following formation of a conditioned taste aversion to linoleic acid, O–M rats were "more" responsive than S5B/Pl rats to fatty acids. There are two possible explanations to these apparently conflicting results. First, there may be a disconnect between the ability of fatty acids to act as taste modulators (Figure 3.1) in behavioral assays (Gilbertson et al., 2005) and those behavioral assays in which fatty acids are applied

in the absence of other stimuli (Pittman et al., 2008). That is, the molecular and cellular differences reported in the taste systems between S5B/Pl and O–M rats have been at the level of DRK channels. It is plausible that the mechanisms involving fatty acid inhibition of DRK channels is more important for enhancing/modulating the response to other tastants and is less important as the primary receptive mechanism for fatty acids (see Section 3.3.1). Following this line of reasoning, a second possibility is that there may be additional mechanisms for the transduction of fatty acids that differ between S5B/Pl and O–M rats that result in the obesity-prone strain being more sensitive to dietary fatty acids. It should be noted that these two possibilities are not mutually exclusive. Contrary to our original hypothesis linking fatty acid chemosensitivity and dietary fat intake, this would imply that there is a positive relationship between taste sensitivity to fatty acids (as taste primers) and intake of dietary fat. Clearly, more research is needed to discriminate between the roles of fatty acids as primary taste stimuli and modulators of peripheral gustatory function.

3.3.1 The "Problem" with DRK Channels as Fatty Acid Receptors

As alluded to above, our data on the role of DRK channels in fatty acid transduction are consistent with there being dual roles for this pathway in the chemoreception of dietary fat. On one hand, these DRK channels play a critical role in the repolarization of taste cells following chemostimulation and their inhibition by fatty acids would inhibit the taste cell's ability to recover from stimulus-induced depolarizations. Stimulation of taste cells in the presence of fatty acids, like linoleic acid, produces greater and longer-lasting depolarizations than in the absence of fatty acids consistent with this interpretation (Gilbertson et al., 1997). Certainly, given the widespread distribution of KCNA5 in taste cells (Liu et al., 2005), we would expect that a significant number of taste cells would express this fatty acid-sensitive DRK channel and the signaling components for other sapid molecules that impart tastes like sweet or salty. We have termed this the modulatory role of fatty acid taste, which may be thought of as being analogous to, but less specific than, the ability of 5′ nucleotides, like inosine monophosphate (IMP), to enhance umami taste (Ninomiya et al., 2000; Kawai et al., 2002).

In addition to a modulatory role, the inhibition of DRK channels by fatty acids in the absence of other tastants (i.e., fatty acids as a taste primer; Figure 3.1) should result in depolarization of taste cells in much the same manner as K^+ channel block results in increased taste cell activity in response to other tastants (Kinnamon et al., 1988; Cummings and Kinnamon, 1992; Cummings et al., 1996; Herness et al., 1997). However, our electrophysiological data demonstrates that fatty acids act as open channel blockers, a finding consistent with that reported in other systems (Ordway et al., 1991; Petrou et al., 1995; Poling et al., 1996; Guizy et al., 2008). Because of this fact, for fatty acids to act as taste primers, it would require that these channels, or at least a portion of them, be open at normal taste cell resting potentials (–35 to –55 mV). We estimate that approximately 5% of DRK channels would be open within this voltage range (cf. Fig. 1B in Liu et al., 2005). While it is feasible that this proportion of open channels could depolarize a taste cell if DRK channels were

inhibited by fatty acids, the modest relative number of open DRK channels at rest led us to query if there were additional receptors upstream of fatty acid-sensitive DRK channels. That is, were there other receptors that played an important role in generating the receptor potential during fatty acid stimulation that contributed to the primary receptive mechanism for fat taste?

3.4 OTHER FATTY ACID RECEPTORS

3.4.1 CD36

At the time we began to explore the ability of fatty acids to activate mammalian TRCs, Fushiki and colleagues presented data showing the immunocytochemical localization of the fatty acid transporter CD36 in posterior taste buds in rodents (Fukuwatari et al., 1997) while it was apparently absent from nontaste epithelium. While the cellular events following fatty acid binding to CD36 were not clear at this time, it pointed out that there may be multiple mechanisms in use by the taste system to recognize and respond to components contained in dietary fat.

Since the initial identification of CD36 in the peripheral gustatory system, a number of studies have validated its expression and functional role in the chemoreception of fatty acids. Studies utilizing CD36-deficient mice have shown that CD36 plays a necessary, but not necessarily exclusive, role in the ability to discriminate fatty acids. Comparison of short- and long-term preference for linoleic acid revealed that mice lacking CD36 had no innate preference for long-chain fatty acid (LCFA) containing solutions in contrast to the normal preference found in wild-type mice (Fushiki and Kawai, 2005; Laugerette et al., 2005). Moreover, the preference for dietary fatty acids is dependent upon there being intact gustatory nerves (Gaillard et al., 2008) linking this behavioral response to activity within the gustatory system. Recent data has shown that free fatty acids are capable of generating a rise in intracellular Ca^{2+} in taste cells (Gaillard et al., 2008; Liu et al., 2008) at concentrations similar to those that inhibit DRK channels. Much, but certainly not all, of the LCFA-induced rise in Ca^{2+} is dependent upon CD36 (Gaillard et al., 2008). In an interesting study, Sclafani et al. (2007) demonstrated the required role of CD36 in preference for dilute fatty acid concentrations, but that at higher concentrations mice lacking CD36 were able to develop preferences for PUFA-rich soybean oil. The authors interpreted these data as evidence that CD36 plays a role in the taste component underlying fatty acid preference, but it was not essential for postoral conditioned fat preferences, despite the fact that CD36 is expressed throughout the enteric nervous system (Chen et al., 2001). An alternative explanation is that CD36 is not the primary receptor for fatty acids but helps facilitate the binding of fatty acids and their presentation to other fatty acid-activated proteins in the cell membrane (DRK channels, fatty acid-activated receptors). Thus, at low concentrations, CD36 may play a critical role in the presentation of fatty acids to other receptive proteins but at much higher concentrations, CD36-independent binding and activation at these other receptors can proceed.

Despite the impressive evidence that CD36 plays a critical role in the fatty acid transduction pathway (Abumrad, 2005; Calder and Deckelbaum, 2006; Mizushige et al., 2007), there is little evidence for the cellular mechanism of CD36 action in

mammalian taste cells. As alluded to above, it may serve as a primary receptor for fatty acids and translocate fatty acids to the cytoplasmic domain where they may activate intracellular signaling pathways. Pathways identified in taste cells to be activated by linoleic acid/CD36 interaction include the release of Ca^{2+} from intracellular stores and the phosphorylation of Src-protein-tyrosine kinases which lead to activation of store-operated channels (El-Yassimi et al., 2008). Alternatively, CD36 may be playing a role as more of a chaperone protein, facilitating the binding and delivery of fatty acids (Figure 3.1). Interestingly, a CD36 homolog in *Drosophila* acts in just the same fashion in the binding and delivery of volatile fatty acids in their correct orientation to pheromone receptors (Benton et al., 2007). Indeed, there is mounting evidence to suggest that the CD36 may not function primarily as a transmembrane transport protein for fatty acids (Doege and Stahl, 2006). The recent development of a functional cell line expressing CD36 may help to discern the role of this important protein in fatty acid taste transduction (Inagaki et al., 2008).

3.4.2 Fatty Acid-Activated GPCR

The deorphanization of several families of GPCRs has led to a significant advance in our understanding of peripheral and central fatty acid signaling (reviewed in Hirasawa et al., 2008). The cognate ligands for these receptors are several classes of fatty acids including the long-, medium-, and short-chain free fatty acids. These receptors are expressed in a wide variety of target tissues throughout the body where they play roles in a variety of cellular processes related to fat (i.e., fatty acid) signaling. While significant progress has been made in elucidating the signaling pathways activated by these receptors, there is little information to date concerning the fatty acid–GPCR interaction in terms of ligand-binding properties.

3.4.2.1 GPR40 Family

Of those previously orphan GPCRs that were identified and found to be activated by a variety of free fatty acids, three were members of a "family" of receptors, the GPR40 family which includes GPR40, GPR41, and GPR43 (Brown et al., 2003, 2005). Ligands for these GPCRs include medium- and long-chain fatty acids (GRP40 (Briscoe et al., 2003; Stewart et al., 2006)) and short-chain fatty acids (GPR41 and GPR43). Of these, GPR40 has been the best characterized to date. One of the cellular locations of GPR40 is in the pancreas where it has been functionally linked with insulin secretion (Itoh et al., 2003; Itoh and Hinuma, 2005; Tomita et al., 2005). Interestingly, a connection between GPR40 activation and DRK inhibition has been proposed to involve cAMP and protein kinase A (Feng et al., 2006). If true in TRCs, this could be a functional link between two putative fat receptors.

GPR41 and GPR43 are closely related receptors that are activated by propionate and short-chain carboxylic acids (Brown et al., 2003; Le Poul et al., 2003). Another member of this family, GPR42, appears to be a gene duplication of GPR41 (Brown et al., 2003). To date, comparatively little is known about these receptors. GPR41 has been found in adipocytes where it has been linked with the release of leptin (Xiong et al., 2004). GPR43, which has a broader distribution, has been shown to play a role, for example, in differentiation of leukocyte progenitors in hematopoietic cells

(Le Poul et al., 2003) and in colon function (Karaki et al., 2008; Tazoe et al., 2008). Both GPR41 and GPR43 couple to inositol 1,4,5-trisphosphate (IP_3) formation via G_i/G_o (GPR41 and GPR43) or G_q (GPR43 only).

3.4.2.2 GPR120

An additional fatty acid receptor has begun to be characterized in enteroendocrine cells of the small intestine. These cells (and a corresponding cell line, STC-1) share many of the same receptors and signaling components as mammalian TRC and have been called "taste cells of the gut" (Raybould, 1998; Rozengurt and Sternini, 2007; Sternini et al., 2008). Commonalities include expression of both the sweet (Dyer et al., 2005, 2007) and bitter receptor families as well as gustducin (Wu et al., 2002; Chen et al., 2006). These cells also express the receptor, GPR120, a GPCR that is activated by LCFAs. Activation of GPR120 in enteroendocrine cells has been shown to induce release of the hormone, glucagon-like peptide-1 (GLP-1) (Hirasawa et al., 2005) as well as inhibit apoptosis induced by serum deprivation (Katsuma et al., 2005).

3.4.2.3 GPR84

Very recently, a novel GPCR has been deorphanized, which is activated by medium-chain fatty acids (C9–C14) and not closely related to any of the previously identified FA-activated GPCRs (Venkataraman and Kuo, 2005; Wang et al., 2006; Bouchard et al., 2007). While its tissue distribution has not been well characterized, it has been suggested that it has a role in regulation of immune cells (Venkataraman and Kuo, 2005). Like some of the other FA-activated GPCRs, GPR84 activation mobilizes Ca^{2+} via an activation of the pertussis toxin-sensitive G_i/G_o pathway.

3.4.2.4 GPR Expression in Taste Cells

Few studies have yet focused on the identification and characterization of fatty acid-activated GPCRs (FA-GPCRs) in mammalian TRCs. Matsumura et al. (2007) have identified the presence of GPR120, but not GPR40, in taste tissue using RT-PCR. Our laboratory has used RT-PCR as the first approach to try and determine the expression profile of all known FA-GPCRs in taste and trigeminal (somatosensory) tissue in rodents. Our data are consistent with a variety of FA-GPCRs being expressed in the taste system with significant differences between the anterior (fungiform papillae) and posterior (foliate and circumvallate papillae) tongue. Table 3.1 summarizes the expression of FA-GPCRs (and CD36) in the taste and somatosensory systems in rodents.

Preliminary studies from our laboratory are consistent with a functional role for FA-GPCRs in fatty acid-induced increase of intracellular calcium in rodent taste cells. Linoleic acid-induced changes in $[Ca^{2+}]$ in rodent taste cells are blocked by inhibitors of G protein activation, like GDP-β-S, a nonhydrolyzable analog of GDP (Liu et al., 2008). Despite positive preliminary evidence, it is clear that the role of FA-GPCRs in the taste transduction of fatty acids is an open question. At present, however, the only available knockout model for FA-GPCRs remains GPR40 (Latour et al., 2007; Brownlie et al., 2008; Lan et al., 2008) and there have been no published reports of the taste phenotype of these mice.

TABLE 3.1
Expression of Putative Fatty Acid Receptors in Taste Cells

Cell Type	fa-s DRK	CD36	GPR40	GPR41	GPR43	GPR84	GPR120
Fungiform TRCs	+	+	(–)	–	+	(–)	+
Posterior TRCs[a]	+	+	+	+	+	+	+
Trigeminal neurons	+	+	+	+	+	+	+

Expression was determined by RT-PCR in C57Bl/6 mice. A + sign indicates the target was reliably found by PCR whereas (–) indicates this target was not found.

[a] Posterior TRCs includes both foliate and vallate taste buds though they were analyzed separately and found to be similar in their expression. Trigeminal neurons are shown for comparison. fa-s DRK, fatty acid-sensitive delayed rectifying K channels.

3.5 FAT TASTE TRANSDUCTION: A UNIFYING MODEL

There is a general consensus that the peripheral gustatory system is responsive to the chemosensory cues for dietary fat. While the role of "fat taste" as a primary taste, like sweet, bitter, salty, sour, and umami, and/or as a taste modulator remains to be elucidated, there is solid evidence pointing to the role of free fatty acids as the prototypical fat stimulus. While early research pointed to the importance of *cis*-PUFAs (essential fatty acids) as gustatory stimuli (Gilbertson, 1998b, 1999; Gilbertson and Boughter, 2003), the recent identification of additional receptive proteins and cutting edge behavioral assays reveal that the taste system is likely to respond to a wider variety of fatty acids. Nonetheless, the vast majority of the current research has focused on the *cis*-PUFAs, particularly linoleic acid. Clearly, to further our understanding of the peripheral cues for dietary fat will require similar emphases being placed on the effects of other ligands for the various fatty acid-receptive proteins identified (cf. Table 3.1).

Our understanding of the transduction pathway for fatty acids is far from complete. While DRK channels have been implicated in this pathway, the role of these fatty acid-sensitive channels remains unclear, especially in light of the emerging data that suggests that there are FA-activated GPCRs and the fatty acid-binding protein, CD36, may also contribute to this signaling cascade. The functional role of DRK channels, like KCNA5, in fatty acid taste transduction is hampered by their ubiquitous expression in peripheral tissues and, hence, the lack of an available, viable knockout model. The generation of a taste-specific KCNA5 knockout or taste-specific knockdown of this channel using RNA interference would answer lingering questions regarding the functional importance of fatty acid-sensitive DRK channels.

Conversely, the importance of CD36 in the transduction and recognition of fatty acids is unequivocal. Data on CD36 knockout mice using a variety of approaches (Laugerette et al., 2005; Sclafani et al., 2007; El-Yassimi et al., 2008; Gaillard et al., 2008) have consistently demonstrated that this protein is important for fatty acid signaling in the gustatory system. How CD36 directly contributes to the signal transduction pathway, however, is an open question. While it may act to transport fatty acids across the taste cell membrane where they could directly affect intracellular

signaling, an equally likely scenario implies that it may act as a binding or chaperone protein to facilitate fatty acid interactions with receptors or channels. Specific experiments designed to distinguish among these possibilities are critical to determine the role of this important protein.

The identification of multiple subtypes of fatty acid-activated GPCRs in the taste system, each with unique ligand profiles, promises to further expand our understanding of the mechanisms surrounding the "taste of fat." While there has been precious little functional data to date on these receptors and their role in taste, they may provide the explanation for the ability of the taste system to respond to short- and medium-chain saturated fatty acids, such as lauric acid (Pittman et al., 2008).

In about a decade, we have gone from the generally accepted notion that fat is tasteless to one that the ability of fatty acids to elicit cellular and behavioral gustatory responses is well established. Further, there appears to be a wealth of putative fatty acid-receptive proteins including CD36, fatty acid-sensitive DRK channels and fatty acid-activated GPCRs. Given that few DRK channels are open at resting potentials in taste cells and that fatty acids act as open channel blockers, we have long proposed that there may be something upstream (other FA or taste receptors) of these FA-sensitive channels to enable their opening and subsequent inhibition by fatty acids. This fact, coupled with the distinct possibility that CD36 is playing a role as a binding protein and not as a transporter (Benton et al., 2007), leads us to hypothesize a single model for fatty acid transduction involving these three distinct fatty acid-responsive proteins based upon available data. As illustrated in Figure 3.2, our working model for fatty acid (linoleic acid) transduction involves an initial binding of free fatty acids, generated by the action of lingual lipase or available in fat-containing food, to CD36. In this scheme, the role of CD36 would be to bind and orient linoleic acid for presentation to fatty acid-activated GPCRs, such as GPR120, and fatty acid-sensitive DRK channels. It is equally plausible that CD36 facilitates the transport of linoleic acid across the membrane where it may interact with GPR120 (the binding site for fatty acids on FA-GPCRs is currently unknown). On the other hand, linoleic acid only inhibits DRK from the extracellular face of the channel (Gilbertson et al., 1997; Liu et al., 2005). The activation of GPR120, in turn, leads to the production of the second messenger, phospholipase C (Fukunaga et al., 2006; Iakoubov et al., 2007) and the eventual release of Ca^{2+} from intracellular stores. Matsumura and colleagues have shown that expression of GPR120 overlaps with expression of PLCβ2 and α-gustducin, lending support to this idea (Matsumura et al., 2009). As with other taste complex stimuli (Roper, 2007), this release of Ca^{2+} is coupled to activation of store-operated channels, like Ca-release-activated cation (CRAC) channels or TRPM channels. Interestingly, a recent paper suggests that specific *cis*-PUFAs can directly activate TRPM5 intracellularly (Oike et al., 2006), which may represent an additional pathway independent of FA-GPCRs, linking CD36 and TRPM5 directly. These transduction channels allow the depolarization and development of the receptor potential during fatty acid stimulation. This depolarization, in turn, would be expected to open DRK channels of the KCNA and KCNB families (i.e., fatty acid sensitive) that can be blocked by linoleic acid to enhance and prolong the depolarization. The ratio of fatty acid-sensitive:fatty acid-insensitive channels would help determine the overall magnitude of the response which could be signaled directly onto the afferent nerve or to output cells within the taste bud.

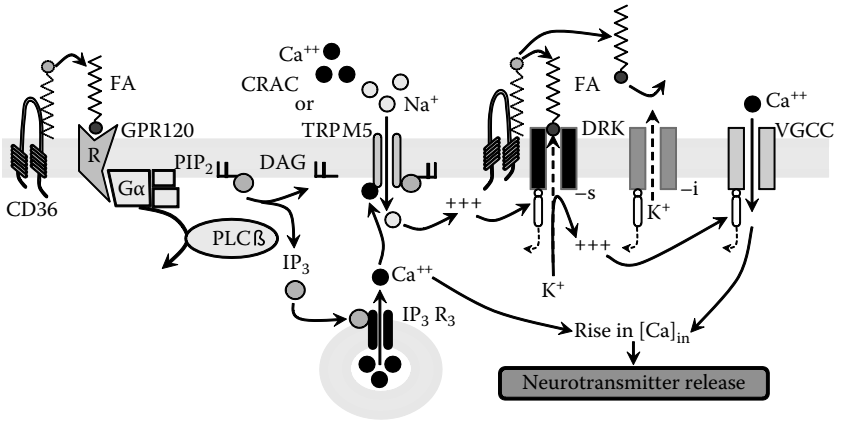

FIGURE 3.2 (See color insert following page 166.) Putative transduction pathway for fatty acids in taste and trigeminal cells. Fatty acids (FA) delivered by the binding protein CD36 activate specific G protein–coupled receptors (like GPR120) to initiate a transduction cascade that in turn produces a second messenger leading to release of calcium from intracellular stores and activation of a store-operated ion channel (CRAC or TRPM-like channel) to produce a receptor potential. This potential opens fatty acid-sensitive DRK channels that are subsequently blocked by FA, leading to an enhanced and prolonged depolarization. The ratio of FA-sensitive:FA-insensitive DRK channels helps to determine the magnitude of the overall chemosensory response to FA stimulation. This depolarization is the impetus for the eventual release of neurotransmitter onto gustatory afferent fibers. It is not known whether the pathway downstream of DRK channels is in the same cell as the upstream elements or if this part of the pathway involves cell-to-cell signaling.

While speculative, this model presents an attractive and testable synthesis of the current data surrounding fatty acid transduction in mammalian taste cells. The specific relationships between FA-GPCRs, CD36, and FA-sensitive DRK channels remain to be elucidated as do the specifics of the pathways activated by other receptor subtypes. Nonetheless, over the past decade it has become apparent that taste cells can and do respond to dietary fat and that this response is translated into the animal's (including human's) behavior. The understanding of how the gustatory system and the body as a whole recognizes and responds to dietary fat holds promise for not only the design of fat substitutes but also the identification of putative targets in the fight against obesity through the control of dietary fat intake.

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4 Role of the Gustatory System in Fatty Acid Detection in Rats

David W. Pittman

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4.1 INTRODUCTION

The prevalence of obesity has provided a strong incentive to understand the motivation to consume high-fat foods. Taste palatability of high-fat food is cited as the primary influence in the over consumption of dietary fat (Drewnowski, 1997; Drewnowski and Greenwood, 1983; McCrory et al., 1999, 2000; Warwick and Schiffman, 1990) and there is a strong positive correlation between obesity and daily high-level intake of dietary fat (McCrory et al., 1999; Miller et al., 1990, 1994). Traditionally, the sensory perception of dietary fat during ingestion has been characterized in terms of textural and postingestive effects. Certainly, dietary fat contributes to the mouthfeel of foodstuffs and can act as a positive reinforcer through fatty acid receptors found in the digestive tract that stimulate pathways leading to the natural reward centers of the brain. However, across the last decade, accumulating evidence from both molecular and behavioral research has begun to challenge the traditional perspectives on dietary fat perception by introducing the concept of chemoreception of dietary fat within the oral cavity, specifically through the gustatory system, as providing immediate and selective signals during dietary fat consumption. Like humans, rodents demonstrate a robust preference for high-fat foods (Greenberg and Smith, 1996; Smith et al., 2000; Warwick and Synowski, 1999; Warwick et al., 1990) and represent the most typical animal model in which the reception, transduction, and neural signaling of the peripheral gustatory system has been explored. This chapter reviews the behavioral evidence supporting a role of the gustatory system in the chemoreception of fatty acids, the principle chemical component of dietary fat.

4.2 INNATE PREFERENCE FOR DIETARY FAT AND FATTY ACIDS

Corn oil and mineral oil are the two prototypical oils respectively representing nutritive and nonnutritive stimuli in research examining dietary fat intake. Sham-feeding paradigms using a gastric fistula to eliminate postingestive feedback are useful in the analysis of orosensory influences on consumption. For example, sham-fed rats unanimously preferred corn oil over mineral oil in two-bottle forced choice tests (Mindell et al., 1990). The salient orosensory cue underlying this innate dietary fat preference does not appear to be textural in nature as rats systematically preferred corn oil to control solutions containing either mineral oil or xanthan gum when the viscous stimuli were presented either in isolation or mixed with other taste stimuli (Elizalde and Sclafani, 1990; Greenberg and Smith, 1996; Mindell et al., 1990; Ramirez, 1992; Smith, 2004; Smith et al., 2000; Takeda et al., 2001). When an aversion was conditioned to a solution of glucose, saccharin, and corn oil, the aversion did not generalize to either sweet stimulus, rather rats selectively avoided all test solutions containing the dietary fat during brief-access (30 s) trials which effectively eliminated postingestive feedback signals (Smith, 2004). Collectively, behavioral research suggests that a salient, nontextural, orosensory cue contributes to the detection and subsequent innate preference for dietary fat.

To effectively explore the chemoreception of dietary fat, we must first identify the constituent chemicals of dietary fat that could act as stimuli within the oral cavity. Vegetable oils representing prototypical dietary fats consist of triacylglycerides, the combination of three fatty acids with an ester. Linoleic acid (52%) and oleic acid (31%) are the principal fatty acids found in corn oil, the quintessential vegetable oil (Gunstone, 1995). Analysis of triacylglyceride hydrolysis by lingual lipase in the rodent oral cavity has shown that millimolar concentrations of dissociated fatty acids can be produced within 1–5 s of exposure to the lingual epithelium (Kawai and Fushiki, 2003). In support of orosensory detection of fatty acids as opposed to triacylglycerides, two-bottle forced choice tests show increased preferences for fatty acids such as oleic acid over triacylglycerides such as triolein, an oleic acid triacylglyceride (Fukuwatari et al., 2003; Tsuruta et al., 1999). Furthermore, the presence of orlistat, a potent lipase inhibitor, significantly reduces the preference for triacylglycerides but does not affect the preference for fatty acids themselves (Kawai and Fushiki, 2003). Conditioned taste aversions (CTAs) to a sucrose–corn oil mixture result in the generalized avoidance of a nonviscous sucrose–linoleic acid mixture, but do not generalize to a viscous sucrose–mineral oil mixture (Smith et al., 2000) providing additional evidence that the likely salient cue for fat detection is the chemoreception of fatty acids as opposed to textural cues. The identification of fatty acids, particularly linoleic and oleic acid, as the most likely candidates for the chemoreception of dietary fat has provided the benefit of conducting research using isolated, minute quantities of the fatty acids as chemical stimuli instead of complex lipids. Using fatty acids as stimuli instead of complex lipids allows discrete control over the concentration of the fatty acid stimulus and minimizes textural cues such as lubricity and viscosity, thus eliminating the necessity of vehicle solutions to mask textural cues.

One of the first studies to support the chemoreception of fatty acids by the gustatory system was conducted by Tsuruta and colleagues (1999) using short-term, two-bottle, forced-choice preference tests to demonstrate that rats prefer fatty acids to control solutions when textural cues were masked by xanthan gum in test and control solutions. Additionally, long-chain fatty acids such as linoleic acid were more preferred than medium-chain acids such as oleic acid suggesting that rats could detect and discriminate between fatty acid chemicals (Tsuruta et al., 1999). A similar innate preference for linoleic acid has also been confirmed in the C57BL/6J mouse strain (Gaillard et al., 2008; Laugerette et al., 2005; Sclafani et al., 2007). A specific role for gustatory sensitivity to fatty acids was first supported by evidence from the research of Fukuwatari and colleagues (2003) examining fatty acid preferences in anosmic rats. Anosmia was confirmed through conditioned odor aversion tests in rats having received intranasal zinc sulfate. Even though anosmia increased the threshold for demonstrating a preference for oleic acid suggesting a role of olfaction in the multimodal detection of fatty acids, anosmic rats were still able to detect and prefer concentrations of oleate ≥0.5% even when masked by the textural agent, xanthan gum. Thus, this research confirmed the contribution of a nontextural, nonolfactory orosensory cue, likely gustation, as an immediate sensory signal capable of influencing fatty acid consumption (Fukuwatari et al., 2003).

4.3 FATTY ACID DETECTION BY RATS

In addition to the innate preferential consumption of fatty acids, the orosensory detection of fatty acids has also been shown to stimulate the cephalic phase and secretion of pancreatic enzymes. Similar to innate preferences only for selective fatty acids, oral stimulation with linoleic and oleic acid enhanced pancreatic secretions in esophagostomized rats while oral application of long-chain fatty acid derivatives and middle-chain fatty acids did not elicit a pancreatic response (Hiraoka et al., 2003; Laugerette et al., 2005). Similar pancreatic responses elicited by oral stimulation with linoleic acid have also been replicated in the C57BL/6J mouse strain (Gaillard et al., 2008; Laugerette et al., 2005). The rapid cephalic response within 5 min of fatty acid stimulation suggests that orosensory cues of fatty acid ingestion not only contribute to enhanced consumption of dietary fat but also initiate digestive responses in preparation for fatty acid ingestion.

CTA methodology is a simple yet powerful paradigm used to identify the detection of sensory stimuli through pairing a conditioned stimulus (CS), typically a tastant, with an innately aversive stimulus termed the unconditioned stimulus (US). Often, a single pairing of the CS and US is sufficient for an association to be formed between the CS and the adverse effects created by the US. This association results in future avoidance of the CS until extinction of the learned association occurs following subsequent repetitive, unpaired exposures of the CS. After pairing the ingestion of a chemical stimulus as the CS with a known aversive US, such as the intraperitoneal injection of LiCl which induces gastric malaise in rodents, it is possible to test the ability of rats to detect and avoid the presence of the CS in subsequent behavioral testing. Dr. James C. Smith and colleagues (2000) were the first to employ CTA methodology to assess the detection of fatty acids in a paramount study examining

the similarity between corn oil and its main fatty acid component, linoleic acid. Following conditioned aversions to corn oil, female rats avoided 22 μM linoleic acid in a 1 h, two-bottle preference test and conversely, following a conditioned aversion to 22 μM linoleic acid, female rats avoided corn oil in a similar preference test (Smith et al., 2000). This experiment suggested that rats could detect linoleic acid and that linoleic acid and corn oil shared similar sensory perceptions such that avoidance of one stimulus generalized to the other. However, due to the 1 h testing duration, contributions of postingestive influences on the detection and avoidance of the fatty acid stimuli could not be discounted.

Recognizing the potential of CTAs to characterize the gustatory detection of fatty acids, we began a series of psychophysical experiments designed to measure the detection threshold and similarity between various fatty acids. Our first experiments utilized 1 h, two-bottle preference tests following a single pairing of either 88 μM linoleic acid or oleic acid dissolved in 5 mM ethanol as the CS with either a LiCl (US) or saline (control) injection. After conditioning a taste aversion, during the 1 h preference test session, male rats were able to detect and avoid both linoleic acid and oleic acid at concentrations ≥66 μM (McCormack et al., 2006). Using unesterified free fatty acids as taste stimuli required dissolution in a low concentration of ethanol (5 mM), therefore there was the possibility that an interaction between the free fatty acids and ethanol or another concomitant tastant such as sodium ions in saliva was acting as the detectable cue allowing avoidance of the CS solutions during the testing phase. However, in follow-up testing, when an aversion was conditioned to 88 μM linoleic acid in dissolved 5 mM ethanol, there was no generalized avoidance of either ethanol alone or NaCl concentrations in two-bottle preference testing between these solutions and appropriate controls (McCormack et al., 2006). These tests provided strong evidence that rats were demonstrating avoidance of the CS solutions based on cues from the free fatty acids themselves as opposed to interactions between the free fatty acids and the vehicle solution or saliva. In addition to the unesterified form of free fatty acids, fatty acids can also bind to a sodium ion to form sodium salt fatty acids such as linoleate and oleate for linoleic acid and oleic acid, respectively. Although linoleate and oleate are aqueous in water, thus eliminating the need to add ethanol to the taste stimuli, there is the additional presence of sodium ions in the fatty acid solutions. However, the presence of these sodium ions does not introduce a confounding taste variable as the concentration of sodium ions in the aqueous fatty acid solutions is in the subthreshold, micromolar concentration range far below the millimolar concentration of sodium necessary for salt detection in rodents (Slotnick et al., 1991). To ensure that rats respond equivocally to sodium salt fatty acids and unesterified free fatty acids dissolved in ethanol, we conditioned aversions to both linoleic and oleic acid in ethanol and linoleate and oleate solutions. In subsequent 15 min, two-bottle preference tests, rats demonstrated similar avoidance between the free fatty acids and sodium salt fatty acids regardless of the form of the fatty acid CS (McCormack et al., 2006).

Our series of two-bottle preference tests following a CTA to linoleic or oleic acid provided firm evidence that rats can both detect fatty acids and avoid consumption of fatty acids in short-term tests; however, measuring the amount of solution consumed during a 15 min, two-bottle test session did not allow discrete assessment of licking

behaviors nor could behavioral variables associated with the use of odor cues to avoid consumption of the fatty acids be measured. Additionally, although we believed that 15 min was probably not sufficient time to allow postingestive detection of fatty acids to influence consummatory behavior, we could not exclude postingestive cues as a potential contributor to the avoidance of fatty acids. Therefore, we designed a set of experiments measuring the licking responses to fatty acid stimuli during brief-access testing in an attempt to further isolate the orosensory contributions to fatty acid detection by rats. In these brief-access tests, fatty acid stimuli were presented to the rats in trials ranging from 8 to 30 s in duration following either a single CTA pairing or three consecutive days of CTA pairings of 88 μM linoleic or oleic acid with either saline or LiCl injections. This series of brief-access tests following 1 or 3 conditioning days revealed significant effects of the method of conditioning as well as the method of testing on the ability of the rats to demonstrate a conditioned avoidance to either linoleic or oleic acid.

Following a single conditioning trial using 88 μM linoleic acid, male rats demonstrated avoidance of linoleic acid at 88 and 176 μM during testing with 8 s stimulus durations; however, when the stimulus duration was extended to 30 s, rats were able to demonstrate avoidance of linoleic acid at concentrations as low as 44 μM (Pittman et al., 2006a, 2007). The generalized avoidance of oleic acid following a CTA to linoleic acid showed a similar effect of ultrashort versus brief-access testing with rats not demonstrating a generalized avoidance of oleic acid (up to 176 μM) in 8 s trials but avoiding both 88 and 176 μM oleic acid during testing with 30 s trials (Pittman et al., 2006a, 2007). In contrast to the avoidance of oleic acid in 15 min, two-bottle preference testing, following a single conditioning trial using 88 μM oleic acid male rats did not show conditioned avoidance of oleic acid at any concentration (44, 88, 176 μM) during testing with either 8 or 30 s stimulus trials (Pittman et al., 2006a, 2007).

Troubled by the incongruence of our preference testing and brief-access testing results for the detection of oleic acid, we considered two hypotheses: (1) oleic acid was not detectable through orosensory cues generated during brief-access trials; (2) a sufficient aversion was not conditioned with a single pairing of 88 μM oleic acid with a LiCl injection. Given that rats showed a generalized avoidance of oleic acid in 30 s stimulus trials following a CTA to linoleic acid, we believed that oleic acid was, in fact, detectable during brief-access testing but the weaker sensory saliency of oleic acid may have resulted in a weaker conditioned aversion than when linoleic acid was the CS. To test this hypothesis, we increased the conditioning phase of the experiment to three consecutive daily pairings of 88 μM oleic acid with LiCl injections prior to brief-access testing. Consistent with the results from our previous two-bottle preference testing of oleic acid, rats showed significant decreases in their consumption of the CS oleic acid on conditioning days 2 and 3 (see Table 4.1). In support of our hypothesis, following three consecutive CTA pairings, rats robustly avoided oleic acid at concentrations ≥50 μM in testing using 15 s stimulus durations (Pittman et al., 2007). Collectively, these experiments suggested that oleic acid was a weaker orosensory stimulus than linoleic acid supporting Tsuruta's prior claims that rats show stronger innate preferences for linoleic acid than oleic acid (Tsuruta et al., 1999).

TABLE 4.1
Oleic Acid Consumption (Mean Grams ± SEM) during Conditioning Trials

	Conditioning Day 1	Conditioning Day 2	Conditioning Day 3
LiCl	13.6 ± 0.7	6.2 ± 0.9	4.2 ± 0.4
NaCl	13.3 ± 1.3	14.5 ± 1.5	12.7 ± 0.7
Statistics	$t_9 = 0.3$, $p = 0.74$	$t_9 = 3.8$, $p < 0.01$	$t_9 = 10.1$, $p < 0.01$

Having characterized the responsiveness to fatty acids based on orosensory stimulation in male Sprague-Dawley rats, we expanded our research to assess the responsiveness to linoleic and oleic acid in female Sprague-Dawley rats as well as strains of obesity-prone and obesity-resistant rats. Regardless of strain, female rats consistently showed greater responsiveness to the fatty acid stimuli compared to male cohorts. Following 3 days of conditioning to 88 μM linoleic acid, the threshold for avoiding linoleic acid in brief-access testing using 15 s trials was 50 μM for male Sprague-Dawley rats and 20 μM for female Sprague-Dawley (Pittman et al., 2007). In follow-up tests, 4 days after the final conditioning day, females showed less evidence of CTA extinction avoiding 50 μM linoleic acid while the male threshold for avoidance had increased to 75 μM (Pittman et al., 2007). Female rats also showed less extinction of aversions conditioned to 88 μM oleic acid than their male counterparts by avoiding 75 μM oleic acid 4 days following conditioning compared to the male threshold for avoidance of 100 μM oleic acid. The results from our brief-access tests provided evidence that differences in the orosensory detection of fatty acids likely underlie an earlier report that female rats could detect lower concentrations of linoleic acid than male rats during 10 min preference testing to assess conditioned avoidance (Stratford et al., 2006).

Differences in dietary preferences for fat have been documented between obesity-prone (Osborne–Mendel) and obesity-resistant (S5B/Pl) rat strains such that obesity-prone rats prefer high-fat diets while obesity-resistant rats prefer high-carbohydrate diets (Gilbertson et al., 1998). Differences in the chemoreceptive sensitivity of taste receptor cells to fatty acids had been proposed as a contributing factor in these behavioral preferences for dietary fat. *In vitro* electrophysiological recordings showed that fatty acids produce greater inhibition of delayed rectifying potassium (DRK) currents in taste receptor cells collected from obesity-resistant rats than cells harvested from the obesity-prone rats (Gilbertson et al., 2005). Using our CTA methodology, we also found significant differences in the ability to detect and avoid fatty acids between the obesity-prone and obesity-resistant strains during brief-access testing. Within each sex, the obesity-prone rats showed conditioned avoidances to lower concentrations of linoleic acid compared to the obesity-resistant rats (Pittman et al., 2008). The obesity-prone rats also showed greater generalized avoidance of oleic acid than the obesity-resistant rats (Pittman et al., 2008). Within each strain of rat, the female rats consistently avoided lower concentrations of linoleic acid than their male cohorts (Pittman et al., 2008) confirming our results from tests of Sprague-Dawley rats. Furthermore, the female rats

showed greater generalized avoidance of oleic acid as well as lauric acid, a fatty acid that male rats have not been able to detect (Pittman et al., 2008). Our results confirm an increased sensitivity of female rats to the presence of fatty acids and suggest that in both male and female rats, the obesity-prone strain may be more sensitive to detecting fatty acids than the obesity-resistant strain. These recently published findings represent the initial exploration of differences in fatty acid detection between males and females within specialized strains of rats. While our data suggest the potential for genetic influences on the ability to detect fatty acid, more research is necessary to fully characterize the differences in sensitivity and responsiveness to fatty acids between sexes and strains of rats and furthermore, to relate those behavioral differences to physiological mechanisms.

Through brief-access tests following conditioned avoidances to fatty acids, we had clearly identified the ability of rats to detect and avoid fatty acids on the basis of orosensory cues. We postulated that the salient orosensory cue was likely a gustatory signal based on the minimal textural cues associated with micromolar quantities of fatty acids and the lack of evidence to support a role of olfactory-mediated detection of the fatty acids such as differences in the latencies to lick between avoided and nonavoided stimuli during our testing sessions. If we entertain the theory that rats use a gustatory signal to detect fatty acids in our CTA experiments, then there is the question of whether gustatory signals induced by fatty acids are perceptually unique or perceptually similar to known taste qualities such as sweet, sour, bitter, or salty perceptions. In a previously unpublished study, we attempted to address this question by assessing the ability of a CTA to linoleic acid to produce generalized avoidance of prototypical taste qualities. Water-restricted, adult, male Sprague-Dawley rats consumed 88 µM linoleic acid followed by an intraperitoneal injection of either 150 mM LiCl ($n = 27$) or saline ($n = 27$) at a dosage of 20 mL/kg body weight. On the following day, the licking responses of the rats were measured in an MS-160 Davis Rig (DiLog Instruments, Tallahassee) during randomly ordered, 30 s stimulus presentations of 88 µM linoleic acid, 0.2 M sucrose, 0.2 M NaCl, 0.01 M citric acid, 0.0002 M qunine–HCl, and water. All responses were standardized to the mean number of licks during water trials (licks stimulus/licks water). A mixed factorial ANOVA compared the effect of the between-subject variable, injection, and the repeated measures variable of stimulus on licking behavior. Post hoc *t*-tests identified significant differences between the licking responses of the injection groups to specific stimuli. As expected, the rats responded differently to the fatty acid, sweet, salty, sour, and bitter stimuli with a main effect of stimulus type ($F_{(4,208)} = 38.1$, $p < 0.01$). There were both, a main effect of injection ($F_{(1,52)} = 5.2$, $p < 0.02$) and an interaction between stimulus and injection ($F_{(4,208)} = 6.6$, $p < 0.01$) indicating that the LiCl and saline-injected animals respond differently to some but not all of the stimuli. As shown in Figure 4.1, a CTA to linoleic acid was formed by the rats receiving the LiCl injection compared to the saline-injected rats ($t_{52} = 4.2$, $p < 0.01$). There were no other significant differences between the licking responses of the injection groups to the sweet, salty, sour, or bitter stimuli (all $t_{52} < 1.7$, $p > 0.09$). The lack of a generalized avoidance between linoleic acid and any of the representative tastants suggests that orosensory fatty acid detection is perceptually independent from sweet, salty, sour, or bitter taste qualities.

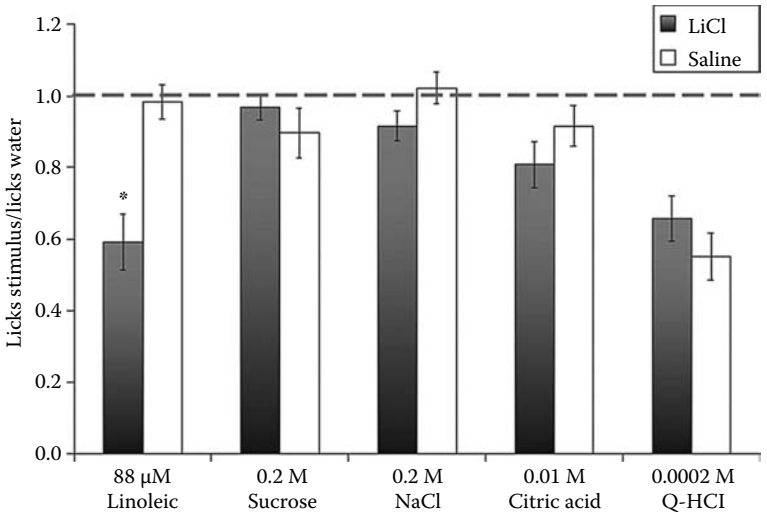

FIGURE 4.1 Mean (±SEM) standardized licking responses to linoleic acid, sucrose, NaCl, citric acid, and quinine (Q-HCl) compared between groups receiving a CTA to linoleic acid (LiCl) and control groups (saline). All responses are relative to the mean licking response to water (1.0). Stars represent significant ($p < 0.01$) differences in licks between injection groups.

An alternative hypothesis to the gustatory detection of fatty acids following a CTA could be the use of olfactory cues to detect and avoid fatty acids during subsequent brief-access testing. In a preliminary, yet-to-be published study, we have attempted to test whether or not olfactory cues were sufficient to allow detection and avoidance of a stimulus tube during brief-access tests following a CTA to linoleic acid. Water-restricted, adult, female Long–Evans rats received two consecutive conditioning days receiving either 150 mM LiCl ($n = 5$) or saline ($n = 6$) injections (20 mL/kg body weight) 20 min after consumption of 100 μM linoleic acid. Consumption of the linoleic acid CS did not differ between injection groups on day 1 (saline group, 9.5 ± 0.7 g; LiCl group, 9.2 ± 0.8 g); however, CS consumption significantly decreased for the LiCl-injected group compared to the saline-injected group on conditioning day 2 (saline group, 10.2 ± 0.8 g; LiCl group, 4.1 ± 0.5 g; $t_9 = 6.8$, $p < 0.01$), indicating successful formation of a CTA to linoleic acid. In order to assess the effect of fatty acid olfactory cues on avoidance of stimuli during brief-access testing, cotton gauze soaked in either distilled water or 100 μM linoleic acid was placed directly above and below each stimulus sipper tube in the MS-160 Davis Rig gustatory behavioral apparatus. Using this combination of stimulus solutions paired with fluid-soaked gauze to generate olfactory cues, we measured the licking responses to the following combinations of stimuli: (1) water stimulus + water olfactory cue, (2) water stimulus + linoleic acid olfactory cue, (3) linoleic acid stimulus + water olfactory cue. Each stimulus combination was presented once in five sets of randomly-ordered stimulus blocks. Stimulus presentations were 15 s in duration with 15 s interstimulus intervals between trials. Rats were given 30 s to initiate the first lick of a trial before the trial period was terminated.

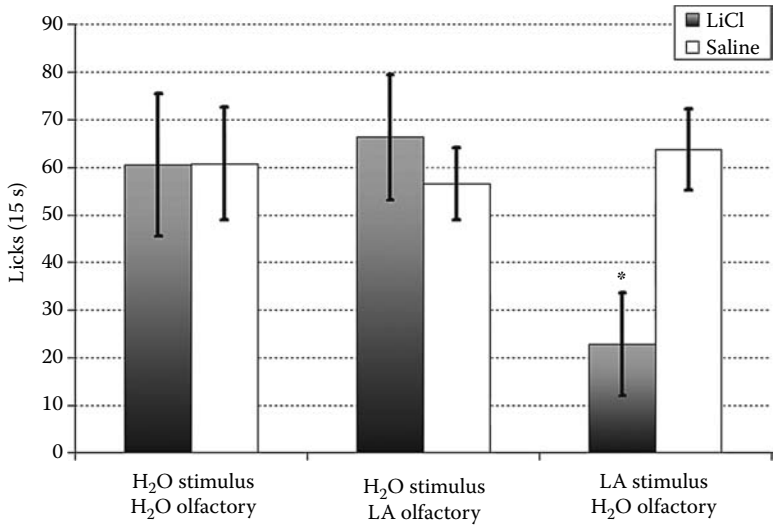

FIGURE 4.2 Mean (±SEM) licks during 15 s trials to stimulus tubes containing linoleic acid or water paired with either water olfactory cues or linoleic acid olfactory following a CTA to linoleic acid. Stars represent significant ($p < 0.01$) differences in licks between injection groups.

The licking responses for each stimulus combination were averaged across the five sets of stimulus blocks and are shown in Figure 4.2. A mixed factorial ANOVA revealed no overall effect of injection group but a significant interaction between the stimulus type and injection group ($F_{(2,18)} = 13.428$, $p < 0.01$) meaning that overall, the LiCl- and saline-injected animals did not treat all stimuli differently, rather, the injection condition affected the licking response to specific stimuli. Posthoc *t*-tests revealed a significant difference between the licking responses to the linoleic acid stimulus + water olfactory cue combination ($t_9 = 3.3$, $p < 0.01$) for LiCl-injection ($M = 22.8$) and saline-injection ($M = 63.8$) groups. There was no significant difference in the licking responses to the water stimulus + linoleic acid olfactory cue between the LiCl-injected and saline groups. Within the LiCl-injected group, the licking response to the linoleic acid gustatory stimulus ($M = 22.8$) was significantly below the licking response to both water alone ($M = 60.5$, $t_8 = 2.3$, $p < 0.05$) and the water stimulus paired with linoleic acid olfactory cues ($M = 66.3$, $t_8 = 2.9$, $p < 0.02$).

In all the brief-access tests conducted in our laboratory, we assess the latency until the first lick in each trial as a measurement of olfactory influences on consummatory behavior. If olfactory cues are being used to detect and avoid conditioned stimuli, then longer latencies until the first lick would be expected for stimuli with lower lick responses than stimuli that are not avoided. Across all of our behavioral experiments, we have consistently measured no difference in the latency until the first lick between experimental groups. In the experiment described above, rats were given a 30 s window in which they must lick the stimulus tube in order to initiate a trial. Figure 4.3 displays the mean latencies until first lick across the three stimulus combinations for the two injection groups. Similar to the findings in our previous

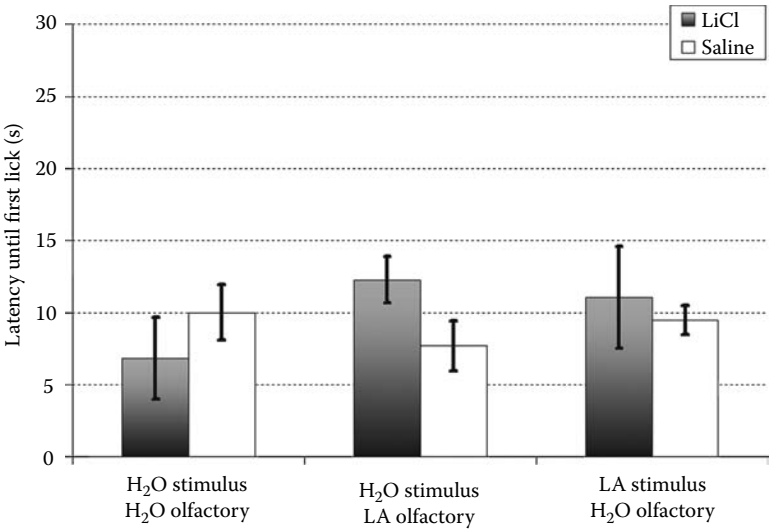

FIGURE 4.3 Mean (±SEM) latency until the first lick during brief-access testing of licking responses to stimulus tubes containing linoleic acid or water paired with either water olfactory cues or linoleic acid olfactory following a CTA to linoleic acid. Rats had 30 s to initiate a lick before trial termination.

studies, there was no effect of injection nor was there an effect of stimulus type on the mean latency until the first lick. On average, rats initiated a lick within the first 10 s of stimulus tube availability with a 95% confidence interval that the first lick would occur within a range of latencies from 7.1 to 12.1 s in duration.

4.4 GUSTATORY CHEMORECEPTION OF FATTY ACIDS

Three gustatory nerves transmit afferent neural signals from the rodent oral cavity to the first gustatory synapse in the nucleus of the solitary tract (NST). The greater superficial petrosal branch of the facial nerve innervates taste buds located in the nasal incisor ducts and palatal regions, the glossopharyngeal nerve innervates the taste buds of the circumvallate papilla on the posterior tongue, and the chorda tympani branch of the facial nerve innervates taste buds found in the fungiform papillae on the anterior tongue. The evidence from CTA studies implies a role for the gustatory system in the orosensory detection of fatty acids; therefore, we hypothesized that bilateral transection of a gustatory nerve could compromise the ability of rats to detect and avoid fatty acids following a CTA. In 2000, we presented the first evidence to support a role of the chorda tympani nerve in the detection of linoleic acid demonstrating that rats with intact chorda tympani nerves were able to form a CTA and subsequently avoid 28 μM linoleic acid in a 1 h, two-bottle preference test; however, rats with bilateral chorda tympani transections did not form CTAs to linoleic acid (Pittman et al., 2000). Using more refined, brief-access testing (8 and 30 s trials), we later confirmed a role for the chorda tympani nerve in

the detection of linoleic acid by providing evidence that rats with bilateral chorda tympani transections showed elevated thresholds for detecting linoleic acid compared to sham-operated controls (Harris et al., 2005). In these preliminary investigations, the transection of the chorda tympani nerve preceded the conditioning and testing phases of the experiment. Therefore, we conducted a final experiment to demonstrate that chorda tympani nerve transection could impair the detection of fatty acids following a known, preexisting CTA to either linoleic or oleic acid. Male and female Sprague-Dawley rats received CTAs to either 88 μM linoleic or oleic acid prior to nerve transection. Having confirmed a CTA to the CS, either linoleic or oleic acid, half of each subject group received bilateral chorda tympani nerve transections. Following three recovery days, brief-access tests with 15 s trials showed a persistent avoidance of linoleic acid in the sham-operated male (threshold ≥75 μM) and female (threshold ≥50 μM) rats, while bilateral chorda tympani transections eliminated any avoidance of linoleic acid up to 100 μM concentration for both male and female rats (Pittman et al., 2007). A similar effect of chorda tympani transection was observed for the conditioned aversions to 88 μM oleic acid with lesioned animals showing no avoidance of oleic acid (≤100 μM) and sham-operated male and female rats demonstrating 100 and 75 μM thresholds, respectively for oleic acid (Pittman et al., 2007). One other publication to date has confirmed a role of the chorda tympani nerve in the detection of fatty acids by rats demonstrating that bilateral chorda tympani nerve transection prior to conditioning an aversion to linoleic acid produced elevated thresholds for linoleic acid detection during longer, 10 min, two-bottle preference testing (Stratford et al., 2006).

A similar impairment in linoleic acid detection following gustatory nerve transection has recently been shown in the C57BL/6J mouse strain (Gaillard et al., 2008). This study showed that transection of the glossopharyngeal nerve reduced short-term (0.5 h) and long-term (48 h) innate preferences for linoleic acid and combined transections of both the glossopharyngeal and chorda tympani nerves eliminated the innate preference for linoleic acid. After conditioning a strong aversion to 2% (75 mM) linoleic acid in C57BL/6J mice, combined glossopharyngeal and chorda tympani transections eliminated the detection and avoidance of linoleic acid in subsequent 1 h, two-bottle preference tests. In addition to impaired consummatory responses related to the detection of linoleic acid, glossopharyngeal nerve transection also reduced pancreatic-bile flux following oral stimulation with linoleic acid. Combined glossopharyngeal and chorda tympani nerve transections resulted in even greater reductions in pancreatic-bile secretions in response to oral application of linoleic acid (Gaillard et al., 2008). Collectively, nerve transection research has shown that disrupting either the chorda tympani or glossopharyngeal afferent taste pathways impairs the ability of rodents to express innate ingestive preferences for fatty acids, to detect and avoid fatty acids following a CTA, and reduces the cephalic responses to oral stimulation by fatty acids. This is the strongest evidence to date supporting a role for fatty acid taste signals as immediate sensory cues capable of influencing ingestive behaviors.

Several molecular mechanisms have been proposed to be involved in the oral detection of fatty acids. Localized expression in gustatory cells and neurophysiological evidence supports a role for the lipid-binding protein CD36 (Fukuwatari

et al., 1997; Gaillard et al., 2008; Laugerette et al., 2005; Sclafani et al., 2007) and the DRK channel (Gilbertson et al., 1997, 1998, 2005) as potential transduction mechanisms for fatty acids within the gustatory system. Furthermore, there is emerging evidence that a subset of G protein–coupled receptors, specifically GPR120 (Matsumura et al., 2007) and GPR40 (Hansen et al., 2006), may also have the potential to transduce fatty acid stimuli within the gustatory system. Behavioral evidence also supports a role for CD36 in the detection of fatty acids utilizing a CD36 knockout (KO) mouse model in comparison with responses of C57BL/6J wild-type (WT) mice. Removal of the CD36 protein in the KO mice eliminated the innate preference shown by WT mice for 2% (75 mM) linoleic acid in 0.5 and 48 h preference tests (Laugerette et al., 2005). Also contrary to the preference of WT mice, CD36 KO mice showed indifference between consumption of a 5% linoleic acid-enriched solid diet compared to a paraffin oil-enriched diet in a 1 h meal preference test (Laugerette et al., 2005). Sclafani and colleagues (2007) provided independent confirmation that naïve CD36 KO mice show indifference to fatty acids and do not prefer soybean oil or Sefa Soyate oil at dilute concentrations as compared to WT mice which preferred the fatty acid stimuli. Sclafani et al. (2007) also demonstrated that postingestive exposure to fatty acids was sufficient to condition a learned preference for fatty acids and oils in the CD36 KO mice although this "rescued" preference was less than the innate preference for fat demonstrated by the WT mice. The most compelling evidence thus far that CD36 is involved in the transduction of fatty acids was recently published by Gaillard and colleagues. In this study, oral stimulation with linoleic acid induced c-fos activity in NST neurons compared to either a control xanthan gum solution or water stimulation in WT mice, and a lack of differential expression of c-fos between the viscous xanthan gum and water control solutions in the WT mice indicated that textural cues are not activating neurons within the gustatory zones of the NST (Gaillard et al., 2008). In contrast to the WT mice, CD36 KO mice showed no increase in the number of Fos-immunoreactive neurons between linoleic acid versus control solutions indicating that CD36 is a necessary protein for fatty acid activation of neurons within the gustatory zone of the NST in the C57BL/6J mouse (Gaillard et al., 2008).

The mechanisms of action for CD36 and DRK following fatty acid stimulation act to depolarize taste receptor cells, therefore, increasing the responsiveness of the taste receptor cells. As supported by nerve transection research and observed c-fos activation in the gustatory zone of the NST, in absence of other taste chemicals, the depolarization of taste receptor cells in response to fatty acid application appears sufficient to stimulate afferent neural signals in the gustatory nerves. Given the ability of fatty acids to depolarize taste receptor cells in theory, adding fatty acids to a solution of known taste chemicals should produce a larger afferent taste signal than the same concentration of taste chemicals in the absence of the fatty acids. To test this hypothesis, we characterized the licking responses of male, Sprague-Dawley rats across varied concentrations of both innately appetitive and aversive taste stimuli with and without fatty acids added to the taste solutions (Pittman et al., 2006b). Appetitive taste stimuli were defined as innately preferred tastants such as the sweet stimuli, sucrose and glucose. Whereas, aversive taste stimuli such as

sour, bitter, and salty tastants produce innate rejection behaviors and are naturally avoided unless animals are sufficiently motivated to consume the tastants such as due to thirst. Using a 23 h water-restriction paradigm and brief-access stimulus testing, rats are sufficiently motivated to consume low to moderate concentrations of salty (NaCl), sour (citric acid), and bitter (quinine–HCl) taste stimuli at a maximal lick rate similar to water; however, in spite of the thirst motivation, moderate to high concentrations of these aversive taste stimuli are not tolerated and thus licking responses decrease to near total avoidance of strong concentrations. In contrast, in similar brief-access testing procedures, increasing the concentration of the unadulterated, appetitive taste stimuli produces s-shaped concentration-dependent functions of licks per 20 s trial for water-replete rats. When either 88 μM linoleic or oleic acid was added to the appetitive solutions, the animals increased their licking responses resulting in upward shifts in the concentration-dependent functions (Pittman et al., 2006b). This characteristic change in licking behavior is reflective of an increase in the responsiveness to previously less preferred concentrations of the sweet stimuli. Conversely, when either 88 μM linoleic or oleic acid was added to the aversive stimuli, licking responses decreased, producing downward shifts in the concentration-dependent licking responses (Pittman et al., 2006b). In effect, the breakpoint of thirst-motivated tolerance of the moderate concentrations of aversive stimuli was lowered by the addition of fatty acids resulting in avoidance of previously tolerated concentrations of salty, sour, and bitter stimuli. The addition of fatty acids to the tastant solutions produced the greatest effects on middling concentrations of both the appetitive and aversive taste stimuli. These middling concentrations of tastants in absence of fatty acids were in the range of stimuli that elicited a transition in licking responses from tolerance to avoidance of aversive stimuli or in the case of appetitive stimuli, from minimal towards maximal licking responses. We interpret these characteristic changes in licking responses when fatty acids were indicative of an increase in the perceived intensity of the tastants at concentrations that in absence of fatty acids elicited neither minimal nor maximal licking responses. Figure 4.4 compares the magnitude of significant effects collapsed across concentrations for each of the taste stimuli tested in the original publication (Pittman et al., 2006b). On average, the addition of either linoleic acid or oleic acid elicited >60% more licks to appetitive stimuli, while the presence of fatty acids typically suppressed the licking responses to aversive stimuli by approximately 30%. These results support the hypothesis that fatty acid-induced depolarization of taste receptor cells may act to increase the afferent taste signals elicited by concomitant taste chemicals such that in the presence of fatty acids weaker concentrations of appetitive or aversive stimuli produce behavioral responses previously associated with higher concentrations of the tastants in absence of fatty acids.

In addition to assessing the effects of 88 μM linoleic or oleic acid on prototypical taste stimuli, we also measured the effect of adding a fatty acid mixture of 55 μM linoleic acid and 33 μM oleic acid. This 88 μM combined-concentration of fatty acids approximates the ratio of linoleic to oleic acid found in the triacylglycerides of corn oil, a prototypical dietary fat. As shown in Figure 4.4, this fatty acid mixture did not produce synergistic changes in licking responses, but elicited similar effects in magnitude on the middling concentrations of both appetitive

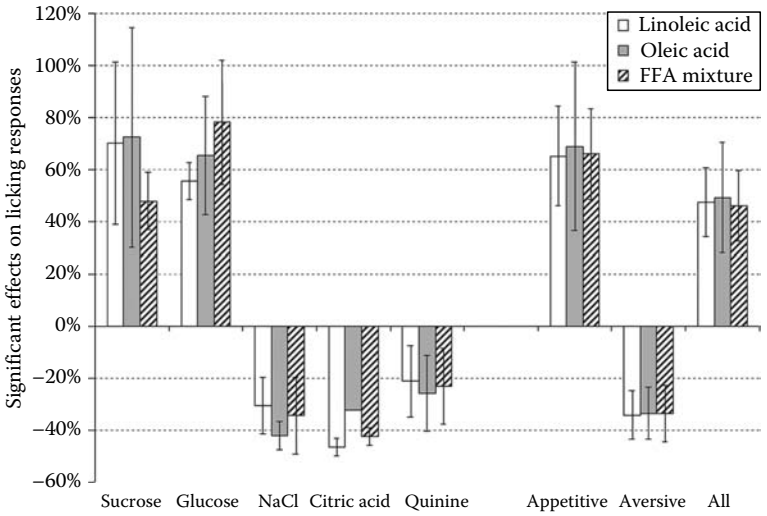

FIGURE 4.4 The magnitude of significant effects by fatty acid stimuli on the licking responses to appetitive (sucrose and glucose) and aversive (NaCl, citric acid, and quinine) tastants as reported in Pittman et al. (2006b).

and aversive stimuli as either 88 μM linoleic or oleic acid when tested separately (Pittman et al., 2006b). Although the lack of synergistic increase in the magnitude of the responses to the fatty acid mixture supports common reception mechanisms and molecular pathways within the gustatory system for both linoleic and oleic acid, in general, linoleic acid did produce more robust changes in licking behavior to the tastants than oleic acid. Similar to previous reports of lower spontaneous preference for oleic acid compared to linoleic acid and evidence that oleic acid is a less effective CS than linoleic acid, oleic acid produced changes in the licking responses to fewer tastant concentrations (31% of tested concentrations) than linoleic acid (48% of tested concentrations). However, when oleic acid did produce a significant change in the licking response to either appetitive of aversive stimuli, the magnitude of the effect was similar to linoleic acid as shown in Figure 4.4.

Research from other laboratories has corroborated the evidence supporting the ability of fatty acids to increase the perceived intensity of appetitive stimuli. Gilbertson and colleagues demonstrated that while neither 20 μM linoleic acid nor a subthreshold concentration of the non-nutritive sweetener, saccharin, produced spontaneous preference in isolation, when 20 μM linoleic acid was added to the subthreshold saccharin solution, an ingestive preference was elicited (Gilbertson et al., 2005). A similar effect on the preference of sucrose was reported by Stratford et al. (2006) with both 44 and 88 μM linoleic acid increasing the number of licks to 0.0375 M sucrose during brief-access 10 s trials.

4.5 SUMMARY AND CONCLUSIONS

The ability to detect dietary fat during feeding represents an advantageous adaptation facilitating the ability to consume high-caloric foods and essential fatty acids. This chapter reviewed the recent evidence supporting the role of the gustatory system in providing an immediate sensory signal allowing the detection of the fatty acid components of dietary fat during consumption by rats. Initial research supported an innate preference for dietary fat utilizing methodology that controlled textural and olfactory cues, leading to the discovery that rats could detect the fatty acid components of dietary fat through orosensory cues likely mediated by the rodent gustatory system. Subsequent research began characterizing the ability of rats to detect fatty acids through orosensory cues. As detection thresholds and fatty acid discrimination was examined through CTA studies, greater female sensitivity in detecting fatty acids was identified along with differences between strains of rats such as obesity-resistant rats showing stronger aversions to fatty acids than obesity-prone rat strains. New evidence was presented in this chapter suggesting that orosensory signals generated by fatty acids are likely to be unique from sensations associated with the prototypical tastes of sweet, sour, salty, and bitter chemicals and that olfactory cues are most likely not sufficient to allow detection and avoidance of fatty acids following a CTA. Finally, research demonstrating fatty acid influences on the innate ingestive behavior for prototypical tastants and impairments in fatty acid detection following gustatory nerve transections and genetic KOs of specific fatty acid receptors in the gustatory system were discussed as quintessential evidence for the chemoreception of fatty acids within the gustatory system.

The ingestion of fatty acids, the chemical components of dietary fat, produces a multimodal sensory perception. Perhaps the adaptation of multiple sensory systems to detect fatty acids is not surprising, given the high-caloric value of dietary fat and the importance of ingesting essential fatty acids such as linoleic, linolenic, eicosapentaenoic, and docosahexaenoic acids. Historically, the predominant sensory perceptions associated with fatty acids have been somatosensory and olfactory orosensory signals as well as postingestive mechanisms allowing the detection of fatty acids and subsequent activation of the intrinsic reward system in response to dietary fat consumption. This chapter outlined a preponderance of recent evidence suggesting that the gustatory system also plays a significant role in the immediate detection of fatty acid consumption. Furthermore, the effect of fatty acids on the gustatory system appears to be sufficient to influence consummatory ingestive behavior of fatty acids alone or concomitantly mixed with tastants as well as being sufficient to stimulate cephalic responses in anticipation of fatty acid digestion. Recent reports of the detection of fatty acids on the basis of gustatory cues in humans (Chale-Rush et al., 2007a,b; Mattes, 2001a,b, 2005, 2007) has given increased importance to the development of an animal model in which we can explore the ability of fatty acids to elicit gustatory sensations capable of influencing dietary fat consumption. A comprehensive understanding of the chemoreception and neural coding of fatty acids by the gustatory system holds the promise of being able to manipulate high-fat foods in the future such that the palatability associated with high-fat content can be retained

while reducing the high-caloric density of dietary fat that is contributing to the human obesity epidemic. This chapter provides a historical review of the research to date that has led to the development of a rodent model allowing the characterization of the taste of dietary fat.

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5 Peripheral Gustatory Processing of Free Fatty Acids

Jennifer M. Stratford and Robert J. Contreras

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5.1 INTRODUCTION

It is well known that obesity is a major health problem, with approximately 66% of adults in the United States considered overweight and more than 1 billion overweight adults worldwide (Ogden et al., 2006) (World Health Organization). In addition to the impact on the joints and bones caused by increased body mass, obesity can also lead to heart disease, hypertension, diabetes, and stroke (Wong and Marwick, 2007). Given the severity and consequences of these conditions, it is not surprising that there is a large body of research exploring factors that contribute to the development of obesity, including diet and, more specifically, the proportion of certain foods in the diet.

Vilified in the media as "Public Enemy Number One in the Battle of the Bulge," dietary fat, and in particular the over consumption of fat, is considered by many to be the greatest contributing factor to obesity. Yet, is fat the enemy? Clearly, high fat ingestion, along with lack of exercise, has the potential to negatively impact a healthy lifestyle. At the same time, however, fats are critical for many biological processes.

As the lipid bilayer of cells, fat is a building block for life and, in the form of myelin, enables fast electrical communication between neurons. Fat provides insulation that helps conserve body heat in cold climates and also can protect organs, like those necessary for reproduction, from damage. Fats and the main component of fats, free fatty acids, are essential for the growth and development of vital organs, including the brain (Spector, 2001). Clearly, fat is crucial for life, yet the body cannot synthesize certain kinds of fats. Rather, these fats are obtained from ingested food. Thus, the ability to detect certain kinds of fat in food sources is necessary for survival.

Fortunately, there is strong motivation to find and subsequently consume fat because fat is preferred by many animals, including humans. What is it about fat that is so alluring? In the past, the palatability of fat was thought to be the result of smell and/or texture. For example, impairment of the ability to smell (either by bilateral transection of the olfactory nerve or by destruction of the olfactory mucosa with $ZnSO_4$) eliminates the preference for high-fat foods in mice (Mela, 1988; Kinney and Antill, 1996). Moreover, increasing the texture of low-fat dairy products also increases the perceived fat content. Interestingly, sensitivity to the texture of fat seems to be related to the number of functional taste buds on the tongue, as people with the greatest number of taste buds (i.e., so-called "super tasters") are the best at discriminating between solutions with varying fat contents (Bartoshuk et al., 1994). Moreover, there are even cells in a specialized primate brain area called the orbitofrontal cortex that respond only to the texture of fats (Verhagen et al., 2003). Clearly, smell and texture are important for fat perception.

However, rats can discriminate between different kinds of oils that, presumably, have a similar texture and continue to prefer fat solutions when texture and smell are minimized in behavioral tests (Larue, 1978; Fukuwatari et al., 2003). Furthermore, ingested fats are rapidly (within 1–5 s) broken down into free fatty acids in the oral cavity by lingual lipase (Kawai and Fushiki, 2003). In fact, rats have a robust preference for free fatty acids; however, prevention of the breakdown of fats into free fatty acids by the addition of a lingual lipase inhibitor greatly reduces rats' preference for fat solutions. Thus, fat and in particular the building blocks of fats—free fatty acids—have a taste component that plays a strong role in our fat preference.

5.2 INTRODUCTION TO FREE FATTY ACIDS

Free fatty acids are organized into three broad categories with respect to their saturation degree: (1) monounsaturated, (2) polyunsaturated, and (3) saturated. Monounsaturated free fatty acids, such as oleic acid and palmitoleic acid, and polyunsaturated free fatty acids, including linoleic acid (LA) and arachidonic acid, together are classified as essential free fatty acids—such named because these fats are not synthesized by the body, but rather must be obtained from the foods we eat.

On the other hand, saturated free fatty acids (such as lauric acid and stearic acid) are easily made by the body and, thus, do not need to be consumed (Galli and Patrizia, 2006). Therefore, the body must discriminate between different types of free fatty acids in order to detect, and subsequently consume unsaturated, essential free fatty acids.

In fact, it appears that the taste of free fatty acids may be important for this discrimination because essential free fatty acids act on taste cells by inhibiting delayed rectifying potassium channels (Gilbertson, 1998; Gilbertson et al., 1998); nonessential free fatty acids do not. These potassium channels are important because they are responsible for bringing taste cells back to resting state after activation (Chen et al., 1996). However, the effect of this inhibition on taste processing and perception remains not fully understood (see Section 5.7).

5.3 LINOLEIC ACID AS A TASTE

Although there are many essential free fatty acids, gustatory processing of LA is the most studied of all. One reason that LA is the focus of so much taste research is because, as the main component of corn oil, this polyunsaturated essential free fatty acid is a large component of the American diet (e.g., fried foods, baked goods, etc.). Surprisingly, little is known about whether and how the gustatory system detects LA. In fact, do animals (including humans) use their taste receptors to respond to and consume LA?

Historically, taste perception has been studied since the time of the ancient Greeks when Aristotle first described taste as a form of touch—thus called because taste molecules must directly act on (i.e., "touch") taste receptors in the tongue (Johansen, 1997). In modern times, the advancement of technology led to a sundry of ways to explore taste perception in depth, including the development of ways to measure the detection threshold for tastes, such as LA. One way to measure the detection threshold for LA is by using a conditioned taste aversion design, which has the advantage of being comparatively straightforward to administer and requiring little animal training as compared to other training—intensive behavioral paradigms, such as conditioned shock avoidance (Spector et al., 1995). In a conditioned taste aversion protocol, rats are placed on a water restriction schedule that gradually gives them access to 10 min of water in the morning (training) and 30 min of water in the afternoon daily. Once they drink reliable quantities of deionized water (≥7 mL) in the 10 min training sessions, all rats are given 88 μM LA in a graduated drinking tube (conditioning day). After 10 min, fluid intake is recorded and half of the rats are then injected with lithium chloride (LiCl) and the other half are injected with physiological saline.

Rats injected with LiCl experience general malaise (i.e., feel "sick") and associate this with the taste of LA. Thus, these animals will not consume LA solutions in which they can taste LA. Rats are given a 10 min two-bottle preference test between water and 88 μM LA to verify that the LiCl-treated animals developed a conditioned aversion to LA. The generalization of the conditioned aversion to less concentrated LA solutions (44, 22, 11, and 5.5 μM) is tested using additional two-bottle (LA and water) tests. During these generalization tests,

one LA concentration is given each day with presentation in descending order of concentration. Preference scores are calculated as intake of LA/total fluid intake. A preference score of 0.5 indicates that animals consumed equal amounts of both solutions and preference scores >0.5 indicate that animals consumed more LA than water; whereas preference scores <0.5 indicate that animals consumed less LA than water. The point at which LiCl-treated animals consume significant amounts of LA, suggests that these animals cannot detect LA at this concentration and, thus, indicates the approximate LA detection threshold. To ensure that the results obtained do not reflect an extinction of the conditioned aversion, the aversion to 88 μM LA is again assessed after the final day of generalization testing. Using this procedure Stratford et al. (2006) found that the LA detection threshold is ~11 μM LA (Figure 5.1, top). It is important to note that,

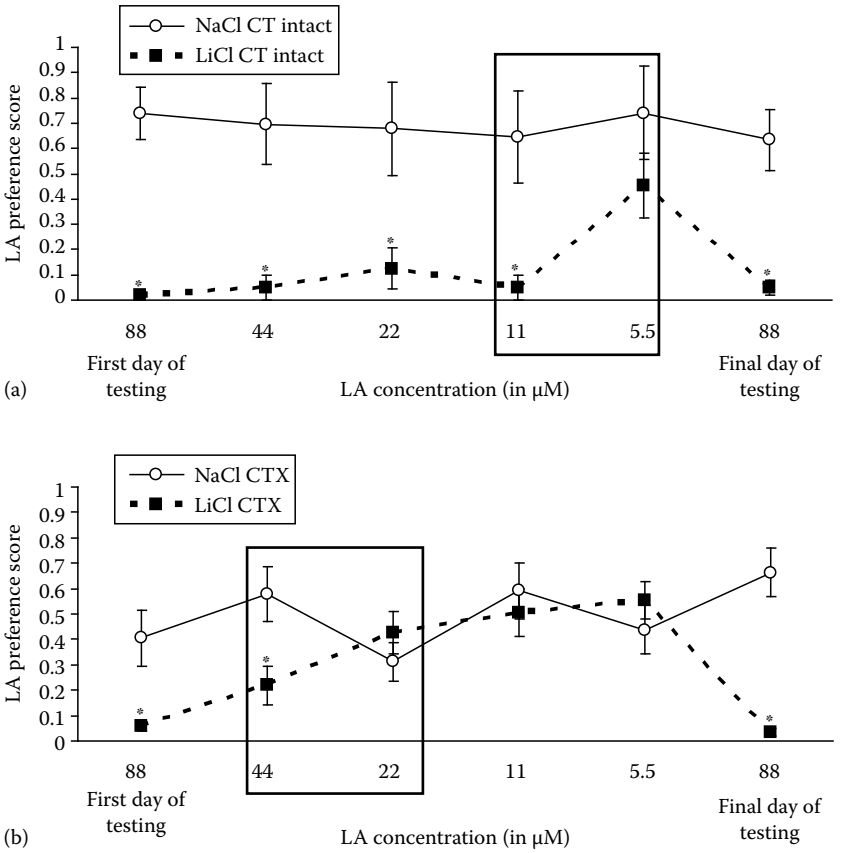

FIGURE 5.1 LA taste discrimination threshold by CT-intact (a) and CTX (b) male rats. Open circles, NaCl treated closed squares, LiCl-treated. (*) LiCl treated significantly different from NaCl treated ($p < 0.05$). Black boxes indicate the approximate LA taste discrimination threshold.

olfaction and texture were not explicitly controlled in this experiment, suggesting that these sensory attributes may contribute to LA detection in this protocol. However, this is unlikely because free fatty acids have minimal viscosity (McCormack et al., 2006) and impairment of the ability to smell by removal of the olfactory bulbs does not prevent the discrimination of free fatty acids even at very low concentrations (Smith, 2004).

5.4 OVERVIEW OF PERIPHERAL GUSTATORY NERVES

Clearly, rats are able to develop a conditioned aversion to LA and generalize this aversion to weaker LA concentrations until they reach a concentration they no longer can detect. However, if LA's taste is indeed its salient component, through what neural pathways is this information transmitted from chemoreceptors on the tongue to the brain? Three sensory nerves carry information about taste from the tongue and palate to the brain. Specifically, two gustatory branches of the facial nerve, the chorda tympani (CT) and the greater superficial petrosal nerves, innervate chemoreceptors on the anterior 2/3 of the tongue and palate, respectively. The glossopharyngeal nerve innervates taste receptors on the posterior 1/3 of the tongue. Together, these nerves convey different sensory information about taste quality from the tongue and palate (Frank and Pfaffmann, 1969; Krimm et al., 1987; Frank, 1991; St John and Spector, 1996) to the nucleus of the solitary tract, located in the hindbrain which then relays this information to higher brain areas such as the parabrachial nucleus, thalamic taste area, and gustatory cortex (Norgren and Leonard, 1973; Norgren, 1976; Norgren et al., 1989).

5.4.1 Role of the Glossopharyngeal and Greater Superficial Petrosal Nerves in Linoleic Acid Taste Processing

Based on numerous factors, the glossopharyngeal nerve appears to be ideally suited to transmit fat taste information from chemoreceptors to the brain. First, lingual lipase (the enzyme that breaks triglycerides into free fatty acids in the mouth) is secreted by the von Ebner's glands found within circumvallate papillae in the back of the tongue. Second, CD36 (a fatty acid transporter/translocase) is highly expressed in circumvallate papillae which are also located in the back part of the tongue and are innervated by the glossopharyngeal nerve (Doty, 2003; Laugerette et al., 2005). However, little is known about the role of the glossopharyngeal nerve in free fatty acid recognition. For example, bilateral transection of the glossopharyngeal nerve impairs the ability of mice to discriminate 2% LA from a control solution, using a 30 min, conditioned aversion two-bottle choice test (Gaillard et al., 2008). Unfortunately, this is the only behavioral study conducted so far and there has been only one electrophysiological investigation exploring the role of the glossopharyngeal nerve in fat taste. Kitagawa et al., 2007, found that the pharyngeal branch of the glossopharyngeal nerve responds to oleic acid ~20 times better than it does to safflower oil (an oil rich in LA). However, this branch of the glossopharyngeal nerve innervates receptors in the pharynx that are most likely important for the control of reflexes, rather than for the identification of taste stimuli (Kitagawa et al., 2007). To date, no study has explored the effect of transection of the pharyngeal branch of the glossopharyngeal

nerve on LA detection. Moreover, no one has conducted an electrophysiological study of the LA responses of the lingual (gustatory) branch of the glossopharyngeal nerve that innervates the chemoreceptors located in the circumvallate papillae on the posterior tongue. Finally, the role of the greater superficial petrosal nerve in free fatty acid taste sensitivity has not been explored.

5.4.2 Role of the Chorda Tympani in Linoleic Acid Taste Processing

5.4.2.1 Behavioral Studies

There is strong evidence that the CT is important for fat taste detection, focusing primarily on the polyunsaturated free fatty acid, LA. Using a conditioned aversion protocol (see 5.3), two recent studies found that the detection threshold for LA is ~10 μM (Figure 5.1A), but that bilateral transection of the CT (CTX) prior to development of a LA conditioned aversion prevents rats from discriminating LA from water at concentrations more dilute than 44 μM (Figure 5.1B) (Stratford et al., 2006; Pittman et al., 2007). Thus, these two nerve cut experiments indicate that the CT is important for LA detection. Importantly, transection of the CT does not completely eliminate the ability to detect LA, suggesting that other gustatory nerves are also involved or that rats can detect LA using sensory attributes other than taste (i.e., texture or smell). However, the latter two possibilities are unlikely because free fatty acids have a minimal viscosity (only about 1.5% greater than that of water) and little smell as bulbectomized rats can discriminate LA even at 10 μM (Smith, 2004; McCormack et al., 2006).

5.4.2.2 Electrophysiological Studies

Removal of sensory input by CTX suggests that the CT is important for the detection of LA, but does not provide the resolution to determine what kind of information the CT carries. One approach to address this question is to record electrophysiological responses from the CT of anesthetized rats in response to LA stimulation of the tongue. By using a solenoid fluid delivery system that delivers taste solutions at a constant flow rate of 50 μL/s, this approximates the fluid volume consumed by a rat licking from a drinking spout obtaining ~5–7 μL/lick at a rate of 6–7 licks/s (Lundy and Contreras, 1999). A custom computer program controls input to a mixing platform that allows rapid switching and/or mixing while maintaining continuous solution flow. Between stimuli, the tongue is continuously rinsed to minimize transient thermal or tactile responses and each taste stimulus is followed by a 90 s rinse to ensure that nerve activity returns to stable baseline levels.

Surprisingly, application of LA to the tongue does not produce a detectable CT response (Figure 5.2). Moreover, individual neurons in the geniculate ganglion (the location of the cell bodies of chorda tympani gustatory sensory neurons) are also unresponsive to LA stimulation (Breza et al., 2007). What could account for this discrepancy? LA detection may depend upon the interaction of multiple gustatory sensory nerves. This is supported by the fact that removal of CT sensory input does not completely prevent the detection of LA (Figure 5.1B). However, the role of other gustatory nerves—individually or in combination—in LA detection remains unknown.

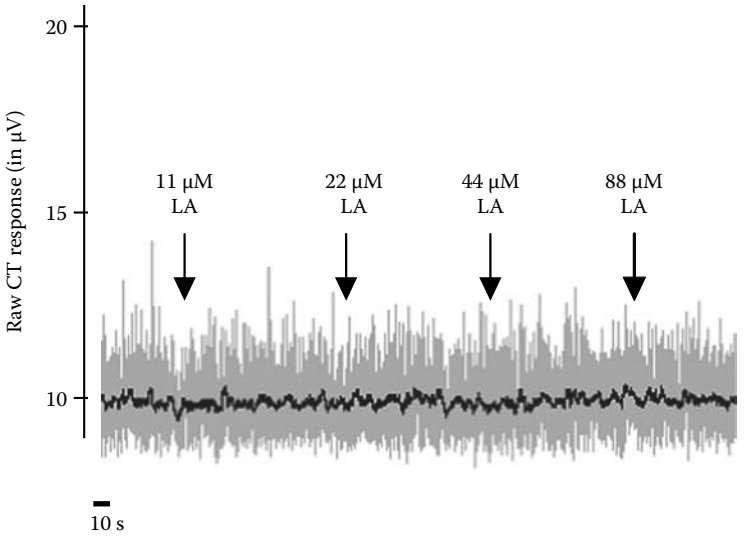

FIGURE 5.2 CT whole nerve activity (μV) in response to lingual application of LA (11, 22, 44, and 88 μM). Gray, raw nerve activity; black, integrated, rectified activity.

5.5 ROLE OF SALIVA IN LA DETECTION

An intriguing possibility is that fat is not an effective taste stimulus when presented alone. Rather, fat is a taste stimulus only when there is an "active background," such as in the presence of saliva in the mouth. Importantly, this could explain the discrepancy between behavioral CTX data (in which saliva is present) and electrophysiological data (in which saliva is washed off during rinses). Moreover, CTX secondarily results in a decrease in saliva (via denervation of the submaxillary and sublingual salivary glands). Thus, impairment of LA taste discrimination seen after CTX (see Sections 5.4.2 and 5.4.2.1) may result from transection of the chorda tympani nerve itself, a secondary decrease in saliva, or both. What in saliva could be important for LA taste processing? Saliva is made of a large number of proteins, ions (e.g., K^+ and Cl^-) and enzymes (Hart, 1998). Thus, at present it is unknown what component of saliva may be important for LA taste processing, but ongoing studies are addressing this idea.

5.6 LINOLEIC ACID-TASTE MIXTURES

If LA taste processing requires the action of another stimulus, such as saliva, this suggests that fats may also exert a powerful influence by complementing, modulating, or enhancing other taste stimuli as well. Indeed, work in isolated taste cells shows that essential unsaturated free fatty acids, but not saturated free fatty acids, inhibit delayed rectifying potassium channels (Gilbertson et al., 1998, 2005). This inhibition presumably leads to a broadening of action potentials and prolongation of

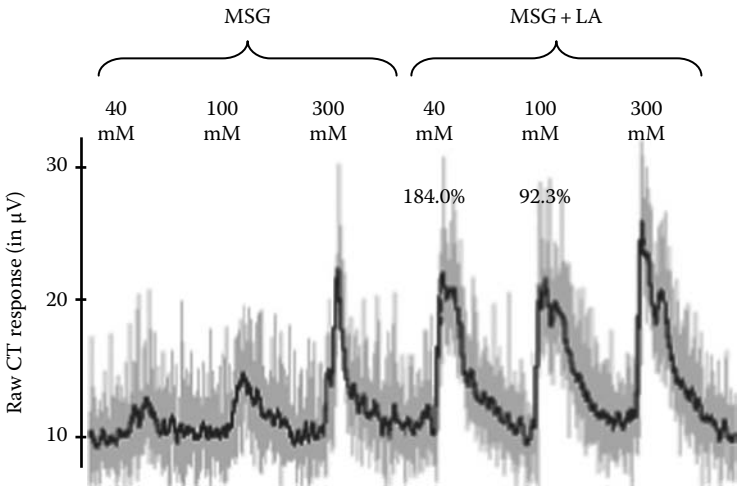

FIGURE 5.3 CT whole nerve activity in response to lingual application of monosodium glutamate MSG (40, 100, and 300 mM) mixed with water (left) or 88 μM LA (right) in a male rat. Gray, raw nerve activity; black, integrated, rectified activity. Percentages reflect increase in CT response from MSG + water to MSG + LA.

the release of neurotransmitters. Thus, unsaturated free fatty acids may increase the perceived intensity of other taste stimuli. In this regard, the addition of LA increases both licking responses to sucrose (Pittman et al., 2006; Stratford et al., 2006) as well as the preference for monosodium glutamate (MSG), especially at lower (40 and 100 mM) concentrations in behavioral studies (Stratford et al., 2008).

These behavioral results are further supported by recent electrophysiological experiments which found that the addition of LA increases CT responses to MSG (Figure 5.3). Importantly, this enhancement occurs at the same MSG concentration whose preference is also increased by LA (i.e., 40 and 100 mM). Because MSG is generally preferred—especially at lower concentrations—it is likely that LA increases the behavioral preference for MSG, in part, by increasing the intensity of MSG. However, whether the effects seen by coapplication of MSG and LA are specific to MSG (or one of its components), or rather, reflects a global enhancement of taste by fats, remains unexplored.

5.7 PROPOSED INTRACELLULAR MECHANISMS: A DUAL PROCESSING THEORY

Given these diverse results, the obvious question is whether free fatty acids such as LA have their own taste or, rather, only increase the intensity of other tastes. In other words, is LA simply a flavor enhancer? Although LA increases behavioral preference for some taste stimuli, fatty acids do not enhance responses to taste stimuli in all conditions. For example, LA increases licking to sucrose and glucose, but decreases licking to sodium chloride, citric acid, and quinine hydrochloride solutions in rats

(Pittman et al., 2006). In humans, the addition of 1% LA significantly decreases the intensity of sodium chloride, citric acid, and caffeine, but does not change intensity perceptions of sweet and sour solutions (Mattes, 2007). Thus, it remains unclear whether LA either (1) requires the action of other taste stimuli to have a behavioral effect, but has its own taste quality, (2) increases only the perception and/or intensity of other taste stimuli, or (3) perhaps, fulfills both roles in certain situations.

An intriguing idea first proposed by Laugerette et al. (2007) suggests that free fatty acids play different roles depending on which part of the tongue they stimulate. Free fatty acids may enhance the intensity of other taste stimuli via taste-free fatty acid interactions on the anterior part of the tongue (as seen in regard to the CT). However, free fatty acids may also directly activate the gustatory system to produce their own "fatty" taste in the posterior oral cavity. In support of this latter idea, free fatty acid stimulation of circumvallate papillae in the back part of the tongue results in increased intracellular Ca^{2+} as well as neurotransmitter release (El-Yassimi et al., 2008). Moreover, these effects may depend on the fatty acid transporter/translocase, CD36, which is highly expressed in circumvallate papillae, as inactivation of the CD36 gene abolishes the preference for free fatty acids in mice (Laugerette et al., 2005).

On the other hand, CD36 is not present in CT-innervated fungiform papillae (Laugerette et al., 2005). Moreover, stimulation of the tongue with LA alone neither activates the CT (Figure 5.2) nor the geniculate ganglion (Breza et al., 2007). However, LA does increase CT responses to MSG (Figure 5.3). Moreover, the addition of LA (but not the saturated free fatty acid, lauric acid) to a subthreshold saccharine concentration makes saccharine detectable (Gilbertson et al., 2005). Furthermore, unsaturated free fatty acids inhibit delayed rectifying potassium channels in the taste cells of fungiform papillae, as mentioned previously, (Gilbertson et al., 1998), which presumably broadens action potentials and prolongs neurotransmitter release.

5.8 SEX DIFFERENCES IN TASTE RESPONSES TO LINOLEIC ACID

To complicate this issue further, there are sex differences in the detection of LA. Female rats discriminate a weaker (more dilute) concentration of LA from water than can male rats (i.e., ~2.75 vs. 11 μM LA; Figure 5.4a). In addition, female rats also increase their licking to a lower concentration of LA when it is mixed with sucrose as compared to males (Stratford et al., 2006). Moreover, CTX impairs LA taste discrimination in female rats and in fact shifts the discrimination threshold to the same LA concentration as observed after CTX in male rats (Figure 5.4b). However, because females have a lower LA taste discrimination threshold, the magnitude of the shift seen after CTX is greater in females. Together, these results suggest that the CT is important for LA taste discrimination in both male and female rats, but that the CT may play a greater role in LA taste discrimination and fat taste responses by females.

To explore further sex differences in fat taste, Stratford et al. (2008) first recorded electrophysiological responses from the CT in response to application of LA to the tongue of anesthetized rats. Similar to the effect seen in male rats, the CT was unresponsive to LA stimulation (Stratford et al., 2006). Moreover, the addition of

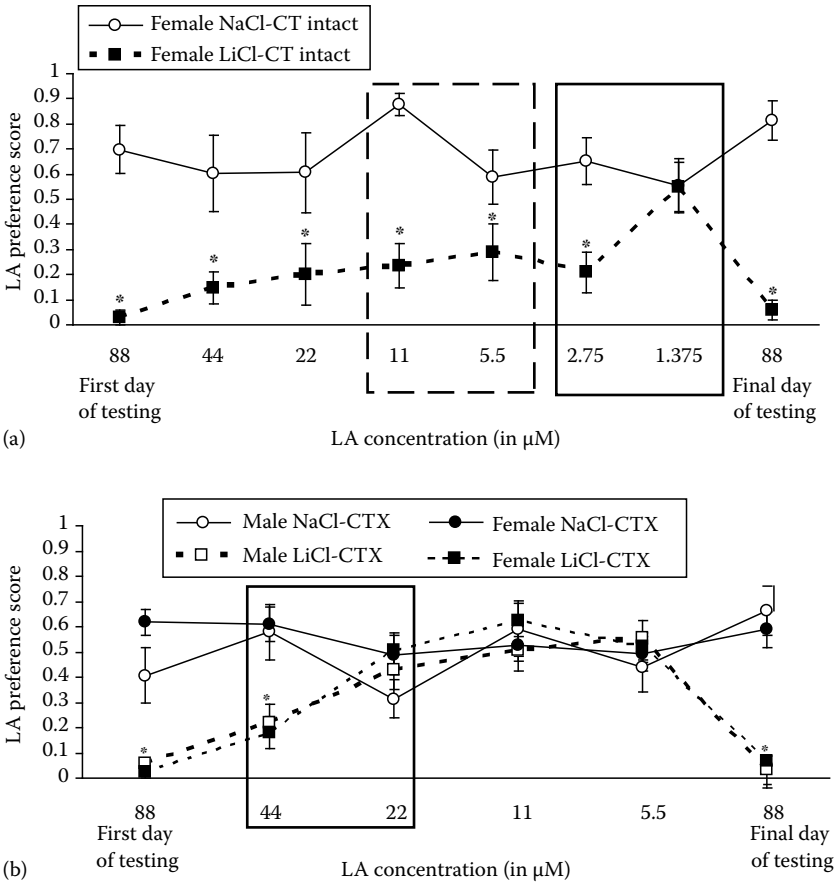

FIGURE 5.4 Sex differences in LA taste discrimination thresholds. (a) LA taste discrimination threshold by CT-intact female rats. Open circles, NaCl treated; closed squares, LiCl treated. Solid lined black box indicates the approximate LA taste discrimination threshold for female rats; dotted lined black box indicates male LA taste threshold (see Figure 5.1A). (b) Effect of CTX on LA taste discrimination thresholds in male and female rats. Open symbols, male rats; closed symbols, female rats. Circles, NaCl treated; squares, LiCl treated. (*) LiCl treated significantly different from NaCl treated ($p < 0.05$). Box indicates the approximate LA taste discrimination threshold for both sexes after CTX.

LA increased CT responses to MSG (Stratford et al., 2008). However, LA increased CT responses to 100 mM MSG in females only (Figure 5.5); whereas it increased CT responses to 40 and 100 mM MSG in males (Figure 5.4). More strikingly, data collected from behavioral preference tests paralleled these results (i.e., LA increased the preference for 100 mM MSG only in females, but increased the preference for 40 and 100 mM MSG in males) (Stratford et al., 2008).

These results are perplexing: females appear to have a lower LA detection threshold and are more sensitive to LA when it is mixed with sucrose, but are less sensitive

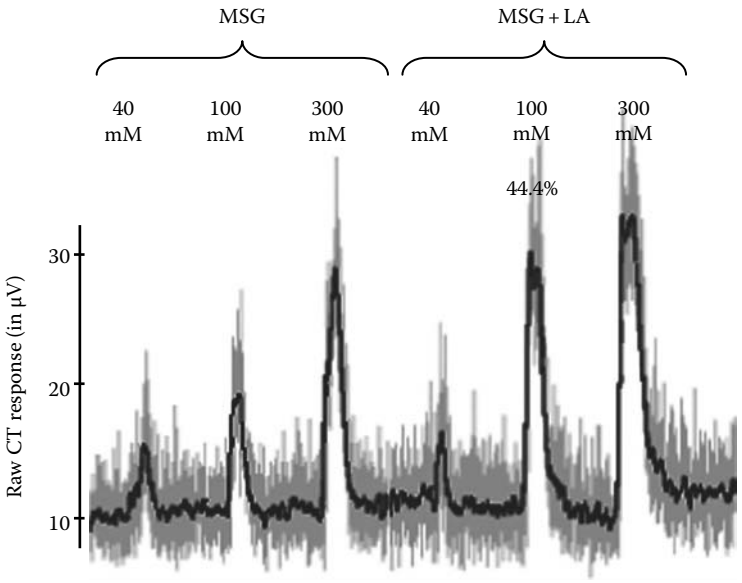

FIGURE 5.5 CT whole nerve activity in response to lingual application of monosodium glutamate MSG (40, 100, and 300 mM) mixed with water (left) or 88 μM LA (right) in a female rat. Gray, raw nerve activity; black, integrated, rectified activity. Percentage reflects increase in CT response from MSG + water to MSG + LA.

to LA mixed with MSG than males. A simple explanation is that sex differences in fat taste may depend on the taste stimulus with which the fat is mixed. For instance, males prefer MSG solutions at lower concentrations than do females, which could explain why LA increases CT responses to and behavioral preference for MSG at a lower concentration in males than in females (Hiji and Masayasu, 1967; Ohara and Naim, 1977). On the other hand, females have a greater preference for sucrose than do males, which may result in females increasing their licking to a lower LA–sucrose concentration than do males. However, what could account for observed sex differences in the detection of LA when it is mixed "alone?" If saliva is important for LA taste processing (see Section 5.5), sex differences in LA detection may be the result of sex differences in the detection of one of the components of saliva. In this regard, there are sex differences in the detection of sodium, as females detect lower concentrations of NaCl than do male rats (Curtis and Contreras, 2006). Thus, sex differences in the detection of LA may result from sex differences in salivary sodium. Moreover, how and to what extent this differing sensitivity to fats affects the perception and subsequent ingestion of other tastes and foods remains unknown.

What evolutionary function could an enhanced sensitivity to certain kinds of fatty foods (such as "sweet-fats") for females serve? It is well known that many evolutionary adaptations promote reproduction, in part, by optimizing survival of the offspring. For example, enhanced sensitivity to environmental stimuli during pregnancy may improve the ability to detect resources necessary to support a viable pregnancy. In this regard, energy dense foods such as fats are essential to good maternal health

during pregnancy (Decsi and Koletzko, 2005; Facchinetti et al., 2005). Thus, the detection of fat may have intrinsic survival value and, in fact, preferences for fats increase during pregnancy. However, although estrogen levels change during pregnancy, it appears that sex differences in LA taste responses is not the result of acute estrogen effects, as estradiol benozoate treatment of ovariectomized rats does not alter licking responses to sucrose–LA mixtures (Stratford et al., 2006). The role of other reproductive hormones (i.e., testosterone and progesterone)—alone or in combination—remains unexplored. Moreover, estrogen may still play a role in sex differences in LA taste responses during the development of the gustatory system. Finally, sex differences in peripheral input do not preclude the possibility of sex differences in the central processing of LA-taste mixtures as well.

5.9 CONCLUSIONS AND WHAT THE FUTURE HOLDS

The field of fat taste is still in its infancy and, as such, much is left unexplored. Several ground-breaking discoveries provided important insight into not only how fat taste information travels from the tongue to the brain, but also what the nature of this sensory information may be. Fat taste detection begins with the initial break down of fat into free fatty acids in the mouth. In turn, free fatty acids, especially the essential unsaturated free fatty acid linoleic acid, activate gustatory receptor cells on the tongue. Moreover, both the glossopharyngeal nerve and the chorda tympani nerve are involved in free fatty acid detection. Yet, the nature of this information remains the subject of debate and may be dependent upon which part of the tongue is stimulated by free fatty acids. Finally, there are sex differences in both behavioral responses and electrophysiological responses to free fatty acids. However, the mechanisms that underlie these differences remain unknown.

More importantly, the implications for this research are far-reaching as taste plays a significant role in food choices. Given the obesity epidemic that many industrialized nations currently face, it is essential to understand and explore every factor that could contribute to the development of this chronic and debilitating disease, including the taste of fat. However, obesity is certainly a multidimensional disorder, which requires a corresponding multifaceted treatment plan. Thus, the future of fat taste research relies on the ability to integrate current and future findings into existing obesity treatments.

ACKNOWLEDGMENTS

This chapter is the culmination of a decade's worth of work. As such, many people contributed to the ideas presented. First, we thank those whose work is presented in this chapter for having the courage to explore interesting questions. We wish you well in all future endeavors. Second, we also thank Dr. Kath Curtis for encouraging the exploration of sex differences in fat taste, which led to many interesting and unexpected discoveries. Finally, we extend a special note of thanks to Dr. Jim Smith for his pioneering work on fat taste at Florida State University as well as providing useful comments on this book chapter. We are indebted to him for all he has done and continues to do. The National Institute on Deafness and Communication Disorders of the NIH supported this research (DC-004785, DC-00044, and DC-008934).

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6 Orosensory Factors in Fat Detection

James C. Smith

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6.1 INTRODUCTION

Fat intake results from both orosensory and postingestive controls. During the past decade, more attention has been given to the study of the orosensory factors in the control of fat intake in rodent models (Gilbertson, 1999; Gilbertson et al., 2005; Sclafani et al., 2007a,b). Understanding the orosensory factors in the ingestion of sweet/fat mixtures is important because in the human diet, fat is most often consumed in conjunction with sweeteners (Elizalde and Sclafani, 1990; Lucas and Sclafani, 1990; Greenberg and Smith, 1997) and is very important in weight control.

The goal of this chapter is to describe the data that have been collected in this laboratory over the past decade involving the taste perception and ingestion of fat by the laboratory rat. Although studies done in other laboratories will be mentioned from time to time, this chapter is not intended to be a comprehensive review of the literature in this area. The research conducted in my laboratory was inspired by Professors Gerry Smith and colleagues at the Bourne Laboratory (Greenberg and Smith, 1996, 1997), Tony Sclafani and colleagues at Brooklyn College (Ackroff et al., 1990; Elizalde and Sclafani, 1990; Lucas and Sclafani, 1990; Ackroff and Sclafani, 1996; Ackroff et al., 2004; Sclafani and Glendinning, 2005; Ackroff et al., 2005; Sclafani, 2007; Sclafani et al., 2007a,b), and Tim Gilbertson's electrophysiological studies (Gilbertson et al., 1997; Gilbertson, 1998; Gilbertson et al., 1998a,b; Gilbertson, 1999; Gilbertson et al., 2005).

6.2 METHODS FOR MEASURING TASTE PREFERENCE AND PERCEPTION

We routinely use three different procedures to collect our data.

6.2.1 Preference Tests

We used two-bottle preference tests ranging in time from 1 h to several weeks. Some of these tests were conducted in standard rat housing and others in our rat "hotel," a modified cage arrangement that allowed for microstructure measurements of ingestive behavior. In the hotel we could study the number and size of ingestive bouts and rates of intake within a bout. In all experiments, animals were individually housed.

The preference tests were conducted in the standard manner with two fluid bottles placed on the home cage. The positions of the bottles were reversed daily to counteract any side preferences. Preference scores were calculated by dividing the consumption in one of the bottles by the total fluid intake.

The tests in the rat "hotel" were conducted in a similar manner. The illustration in Figure 6.1 describes the shoebox plastic cage where the eating and drinking ports are attached to the front and back, respectively. Eight such cages constituted the hotel. The eating port housed a 4 ounce glass jar which held up to 65 g of powdered food. When the rat entered the feeding compartment its head broke a beam between an infrared light emitting diode and a photodetector. Interruptions of the beam were recorded and stored in a microcomputer. In some of the hotels the food jar rested on a load beam which continuously recorded the weight of the jar. The correlation between duration of the beam break and amount of food consumed has averaged about 0.97 over many observations. A stainless steel rack which held two glass water bottles was attached to the back of the cage. Each of the two sipper tubes had contact circuits that counted the individual licks and stored the interlick intervals in the microcomputer. The data were recorded in 6 s intervals, which gave 13,800 data points for each of the three ingestive ports. The day/night cycle was 12/12 with the lights on at 07.00 h. A photoreceptor attached to the top of the cage rack was used to indicate the period when the lights were on. At the end of each daily testing period the data were saved and transferred to an analysis computer for processing. On each

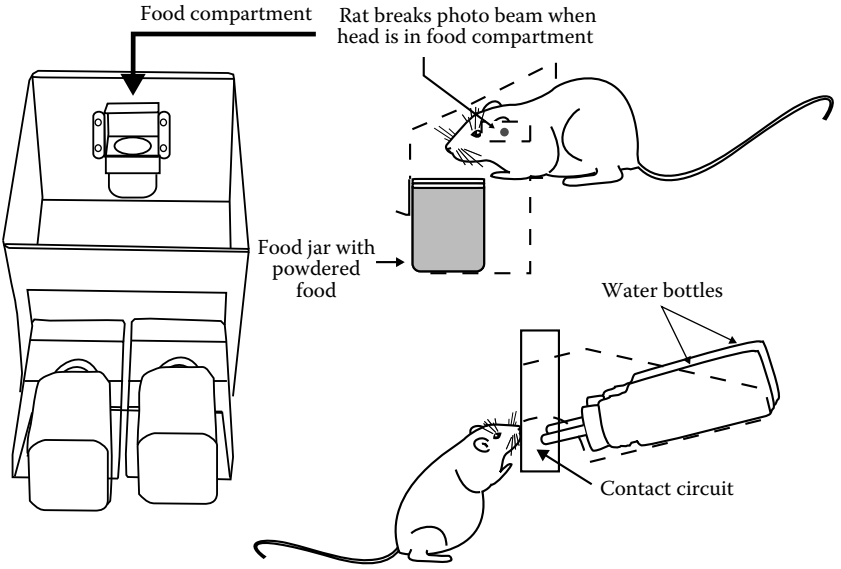

FIGURE 6.1 The "rat hotel" was fabricated from a standard shoebox plastic cage with cutouts in the front for the feeding port and the back for the drinking ports. When the rat entered the food port an infrared beam was broken which allowed for the measurement of food bout duration. Each of the stainless steel sipper tubes was fitted with contact circuits (Dilog Instruments Co.) which enabled the count of each lick during the testing period.

of the water bottles and food jars were bar codes that could be read by a scanner attached to an electronic balance, which enabled rapid determination of the amount of food and liquid consumed (Smith and Bigbie, 1997). The data analysis utilized Windows-based customized software that allowed for the determination of number of eating and drinking bouts, their durations and the intervals between them (inter-bout intervals). In addition, we determined the mean number of licks per second (or volume per second) during each drinking bout. In order to attain this measure we divided the number of licks in each bout by the length of the bout. If the rat licked without pause during a bout, this value was approximately $6\,s^{-1}$. Our overall rate measure reflected the number of pauses that occurred within a bout.

This latter measure has proved to be quite interesting to us in our studies of sucrose intake. It is well known that when the rat is presented with a two-bottle preference test between various concentrations of sucrose and water, an inverted U-shaped curve is obtained for the sweetened solution with the highest sucrose intake at about 0.25 M (Richter and Campbell, 1946; Smith and Wilson, 1988). It is not correct to refer to this peak as the "most preferred" sucrose concentration. Collier and colleagues (Collier and Bolles, 1968) have clearly shown in two-bottle preference tests between concentrations of sucrose ranging from 1.0 M and lower, the rats always drink more of the higher over the lower concentrations.

In short-term preference tests (lasting only a few minutes) or sham-feeding tests across a range of sucrose concentrations, rats always drink more of the higher than

the lower concentrations and do not show a peak at 0.25 M (Smith, 2000). In other words, when ingestion is under the control of primary oral sensory input, because postingestive factors have been minimized, the sweeter the better. Our measure of "licks per second during a drinking bout," correlates highly with other taste tests that eliminate or minimize postingestive factors (Spector and Smith, 1984; Smith, 2000). A comparison of our rate measure with (a) ultra short-term taste tests (to be described in Section 6.2.3), (b) electrophysiological recordings from the greater superficial petrosal (GSP) branch of the facial nerve, and (c) sham-feeding tests can be seen in Figure 6.2. These electrophysiological and behavioral measurements have been equated by fitting them with Beidler's taste equation (i.e., $R = CKRs/1 + CK$) where R is the magnitude of response to concentration C, Rs is the maximum response, and K is the association constant (inverse of the concentration which produces a response, half the magnitude of the maximum response) which is considered a measure of the affinity of the receptor for the ligand (Smith, 1988).

Because of these similarities seen in Figure 6.2, we have concluded that our measure of "licks per second during a sucrose bout" from data collected in the rat hotel is also a measure of taste. Each drinking bout is like a short-term test and by study

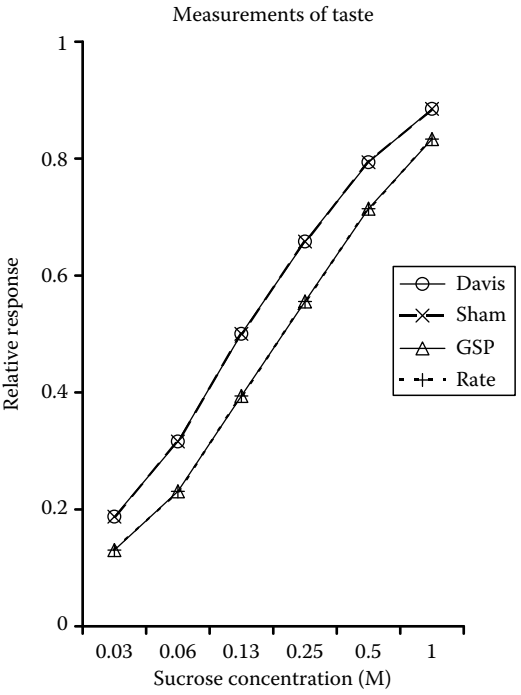

FIGURE 6.2 Each of the four measurements described here was taken for a variety of sucrose concentrations. Licking in the Davis Rig (Davis), consumption by a rat in a sham-feeding test (Sham), electrical activity in the GSP nerve, and overall rate of licking (Rate) within a drinking bout in the rat hotel all increased with an increase in sucrose concentration. Each of these measurements was fitted with Beidler's taste equation in order to give a common *y*-axis. Similarities of these functions are displayed in this graph.

of the details of licking within a bout one can make inferences regarding gustatory experience. We will use this rate measure later in this chapter to infer that the rats "taste" corn oil in a similar manner since their rate of licking in a bout increases as the concentration of the oil is increased in a manner similar to the findings from sucrose testing.

6.2.2 Conditioned Taste Aversion Tests

We also used conditioned taste aversion (CTA) procedures induced by injections of lithium chloride. As such, we could condition an aversion to one substance and test the effects on preferences for other substances, allowing us to infer similarities and differences in the gustatory perceptions of the rats.

The CTAs referred to in this chapter have all been induced by presenting the conditioned stimulus (CS) solution for 10 min followed immediately by an injection of LiCl. The LiCl concentration was 0.6 M, delivered in doses of 5 μL/g of body weight. Nachman and Ashe (1973) recommended this dose for producing a reliable flavor aversion after only one pairing. Control groups were run by injecting rats with isotonic saline at the same dose level following their ingestion of the CS. In order for the rats to drink on conditioning day, they were subjected to a water deprivation regimen for 5 days before the conditioning day. After an overnight water deprivation on the first day of this schedule the rats received water for 60 min followed by a second overnight deprivation regimen. On the following 4 days they received water for 30 min, 20 min and 2 days of 10 min drinking. Using this procedure ensured that all rats would drink the CS solution on conditioning day prior to the lithium chloride injection. The CS is described later in this chapter for each experiment. The pairing of the CS and the LiCl injection was always followed by 24 h of food and water ingestion and the preference tests were initiated at the end of this period when the rats were no longer water deprived. The postconditioning flavor aversion was described with a preference score (i.e., CS Intake/(CS Intake + Solvent Intake)).

When the CS was a mixture of substances, we tested the substances separately to allow inferences about the salient components of the mixture.

6.2.3 Davis Rig Tests

Davis Rig tests were used to take short-term measurements of licking that is based primarily on orosensory stimulation while minimizing the effects of postingestive factors. The Davis Rig has been described in detail elsewhere (Smith et al., 1992; Smith, 2001). Basically it is a small rat-testing cage with a drinking port at the front end. The rat had access to the drinking port only when a motor-driven shutter is opened. Behind the shutter was a tray that held eight drinking tubes with stainless steel sipper tubes. The tray could be moved so that only one of the sipper tubes was available to the rat at a given time. The positioning of a single tube was controlled by a reversible motor and a heliport located under the tray. A computer program controlled the position of the tray and the opening and closing of the shutter. Contact circuits on each of the tubes allowed for measurement of individual licks. A list of interlick intervals was compiled, allowing for subsequent analysis of drinking bursts

and clusters of licks. In addition, the latency of the first lick after the shutter was opened was recorded. This latter measurement proved to be very important in our analysis of sucrose intake. We found that the latency to lick was inversely related to the concentration of the sucrose, leading to the suggestion that the rats could detect the presence of sucrose by olfaction at higher concentrations (Rhinehart-Doty et al., 1994). This latency measurement is used later in this chapter to denote the role of olfaction in the detection of corn oil.

In our experiments with corn oil/sweetener ingestion, we typically ran the Davis Rig with the following parameters:

1. About 1 min after the rat was placed in the chamber the shutter opened. Following the first lick the shutter remained open for 30 s.
2. The shutter was then closed for 30 s while the tray moved to another preselected position.
3. The shutter was then opened and this procedure was followed until access to all eight tubes had been accomplished.
4. On any trial if the rat failed to lick on the tube, the 30 s timer would not start. If no licking occurred for 180 s, the shutter was closed for 30 s and the presentation of the next tube followed.

If a rat licked quickly after the shutter opened, it could complete the session in slightly over 8 min, having a total of only 240 s of licking. This Davis Rig procedure yields a short-term ingestive measure, minimizing the possibility of much postingestive contribution to the intake. The rig allows for the opportunity of automatically measuring the intake of as many as eight different compounds during one short-term test.

6.2.4 Preparation of the Solutions

We chose corn oil as our prototypical fat stimulus and we have most frequently presented it blended with water that was sweetened with sucrose (SU) or a mixture of glucose and saccharin (G + SA). The corn oil (CO) (Mazola Brand) was blended with the sweetened solution by the addition of 5 mL of Tween-80. The sweetened water, corn oil, and Tween-80 were mixed in an industrial blender for 2 min. For example, we mixed 85.6 g of sucrose in 840 mL of deionized water and with the addition of 5 mL of Tween-80; we blended this with 160 mL of corn oil. We called this a 16% corn oil mixture in a 0.25 M sucrose solution. The phase separation of the oil from the water occurs fairly rapidly and our measurements of this have been described elsewhere (Smith et al., 2000). Therefore, in this chapter all of the oil/water mixtures are described as the concentration when they were mixed, acknowledging that the concentration of the oil consumed by the rat diminished over time to about half the original concentration by the end of a 24 h period.

6.2.5 Animal Subjects

All rats used in these experiments were Sprague-Dawley. All were males except in a few cases as noted. In the few cases where females were used, females did not differ

from the males in the intake or preference measures. In all cases the rats ranged in body weight between 325 and 550 g, except where noted.

6.2.6 Statistical Analyses

The analyses reported in this chapter were performed by appropriate *t*-tests and ANOVAs. For the sake of simplicity, *F*, *t*, and *p* values are not reported. Rather, outcomes of statistical tests are reported as significant if they reached the 5% level. Post hoc tests used the Fisher's least significant difference (LSD) test.

6.3 EXPERIMENTAL EVIDENCE SUPPORTING OROSENSORY FACTORS IN FAT DETECTION

6.3.1 Background

Careful observations made by Sclafani and colleagues (Lucas and Sclafani, 1990) compared the intake and weight gain of rats given a sweet/fat mixture (35% corn oil blended with 8% sucrose solution), a sweet solution (8% sucrose) or a fat mixture (35% corn oil blended with water). They found that this mixture of sucrose and corn oil resulted in enhanced caloric intake and a significant increase in body weight. In order to determine if the increased intake of the sucrose–fat mixture was the result of the sweetness, the group that had received only the corn oil mixture was subsequently tested with a 0.2% sodium saccharin solution rather than water mixed with the corn oil. The intake of this sweetened-fat solution over the fat alone increased immediately. Further evidence that the palatability of the sweet-fat solutions played a major role in the enhanced intake was shown in two-bottle preference tests between the oil alone vs. the oil plus sweetener. It was demonstrated that saccharin–oil was preferred over oil, sucrose–oil was preferred over oil and that sucrose–oil was preferred over saccharin–oil mixtures. Since it is well known that sucrose is preferred over saccharin (Collier and Novell, 1967) it seems quite likely that the increase of intake of these two sweet-oil solutions is the result of taste.

6.3.2 Gustatory Cues Play a Role in the Ingestion of a Corn Oil with Glucose + Saccharin Mixture

In our laboratory we have collected similar data where we blended the corn oil with a mixture of glucose and saccharin (Smith, 2004). Valenstein and colleagues demonstrated that when presented with a glucose and saccharin solution (30 g glucose + 1.25 g saccharin in 1 L of water) rats drank excessive amounts of this mixture (Valenstein et al., 1967). In fact, some rats exceeded their own body weight in intake during a 24 h period. They suggested that this solution could be used as a vehicle for getting rats to ingest substances that they normally may not consume in large quantities. In our laboratory we have also replicated this extremely high intake of the G + SA mixture. In fact, we found that when we presented the glucose and saccharin solutions in separate bottles, rats will alternately drink from both bottles, mixing the glucose and saccharin solutions in appropriate quantities. (Smith et al., 1976, 1980,

1982; Smith and Foster, 1980). Hence, we used the G + SA solution as a vehicle for the corn oil. We divided the experiment into three phases. For the first phase, 30 male rats were divided into three groups that were given 24 h ingestion tests for 56 days. The first group received only water and powdered Purina Chow (PC) throughout the testing period. The second group received food and water for 7 days and then received the G + SA mixture for the remaining 49 days. The third group was treated like the second group for the first 14 days (a week of food and water followed by a week of food, water, and G + SA) and then in their third week of testing, 2% corn oil was blended with the G + SA mixture. In the fourth week the corn oil concentration was doubled to 4%. This doubling procedure was continued until the eighth week when the concentration of the corn oil was 64%.

The results of these manipulations can be seen in Figure 6.3. In the upper left panel it can be seen that the total caloric intake from the food, glucose, and corn

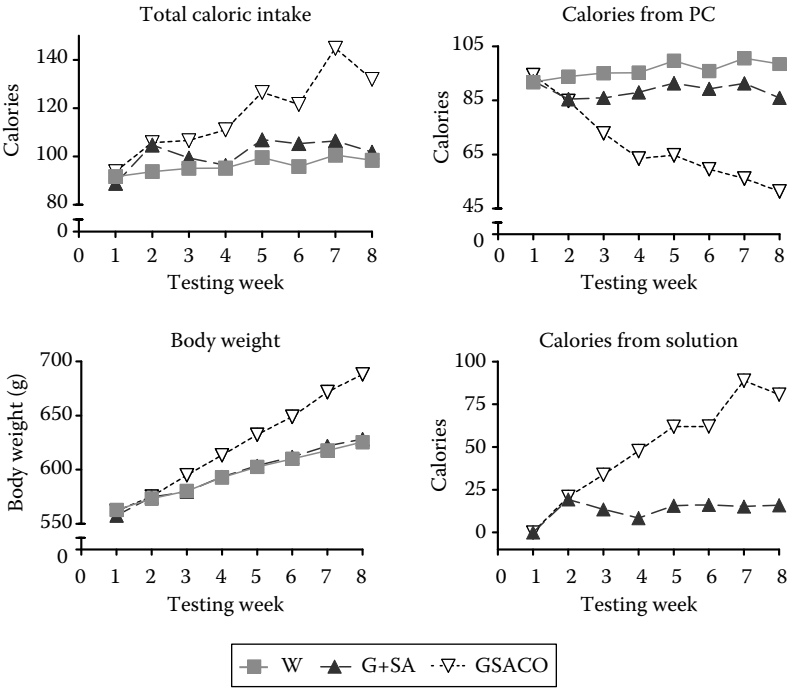

FIGURE 6.3 Fluid and powdered PC were measured daily in these rats over an 8-week period. The first group received nothing but chow and water (W) over the time of testing. Rats in the second group received food and water the first week and then a solution of glucose and saccharin was added for the ensuing weeks (G + SA). Rats in the third group received food and water the first week, then a solution of glucose and saccharin was added the second week, and on the third week corn oil was blended with the G + SA solution (GSACO). In the third week the concentration of the corn oil was 2%. The concentration of the corn oil doubled each week thereafter resulting in a 64% concentration during the eighth week. The upper left panel shows total caloric intake, the upper right panel shows calories from PC, the bottom right panel shows calories from the solutions, and body weight over the 8-week period is given in the bottom left panel.

oil (only group 3 had corn oil) rose as expected as the concentration of the corn oil was increased. The difference between the corn oil group and the other two groups became statistically significant at week 4 when the concentration of the corn oil had reached 4%. Body weight for the corn oil group began to significantly increase after the third week of testing when they first consumed the 2% corn oil mixture (lower left panel). As can be seen in the upper right panel, caloric intake from the PC was significantly lower for the second group (G + SA) as compared to the food–water control group (W) because of the calories from the glucose in the solution. The caloric intake from PC for the corn oil group dropped significantly as the concentration of the oil increased over the 8-week period. However, the rats were still consuming about 50 cal a day from the PC when the oil concentration was at 64%. This was not enough reduction to compensate for the increase in calories from the solution as can be seen in the bottom right panel. Hence, the total calories went up over the course of these observations as a result of ingesting the sweetened corn oil mixture.

We wanted to take some measurements of the "taste" of the sweet–fat mixture of corn oil with G + SA to determine if this blend was more palatable to the rat than the sweetener alone. In order to get a more detailed measure of this intake, we repeated the procedure for the group that received the incremental increases in corn oil mixed with the G + SA solution. In this replication we collected the data in our rat "hotel" where we could get a measurement of rate of eating and drinking during an ingestive bout. These eight male rats received first week of PC and water, a second week of PC, water, and the G + SA mixture, and in their third week of testing they received the G + SA solution with corn oil added. The concentrations of corn oil were doubled each week until it increased to 64%. Similar to the weight increase reported in the earlier study, the rats in this group had a 35% increase in body weight over the 8-week period.

As can be seen in Figure 6.4, the overall rate of drinking during about increased significantly as the concentration of the corn oil went from 2% to 64%. This measure does not reflect any marked increase in the local lick rate (usually around 6 s^{-1}) but points out that the rat is licking more steadily without an excessive amount of pausing. As mentioned earlier in this chapter, when dealing with sucrose ingestion, we inferred that this was a measure of the taste-related motivational response of the rat.

6.3.3 Taste, Textural, and Olfactory Factors Influence the Ingestion of a Corn Oil and Sucrose Mixture

We conducted a study somewhat like the earlier study with G + SA where we mixed sucrose with the corn oil. In this study we divided 30 male rats into three groups. One group received no corn oil, a second group received 4% corn oil, and the third group was given 32% corn oil. Over several days sucrose was added to the solutions in a concentration series including 0.25, 0.5, and 1.0 M. The calories from food and fluid can be seen in Figure 6.5. It can be seen from this figure that when no fat was available (left panel) the total caloric adjustment was quite good. When the solution contained 32% corn oil the rats failed to decrease PC intake and ingested

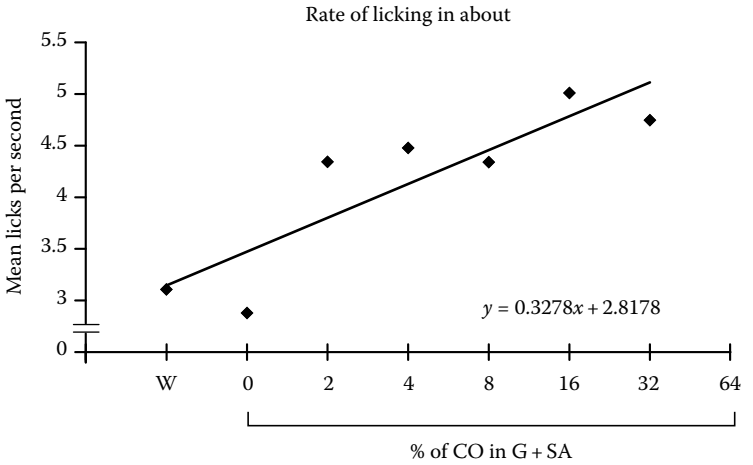

FIGURE 6.4 Licks per second within a drinking bout are plotted as a function of increasing the corn oil concentration that is blended in the G + SA solution starting in the third week of testing. During the first week of testing the rats received only water and chow. The trend line is plotted showing a correlation of 0.86.

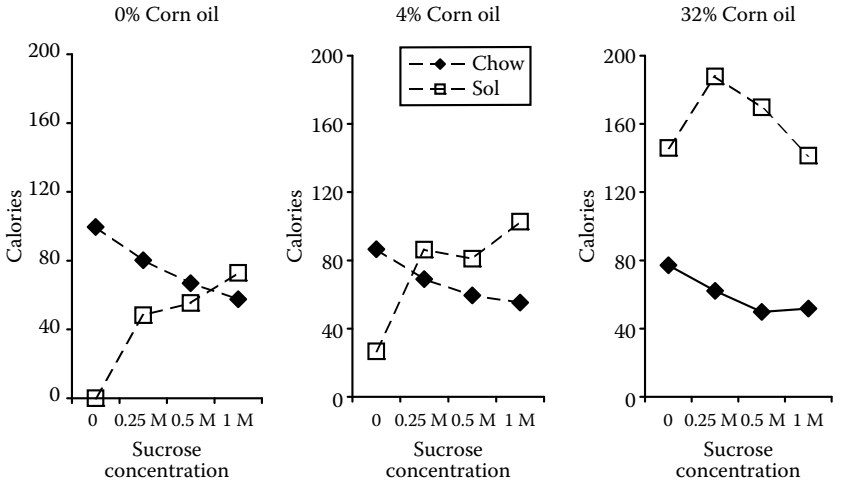

FIGURE 6.5 Mean calories from chow and sucrose solutions are shown for sucrose concentrations of 0, 0.25, 0.50, and 1 M and corn oil concentrations of 0%, 4%, and 32%.

significantly more daily calories. In Figure 6.6 the proportion of total daily calories from food is plotted. The analysis of these data yielded a significant difference between the corn oil groups, a significant difference across the sucrose concentrations, and a significant interaction. Post hoc comparisons show that from 0.25 to 1.0 M there was a significant decrease in food calories for both the 0% and 4% corn oil groups. In contrast, the group receiving 32% corn oil showed no decrease in calories from PC as the sucrose concentration increased.

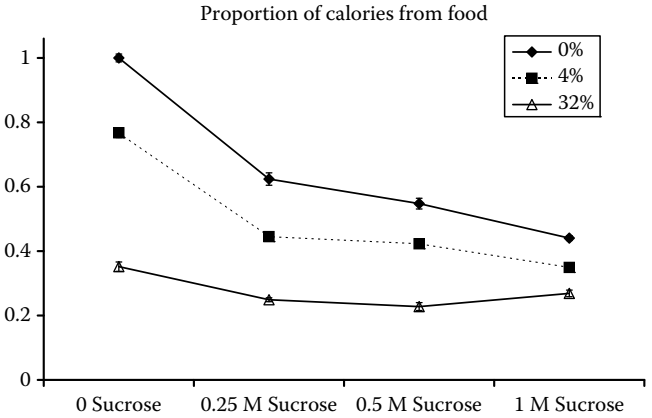

FIGURE 6.6 Proportion of calories from PC are given for each of the sucrose and corn oil combinations indicated in the figure.

When ingesting higher concentrations of sucrose without added fat, rats adjusted their total caloric intake much more efficiently than when given either the corn oil–G + SA mixture or the corn oil–sucrose (Smith, 2000). In fact, in a study where we gave Fisher 344 rats high concentrations of sucrose for a lifetime, they gained very little extra weight. In that longitudinal study, as caloric intake increased with the increase in sucrose concentration, the rats lowered their chow calorie intake appropriately. Rats that were given 1 M sucrose took 50% of their calories from the solution and showed no marked increase in calories over this 28-month span (Smith et al., 1992). As was seen in Sclafani's data and from our present data, if corn oil is added to the sweetener the rats do not appropriately reduce their chow caloric intake as they increase their calories from the sweet-fat solutions. The result is a significant increase in body weight.

The orosensory factors that influence the ingestion of corn oil involve not only taste, but also texture and olfaction. We conducted several experiments designed to further understand why the mixture of sweet and corn oil results in such large caloric intake. Using a CTA design, we used a mixture of sucrose and corn oil as the CS (Smith et al., 2000). Forty-two male and female rats (we found no differences between the sexes) were subjected to mild water deprivation and on the conditioning day were given a 0.25 M sucrose solution blended with 16% corn oil. Twenty-two were then given the LiCl injection and 20 control animals received the saline injection. Forty-eight hours later all animals were given a 90 min preference test between the sucrose–corn oil mixture and water (SUCO vs. W). It can be seen from Figure 6.7 that the LiCl-injected rats showed a profound aversion to the SU + CO mixture. The following day all rats were given a preference test between sucrose and water (SU vs. W). The preference for sucrose in the lithium-injected rats was significantly reduced, but it was not profound. The third preference test was between the corn oil and water (CO vs. W). Here again, there was a significant aversion to the corn oil. The final test was between corn oil and sucrose (SU vs. CO). As can be seen, the saline injected rats consumed both sucrose and corn oil,

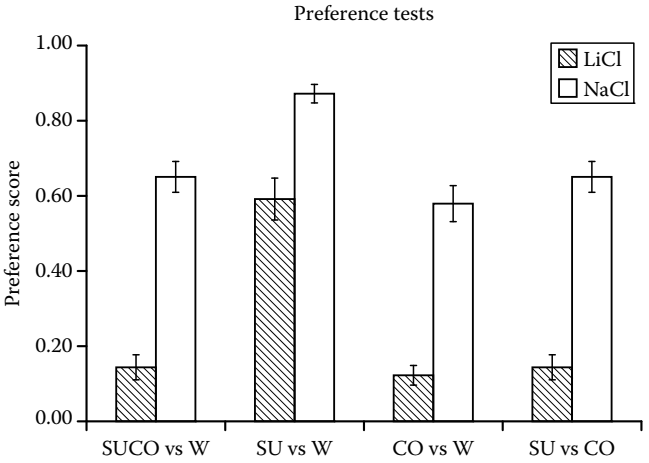

FIGURE 6.7 Preference scores are plotted comparing LiCl- and NaCl-injected rats for preference tests between corn oil + sucrose vs. water (SUCO vs. W), sucrose vs. water (SU vs. W), corn oil vs. water (CO vs. W), and sucrose vs. corn oil (SU vs. CO). For all four tests the differences between the LiCl and the NaCl groups were statistically significant.

but the lithium-injected animals significantly avoided the corn oil. We inferred that the salient feature of the sucrose–corn oil mixture was the corn oil. It could be argued that it is simply easier to get an aversion to corn oil than it is to sucrose. To test for this possibility, we conditioned two more groups of rats to sucrose ($N = 17$) or to corn oil ($N = 20$). Rats conditioned with sucrose showed a profound aversion to sucrose and rats conditioned with corn oil demonstrated a profound aversion to corn oil. The magnitude of these two aversion scores was not different, so it does not appear that it is easier to get an aversion to corn oil than an aversion to sucrose.

We have found that a good measure of a taste aversion is how long it lasts (Spector et al., 1981). We tested the extinction of the aversion to sucrose–corn oil. Twenty-eight male rats were given the 0.25 M sucrose and corn oil mixture for 10 min on conditioning day. All received an injection of LiCl following the drinking period. Forty-eight hours later, a 90 min preference test was initiated and these tests were repeated for the next 9 days. The preference test for the first group was between the sucrose–corn oil mixture and water (SU + CO); for second group the test was between sucrose and water (SU); for the third group the test was between corn oil and water (CO) and the rats in the fourth group received only water (W) during the test. The extinction curves can be seen in Figure 6.8. The rats that received corn oil alone or corn oil with sugar showed little, if any, extinction. The aversion to sucrose was extinguished by the third day of testing. The water group consumed about 25 mL of water during each test. Again we concluded from this more stringent measure of CTA that the corn oil is the salient feature of the sucrose–corn oil mixture.

The next test that we performed was to conduct the CTA protocol in the reverse order from the previous approach. We hypothesized that if corn oil were the salient feature of the sucrose–corn oil mixture, then if we used corn oil as the CS, we would get a good aversion to the mixture of sucrose and corn oil. In contrast, if we used

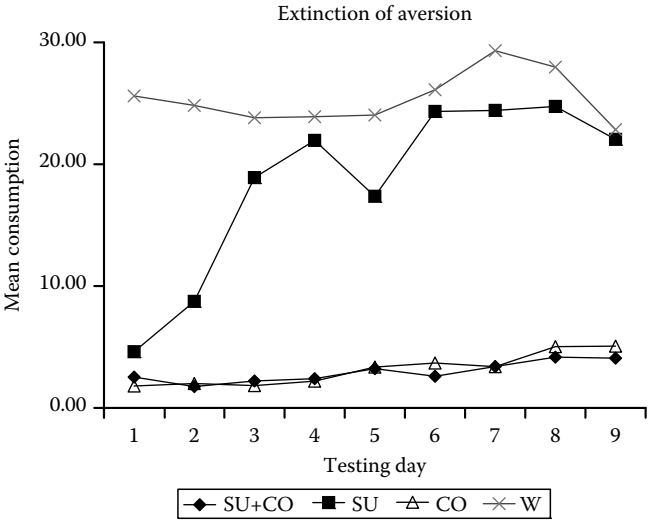

FIGURE 6.8 The intake of solutions is plotted over the 9 days after an aversion was induced by pairing a solution of sucrose (SU) and corn oil (CO) with an injection of LiCl. When tested with water (W), the mean daily intake was about 25 mL and it did not change significantly over the course of testing. There was a strong and significant aversion to both corn oil and corn oil + sucrose. In contrast, the aversion to sucrose was short-lived and complete extinction occurred by the third day of testing.

sucrose as the CS, we would get little or no aversion to the sucrose–corn oil mixture. Twenty-four rats were divided into two groups with one group receiving 0.25 M sucrose as the CS and the other group receiving 16% corn oil as the CS. The 90 min daily preference tests conducted for both groups were between the sucrose–corn oil mixture and water. The rats that received the corn oil as the CS showed a profound aversion to the mixture across 5 days of testing and the group that received sucrose as the CS initially showed a mild aversion to the mixture that extinguished completely by the fifth day of testing.

It is not clear why the corn oil would be the salient feature in the sucrose–corn oil mixture. The rat could be discriminating the corn oil by taste, texture, or olfaction. In order to test the possibility that texture plays a major role, we performed an experiment to see if the rat could discriminate a sucrose–corn oil mixture from sucrose mixed with mineral oil. We hypothesized that the lubricity of corn oil and mineral oil would be quite similar to the rat and would result in the same textural sensations. In this experiment, 20 rats were given a sucrose–corn oil mixture as the CS in a CTA design. After drinking the mixture for 15 min, half of the rats received an injection of LiCl and the other half got a saline injection. On both of the following 2 days the rats received a 1 h two-bottle preference test between a sucrose–corn oil mixture and a blend of sucrose with mineral oil. The sucrose concentration was 0.25 M and both oils were 16%. The control rats drank equal amounts of the two solutions, but the LiCl-injected rats consumed significantly more of the mineral oil blend than of the corn oil. These data are consistent with the data of Greenberg and

Smith (1996) in their procedure with sham-fed rats. They found that rats took an equal amount of the two solutions, but could easily discriminate between mineral and corn oil in subsequent tests.

6.3.4 Linoleic Acid (L) Is Detected in the Mouth but Is Not the Salient Feature of a Linoleic Acid–Sucrose Mixture

Gilbertson and others have proposed that linoleic, oleic, and other fatty acids are cleaved from corn oil in the rat's oral cavity as the result of lingual lipase being secreted from Von Ebner's gland. The rat would then get a gustatory sensation from the fatty acids (Gilbertson, 1998, 1999; Gilbertson et al., 1997, 1988a,b, 2005). We postulated that if we mixed linoleic acid with our sweeteners we should obtain results that are similar to what we found for mixtures of corn oil with sucrose or the glucose and saccharin solutions. The recordings made by Gilbertson showed that the taste cells were sensitive to concentrations of linoleic acid as low as 10 μM. Our first experiment was designed to see if rats could discriminate linoleic acid from water and then to determine the behavioral gustatory sensitivity to this fatty acid. We prepared the solution by dissolving 28 μL of linoleic acid in 1 mL of ethanol using a vortex mixer for 1 min. This solution was mixed into 4 L of deionized water. This linoleic mixture served as the CS in a CTA design. Following our standard water deprivation procedure we gave 20 male rats the linoleic acid solution for 15 min. Immediately after the CS period was over, we gave 10 of the rats a LiCl injection and the other 10 received an injection of NaCl. The preference test was initiated 24 h later and lasted for 1 h each day. An hour after the preference tests, the rats were given a water supplement for an hour in order to maintain good hydration. Rather than pairing the linoleic acid with water during the preference tests, the preference tests were between linoleic acid and ethanol solution (1 mL of ethanol in 4 L of water). The concentration of the linoleic acid was reduced each day of preference testing from 28 to 3.5 μM as can be seen in Figure 6.9. When compared to the NaCl-injected rats, the aversion was significant for the concentration of 28, 14, and 10.5 μM and not significant for the three lower concentrations. By this measurement, the threshold for detection would lie between 10.5 and 7 μM, a value that agrees with the electrophysiological findings of Gilbertson (Gilbertson, 1998, 1999; Gilbertson et al., 1997, 1998a,b, 2005).

In the next experiment we mixed the linoleic acid with the G + SA solution to see if the fatty acid would be a salient feature of that mixture as the corn oil proved to be in the previous experiments. Twenty male rats were given our standard water deprivation procedure and a mixture of linoleic acid in the G + SA solution was given for 15 min as the CS on conditioning day. After a 15 min period of drinking, half of the rats were given an injection of LiCl and the other half served as a control group receiving the saline injection. After a 24 h period with food and water available, a series of seven daily preference tests were administered in the following order: Day 1, G + SA + linoleic acid vs. water; Day 2, glucose vs. water; Day 3, saccharin vs. water; Day 4, linoleic acid vs. water; Day 5, G + SA vs. water; Day 6, G + linoleic acid vs. water; Day 7, saccharin + linoleic acid vs. water.

As can be seen in Figure 6.10, the rats developed an aversion to all combinations of the mixture of glucose, saccharin, and linoleic acid. Unlike the results from the

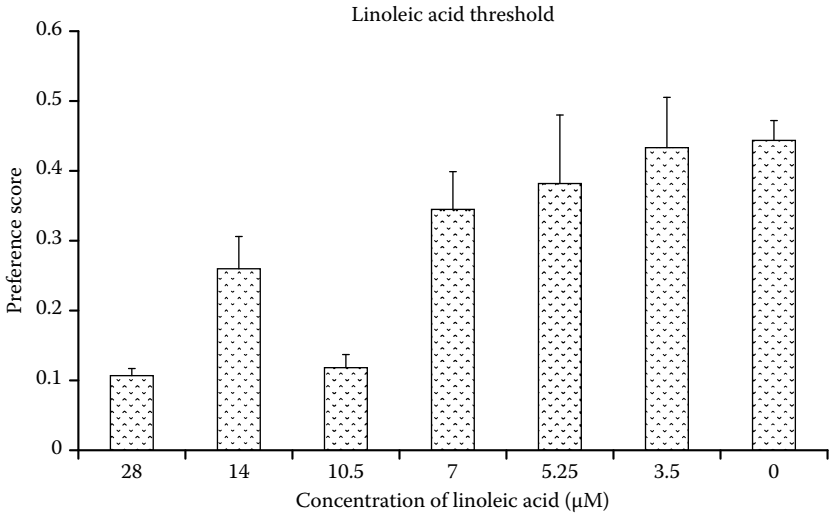

FIGURE 6.9 These rats were conditioned to avoid linoleic acid by pairing a 28 μM solution with a LiCl injection When tested subsequently, they showed a significant aversion to concentrations of 28, 14, and 10.5 μM linoleic acid but no aversion to concentrations of 7 μM and lower.

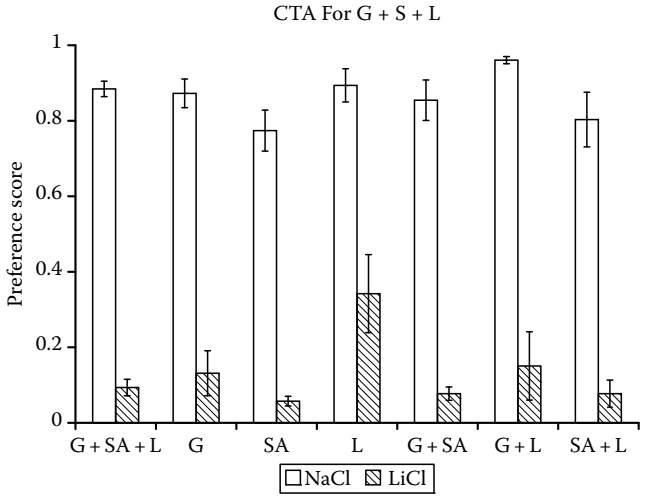

FIGURE 6.10 Linoleic acid (L) mixed with a glucose plus saccharin solution (G + SA + L) was used as the CS in a CTA design with half receiving a LiCl injection and the other half receiving a NaCl injection after drinking the solution. Seven preference tests were run comparing the solution indicated vs. water. Preference for the solution is plotted on the *y*-axis. The difference between LiCl- and NaCl-injected rats was statistically significant for all of the seven preference tests.

experiments with corn oil mixed with the sweetened solutions (see Figure 6.7), linoleic acid does not appear to be the salient feature of the solution when it is mixed with G + SA. However, the rats do show a significant aversion to the linoleic acid when it is tested without the G + SA, indicating that they can detect it in the mixture.

Our next experiment was to see if the rats could detect the linoleic acid when it was mixed with a high concentration of sucrose. In this experiment, 20 male rats were subjected to the standard water deprivation. On conditioning day they all received a 15 min CS period with a mixture of 0.25 M sucrose and 28 μM concentration of linoleic acid. Half the rats received the LiCl injection and the other half were injected with saline. Twenty-four hours later the first of three daily preference tests were given. These two-bottle tests lasted 1 h and the choices were as follows: Day 1, 0.25 M sucrose + 28 μM linoleic acid vs. water; Day 2, 0.25 M sucrose vs. water; Day 3, 28 μM linoleic acid vs. water.

In order to ensure that the small amount of ethanol that was a necessary addition in the solutions containing linoleic acid was not a factor, the same quantity of ethanol (4.3 mM) was added to the sucrose on Day 2 and the water in all of the preference tests. The results of this experiment can be seen in Figure 6.11. The preference in the LiCl-injected group is significantly lower than in the NaCl-injected group on all three tests. Once again we showed that the linoleic acid was not the salient feature of the sucrose–linoleic acid mixture (CS). However, we infer that the rat could taste the linoleic acid in the mixture. We failed to run extended testing in these two

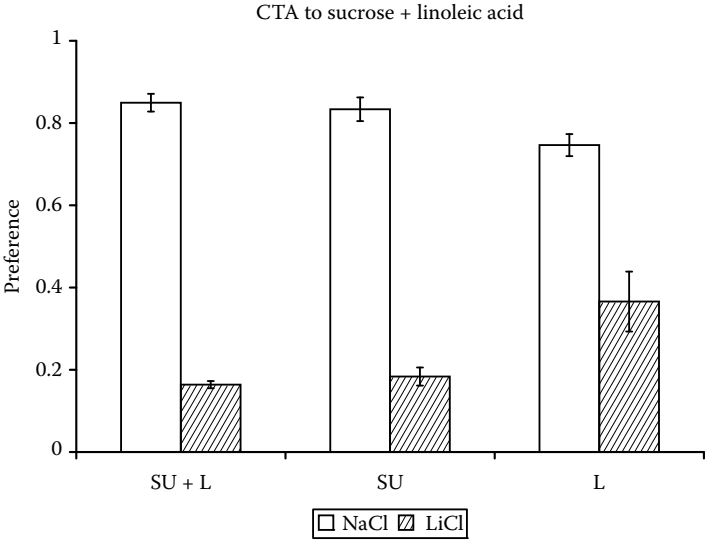

FIGURE 6.11 Linoleic acid mixed with sucrose (SU + L) was used as the CS in a CTA design with half of the rats receiving a LiCl injection and the other half receiving a NaCl injection after drinking the CS solution. Three preference tests were run comparing the solution indicated vs. water. Preference for the solution is plotted on the *y*-axis. The difference between LiCl- and NaCl-injected rats was statistically significant for all of the three preference tests.

experiments which may have revealed a more rapid extinction of the sweeteners than with the linoleic acid as we had seen previously with the corn oil mixtures.

6.3.5 Ethanol Is Detected by the Olfactory System

Because ethanol was used as the solvent for mixing the linoleic acid, we next tested to see if rats could detect the alcohol at the concentrations that we used. The ethanol was tested by adding 0.25 mL of ethanol to 1 L of deionized water (a concentration of 4.3 mM). Twenty male rats were given the standard water deprivation for our CTA design. The CS on conditioning day was 4.3 mM solution of ethanol. Half the rats received a LiCl injection and the other half received the saline injection. Twenty-four hours later, the first of seven daily 1 h preference tests was administered. The fluids available in the preference test were: Day 1, 4.3 mM ethanol vs. water; Day 2, 4.3 mM ethanol vs. water; Day 3, 3.4 mM ethanol vs. water; Day 4, 2.6 mM ethanol vs. water; Day 5, 1.7 mM ethanol vs. water; Day 6, 0.9 mM ethanol vs. water; Day 7, 0.43 mM ethanol vs. water.

It can be seen in Figure 6.12, there is a clear aversion to the ethanol solution at all but the lowest concentrations. The ANOVA for these data gave a significant main effect for LiCl vs. NaCl injection, across the concentrations and the interactions. The Fisher LSD comparisons were significant for all but the 0.43 mM concentration. The sensitivity of the rats to such low concentrations of ethanol was so unusual to us that we replicated this finding two additional times. Because of the controls that we

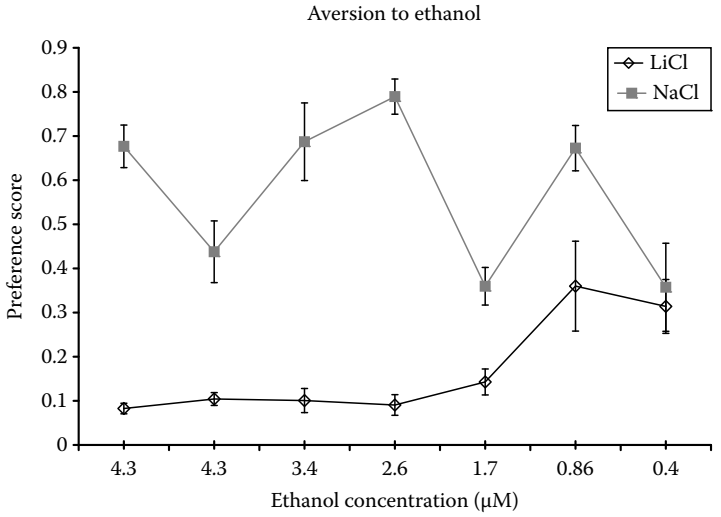

FIGURE 6.12 Ethanol at a concentration of 4.3 mM was used as the CS in a CTA design. After drinking the ethanol solution, half of the rats received a LiCl injection and the other half were given a NaCl injection. The ensuing two-bottle preference tests were given between ethanol and water. After the second day of testing the concentration of the ethanol was lowered each day. Preference for ethanol is plotted on the *y*-axis. The difference between LiCl- and NaCl-injected rats was statistically significant for all concentrations except 0.43 mM.

used in the previous experiments with linoleic acid (which was mixed with ethanol) we still conclude that the rats can taste linoleic acid, but we recommend that care be given in behavioral experiments where ethanol is used as a vehicle. It was not clear at this point if the rats discriminated the ethanol from water in these observations by gustation or by olfaction. Our next experiment was an effort to clarify this issue.

For testing the role of olfaction in ethanol detection, 28 male rats were divided into two groups of 14 each. One group was subjected to a surgical procedure where the olfactory bulbs were ablated (OLFFx) and the second group served as sham-operated controls (SHAM). All rats were given the standard CTA water deprivation procedure and on conditioning day they received 4.3 mM solution of ethanol as the CS for 15 min. The two groups were subdivided into two groups of seven each. Seven rats from the OLFx group received a LiCl injection (OLFx-LiCl) and the other seven received saline injections (OLFx-SHAM). The 14 sham-operated rats were also divided into two groups of seven each. Half of these sham-operated rats received the LiCl injection (SHAM-LiCl) and the other seven received a saline injection (SHAM-NaCl). Twenty-four hours later they were given a 1 h two-bottle preference test between ethanol and water. The results of this preference test can be seen in Figure 6.13. The sham-operated rats showed a significant aversion to the ethanol solution, but the OLFx group exhibited no aversion. We concluded that the rats were detecting the low concentrations of ethanol as seen in the previous experiments by olfaction and not by taste.

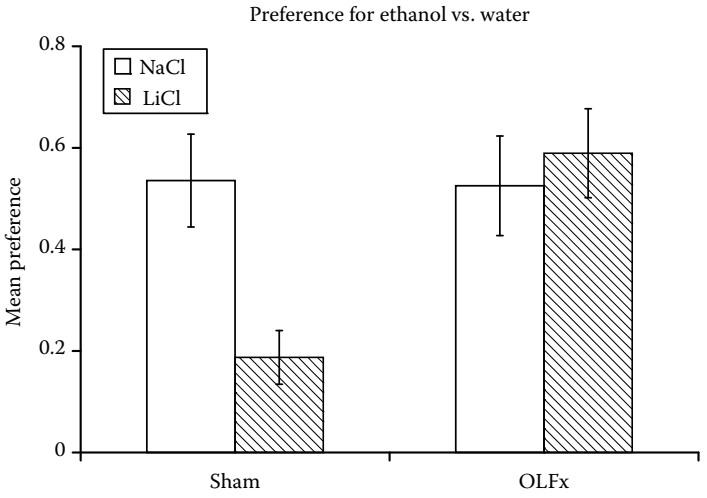

FIGURE 6.13 Half of a group of rats were subjected to bilateral olfactory bulb ablations and the other half were sham-operated. After recovery, ethanol at a concentration of 4.3 mM was used as the CS in a CTA design. After drinking the ethanol solution, half of the rats from the surgery and sham-operated groups received a LiCl injection and the other half were given a NaCl injection. The ensuing two-bottle preference tests were given between ethanol and water. The difference between LiCl- and NaCl-injected rats in the sham-operated group was statistically significant. There were no differences between LiCl- and NaCl-injected rats in the olfactory bulb-ablated rats.

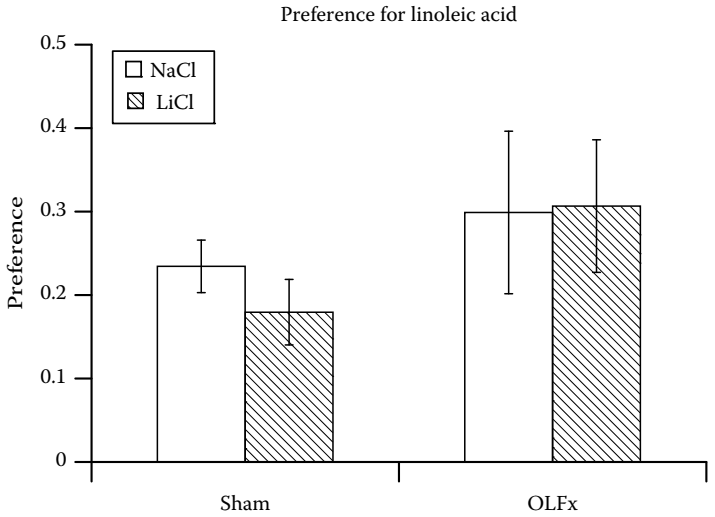

FIGURE 6.14 Half of a group of rats were subjected to bilateral olfactory bulb ablations and the other half were sham-operated. Following recovery from surgery, linoleic acid at a concentration of 28 μM was used as the CS in a CTA design. After drinking the linoleic acid solution, half of the rats from each of the surgery groups received a LiCl injection and the other half were given a NaCl injection. The ensuing two-bottle preference tests were given between linoleic acid and 4.3 mM ethanol. There was no difference between LiCl- and NaCl-injected rats in both the sham-operated and the olfactory bulb-ablated subjects.

We repeated this last experiment using 22 μM linoleic acid as the CS in a CTA design. Fourteen male rats had their olfactory bulbs ablated and an additional 14 were sham-operated. Half of each of these groups were given LiCl and half saline after drinking the linoleic acid mixture for 15 min. The following day they were given a single 1 h two-bottle preference test between 22 μM linoleic acid and 4.3 mM ethanol. The results from this manipulation can be seen in Figure 6.14. Olfactory bulb ablations had no effect on the discrimination of linoleic acid from water in this CTA design. In contrast to the results from the ethanol test above, we concluded that linoleic acid is detected by the gustatory, not the olfactory, system. This finding is in agreement with the work of Pittman and his colleagues (McCormack et al., 2006; Pittman et al., 2006, 2007, 2008) and Stratford and her colleagues (Stratford et al., 2006).

6.3.6 Brief Access Studies with the Davis Rig

"The control of fat intake is the result of interactions between fat palatability under orosensory control and the satiating and metabolic effects of fat under the control of postingestive factors. The relative importance of these factors in the control of intake is unknown" (Greenberg and Smith, 1996). The work described thus far in this chapter from our laboratory does little to separate the role of orosensory factors from the postingestive factors since our preference tests have been at least an hour

in length. It is clear that by the end of an hour, postingestive factors are playing a significant role in the ingestion of fat (Davis et al., 1995). However, most of the early research has emphasized texture and olfaction as the major contributors to the palatability of fat and have ignored the role of gustation. The work described here and in other chapters in this volume supports the contribution of the sense of taste proper in influencing fat intake.

Davis and colleagues (Davis et al., 1995) have thoroughly described the microstructure of corn oil drinking during 30 min tests. Depending on the concentration of the corn oil, the postingestive factors begin to have an effect after a few minutes of licking. Taste tests with the sham-feeding procedure essentially eliminate postingestive feedback and allow for an explicit study of the orosensory factors in fat ingestion. However, Davis' findings would indicate that very short-term taste tests could also allow for the study of orosensory factors while minimizing postingestive feedback. A second advantage to these short-term tests in the Davis Rig is that there was little time for the phase separation of the corn oil from the water in the solutions. Finally, it is possible to measure the latency of the first lick after the Davis Rig shutter is opened, an important dependent variable that is not normally available in the standard two-bottle preference tests. If the rat shows some discrimination of the solutions before taking a lick, it is likely that olfaction is contributing to the oral sensations (Rhinehart-Doty et al., 1994). Thus, we conducted several experiments with short-term ingestive tests using the Davis Rig.

Six rats were placed on our water restriction regimen and trained to lick on the water spouts in the Davis Rig. A range of concentrations of corn oil was mixed as described earlier and loaded in seven of the tubes. The rats were tested with 0%, 1%, 2%, 4%, 8%, 16%, and 32% corn oil. Three daily tests were conducted and the average number of licks per second was calculated. These data are plotted in Figure 6.15. The rate of licking increased as a function of the concentration of the corn oil in a similar manner as we found in the hotels earlier.

In the spirit of an experimental design used by P. T. Young (Young, 1966) to study the effectiveness of binary mixtures to stimulate licking in rats, we then made 30 solution combinations of sucrose and corn oil in order to test the lick rate as concentrations of both were increased. The concentrations of corn oil and sucrose used can be seen in Figure 6.16. When no fat was in the solution the lick rate rose as the concentration of sucrose was increased as was previously shown in Figure 6.2. When only 4% corn oil was added to the sucrose solutions, the rats were rapidly approaching their maximum rate of licking. In these very short-term drinking tests there is little probability that postingestive factors play any role in the rate of licking. We concluded that almost any combination of sucrose and fat is highly palatable to the rat.

With the Davis Rig we replicated some of the CTA experiments where two-bottle preference tests were used to measure the strength of the aversion. When using the Davis Rig, our dependent variables would be the number of lick made in 30 s and the latency to the first lick.

After 10 rats were trained to lick in the apparatus, we filled all of the tubes with 0.25 M sucrose mixed with 16% corn oil. The rats had the opportunity to lick on all eight tubes for 30 s each with a 30 s delay between the presentations of each of

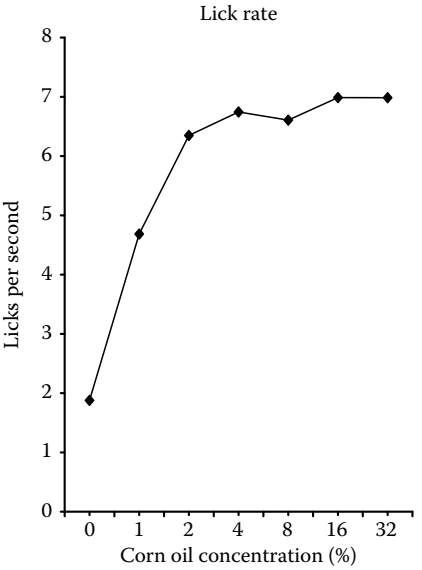

FIGURE 6.15 The number of licks per second as a function of corn oil concentration is plotted here. It can be seen that the rats are licking at a maximum rate when the concentration of the corn is 4% and higher.

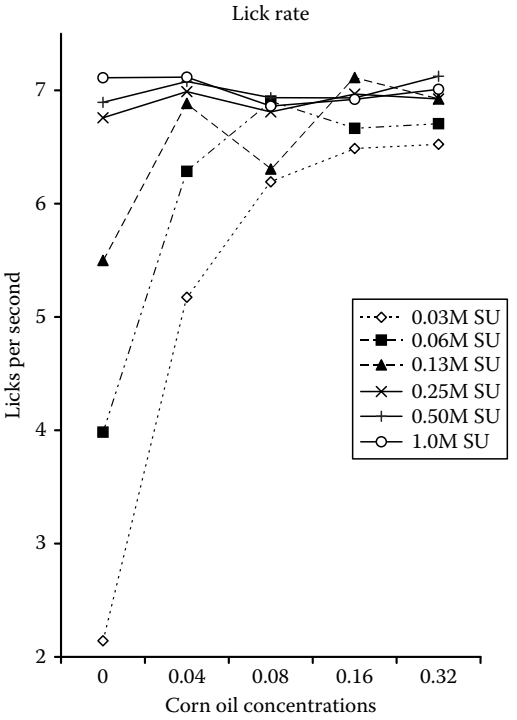

FIGURE 6.16 Licks per second are plotted as a function of the concentration of corn oil (*x*-axis) that has been blended with the concentrations of sucrose indicated in the box on the right-hand side.

the eight tubes. Five of the rats then received an injection of LiCl and the other five received the saline injection. The following day the tubes were filled as follows: Tubes 1 and 5 contained water; Tubes 2 and 6 contained the sucrose–corn oil mixture; Tubes 3 and 7 contained 25 M sucrose; Tubes 4 and 8 contained 16% corn oil.

The tubes were presented to the rats in order of 1–8. This presentation was repeated on the second day of testing. The results can be seen in Figure 6.17. The LiCl-injected rats licked very little when tubes were presented that contained corn oil when it was presented with or without sucrose. Quite like the results from the earlier two-bottle preference tests, the conditioned rats displayed no aversion to the sucrose. Because of the brevity of this test, we concluded that this discrimination of the corn oil was the result of an orosensory cue. When we measured the latency to the first lick for the LiCl-injected group we found that the latency to lick was significantly longer for the two tubes that contained the corn oil (Figure 6.18). This would give some evidence that the rats were sniffing at the opening and not licking until later in the 30 s period. As can be seen in this figure, the variation was quite high and the sample size was quite small for this observation.

We conducted another CTA experiment with the sucrose–corn oil mixture to test for a role of texture in the detection of corn oil. In this experiment, 22 rats were given

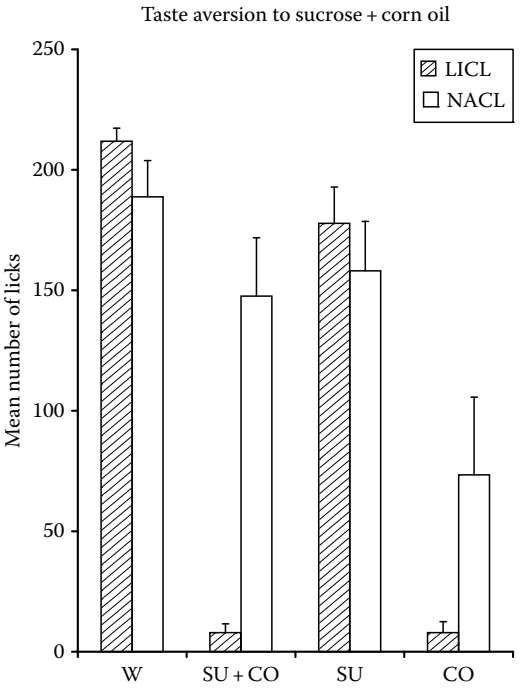

FIGURE 6.17 Taste aversion to a mixture of sucrose and corn oil (SU + CO) presented in the Davis Rig is plotted here. When sucrose plus corn oil or corn oil alone was presented in one of the drinking tubes there was a significant difference between LiCl- and NaCl-injected rats. When either water or sucrose was presented, there were no differences between the groups.

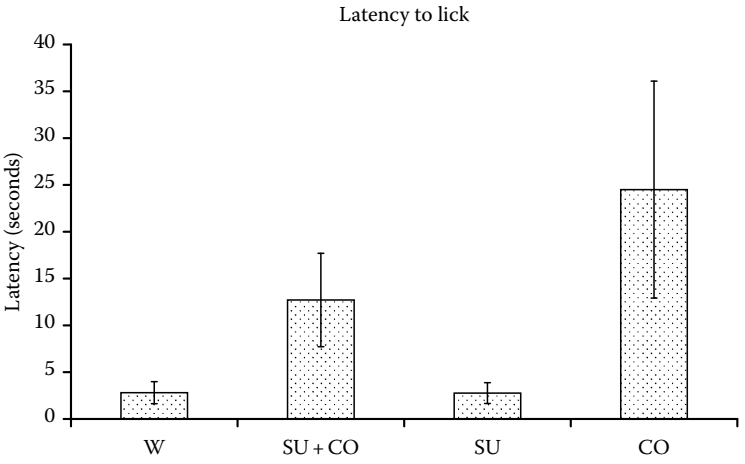

FIGURE 6.18 Latency to the first lick for the solutions indicated is presented here. If the solution presented contains corn oil, this latency is significantly longer than when either water or sucrose is presented.

training to lick in the Davis Rig. On conditioning day, the rats received the mixture of 0.25 M sucrose and 16% corn oil in each of the eight tubes. After drinking it, half of the rats were given an injection of LiCl and the other half received a saline injection. On the following day three tubes were filled with the sucrose–corn oil mixture, three with a sucrose–mineral oil mixture and the other two tubes with water. They were presented in random order. From Figure 6.19 it can be seen that on Day 1 the LiCl-injected rats developed a strong aversion to both corn and mineral oil as compared to water drinking. However, over the next 5 days, the aversion to mineral oil extinguished much faster than it did to the corn oil. The rats were significantly discriminating the corn oil from the mineral oil on the second day of testing. The saline injected rats showed no significant change in licking over the 6 days if testing, with about the same number of licks on the corn oil and the mineral oil tubes. These saline injected rats did lick significantly less on the water tube during the 30 s tests than to both corn oil and mineral oil.

Earlier we presented data showing that by using a mixture of glucose, saccharin, and corn oil as the CS in a CTA design, we could condition an aversion to any combination of these three compounds as long as it contained the corn oil. It was not clear if this conditioning was the result of orosensory factors only or if some postingestive factor played a role. Furthermore, we could not rule out olfaction as part of the orosensory sensation. We repeated that conditioning design by measuring the flavor aversion in a short-term test in the Davis Rig. Thus, we could minimize the role of postingestive factors and by measuring the latency to the first lick in the Davis Rig we could get some idea if olfaction played a role in that discrimination. Fourteen rats were trained to lick on the eight tubes in our Davis Rig. On conditioning day all eight tubes were filled with a mixture of glucose, saccharin, and corn oil (16% corn oil blended with the standard G + SA mixture). Following this drinking session, eight rats received the LiCl injection and the other six received a saline injection.

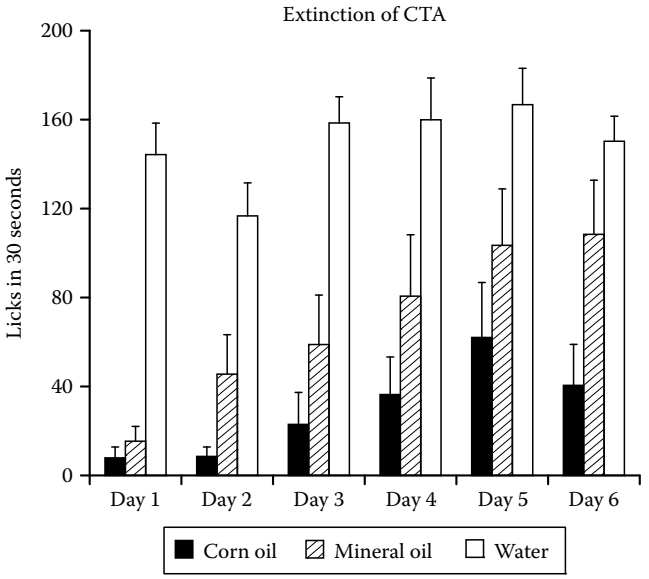

FIGURE 6.19 In this CTA experiment, corn oil was the CS followed by an injection of either LiCl or NaCl. There were subsequently tested with corn oil, mineral oil, or water in the testing tubes in the Davis Rig. It can be seen on the first day of testing that there is an aversion conditioned to corn oil that generalizes to the mineral oil. The aversion to the mineral oil extinguished at a faster rate that the extinction to the corn oil. In fact, there was a significant difference between the corn oil and the mineral oil on the second day of testing and this difference remained significant for the next 4 days.

The following day the tubes were filled as follows: Tube 1, water; Tube 2, glucose + saccharin + corn oil; Tube 3, glucose; Tube 4, saccharin; Tube 5, corn oil; Tube 6, glucose + saccharin; Tube 7, glucose + corn oil; and Tube 8, saccharin + corn oil.

The mean number of licks in each of the 30 s presentations can be seen in Figure 6.20. There was some aversion generalized to the saccharin (Tube 4) and to the glucose + saccharin (Tube 6), but the strong aversions were seen to the tubes that contained the corn oil. It was not surprising that we saw no aversion to the glucose since we previously have shown that the salient feature of the glucose and saccharin mixture is the saccharin. The brevity of this test allowed us to conclude that these aversions were most likely the result of orosensory factors. What we found most interesting was the latency to the first lick when the shutter in the Davis Rig opened. If the tube contained corn oil this latency was significantly longer as can be seen in Figure 6.21. The rats were detecting the aversive characteristics of the CS prior to taking their first lick.

For the final experiment to be reported in this chapter, we replicated the previous experiment using linoleic acid in place of corn oil in the mixtures. As can be seen in Figure 6.22 using G + SA + linoleic acid as the CS in the CTA design, the aversion generalized to all of the components of this mixture. The important result here is that the rats displayed an aversion to the linoleic acid when it was presented alone. It is

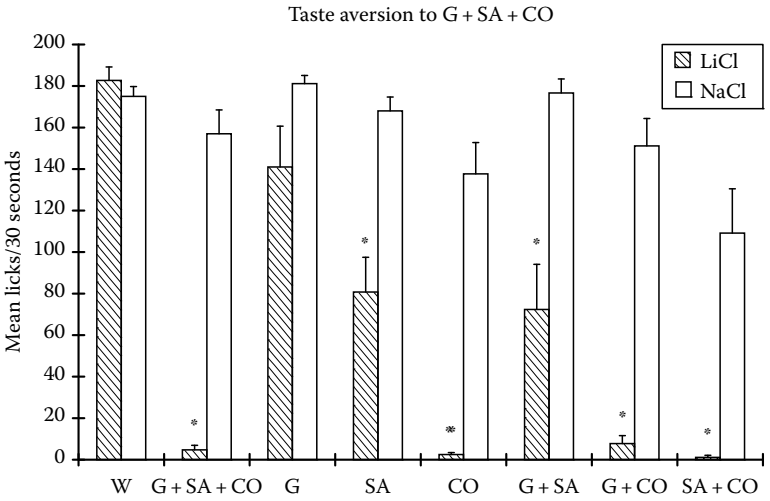

FIGURE 6.20 These rats were conditioned with glucose (G) + saccharin (SA) + corn oil (CO) and tested with the solutions indicated in the Davis Rig. Differences in the number of licks that were statistically significant between LiCl- and NaCl-injected groups are indicated by "*."

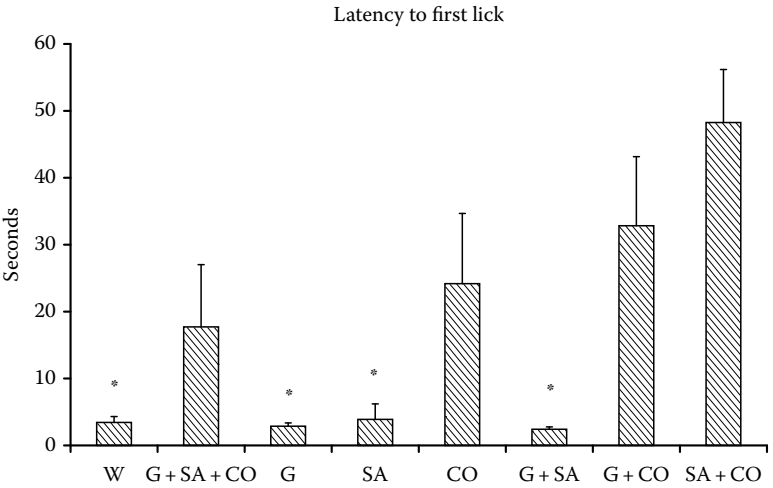

FIGURE 6.21 These rats were conditioned with glucose (G) + saccharin (SA) + corn oil (CO) and tested with the solutions indicated in the Davis Rig. Differences in the latency to the first lick that are statistically significant between LiCl- and NaCl-injected groups are indicated by "*"; water, W.

unfortunate that we did not run extinction trials here since it would have been interesting to see which components extinguished most rapidly. The latency to make the first lick was not different for either the tube containing the G + SA + linoleic acid or the linoleic acid alone. There was no sign that the rats were detecting the linoleic acid by olfaction.

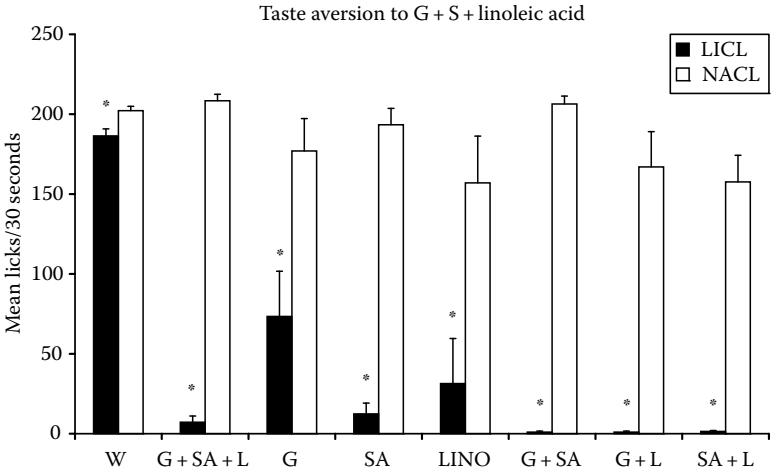

FIGURE 6.22 These rats were conditioned with glucose (G) + saccharin (SA) + linoleic acid (L) and tested with the solutions indicated in the Davis Rig. Differences in the number of licks that are statistically significant between LiCl- and NaCl-injected groups are indicated by "*"; water, W.

6.4 CONCLUDING REMARKS

My goal in this chapter was to report data from our laboratory here at The Florida State University on the orosensory factors involved in the ingestion of corn oil. Some of the data in this chapter have been presented at various professional meetings and some have been published in two papers in *Physiology and Behavior* (Smith et al., 2000; Smith, 2004). Much of the data reported in this chapter, however, have not been presented or published elsewhere.

Our research in my laboratory has been an attempt to contribute to our understanding that corn oil and corn oil mixed with sweeteners is detected by orosensory factors and does not rely solely on postingestive feedback. The data that have been presented support the idea that the orosensory detection of corn oil by the rat involves olfaction, texture, "and" taste. From our Davis Rig studies it can be seen that detection of corn oil is made before the rat takes its first lick on the drinking spout. By analogy with previous research showing that sucrose can be detected by olfaction (Rhinhart-Doty et al., 1994) we concluded that the rats can "smell" the corn oil. However, it does not appear that olfaction is the only route of detection since the rats initially confuse corn oil with mineral oil, presumably by texture. With repeated trials they learn to discriminate between these two oils. If indeed, lingual lipase from Von Ebner's glands sufficiently breaks the corn oil into its fatty acid components (Kawai and Fushiki, 2003), we have shown with behavioral techniques that the rats can easily detect linoleic acid at concentrations that would be present on the oral cavity. The detection of linoleic acid does not appear to be dependent on olfaction and we think it is unlikely that texture plays a role here. We found that rates of ingestion in our short-term behavioral tests, data from sham-feeding tests in other laboratories,

and electrophysiological data (where postingestional factors play no role) correlate well with the rates of licking within a drinking bout in long-term tests for both corn oil and for sucrose. We concluded that this relationship further buttresses the important contribution of gustation in fat ingestion, at least in the rat model.

It was noted that the rat seems unable to regulate total daily caloric control with corn oil as it does with sucrose alone. This led to considerable weight gain over prolonged ingestion of the oil which does not occur with sucrose alone.

We recommend that when using CTA as a measure of similarities among various tastes investigators should use duration of the aversion (extinction) as a dependent variable (Spector et al., 1981). We found this to be important in two cases from our data. When using a combination of sucrose and corn oil as the CS, the aversion generalized to both the sucrose and the corn oil in the initial postconditioning two-bottle test. By measuring the time course of extinction we found that the aversion to sucrose was quite fragile and disappeared in a few days, where the aversion to corn oil was much more robust, lasting much longer. We found a similar result when comparing the aversion to corn oil and mineral oil after using corn oil as the CS. Initially, the rats avoided both oils, but the aversion to corn oil appeared to be much more robust when testing with extinction as a variable.

Our data with the threshold for detecting ethanol proved to be most surprising. It did not seem possible that 1 mL of ethanol in 4 L of distilled water could be detected. We found that we could use this mixture as a CS in a taste aversion design and the rats reliably avoided this solution when compared to water. In fact, they continued to make this discrimination until we reached 0.1 mL in 4 L of water (the 0.43 mM concentration). Although we reported only one experiment on measuring this threshold in this chapter, we repeated this experiment two times. We found the same result except in one of the replications they could only discriminate 0.2 mL of ethanol in 4 L of water. It was pointed out in the chapter as a caution to investigators who may use ethanol as a vehicle for mixing substances for behavioral ingestive tests. We do not feel that this finding compromised any of our data regarding the tests with linoleic acid since we always added ethanol to the water in our two-bottle tests.

Finally, we attempted to emphasize the value of knowing "how" as well as "how much" a rat consumes when conducting behavioral ingestive research. The microstructure of licking in both the long- and the short-term ingestive tests had been invaluable in our previous work in understanding "how" the rat ingested sucrose solutions. In this chapter, we have shown that we can understand much more about the ingestion of corn oil by the addition of the microstructure analysis of licking behavior. In this chapter we have emphasized the rate of licking in a drinking bout, but there is much more to learn by studying the clusters of licking (Davis et al., 1995), the number and size of ingestive bouts (Smith et al., 1992; Smith, 2000) and the day–night patterns of the intake. These measures will ultimately help in understanding the orosensory factors involved in the ingestion of fat.

ACKNOWLEDGMENTS

As stated in the beginning of this chapter, I would like to thank Professors Gerard P. Smith and Anthony Sclafani for the many conversations and consultations over many

years. In my behavioral work with ingestion, I had concentrated on sweeteners and had not ever tested the ingestion of fat until challenged by these two scientists. The electrophysiological work of Professor Tim Gilbertson opened many doors about the possibility of fat detection via the gustatory system. My former graduate student (and now colleague), Alan Spector, has collaborated with me over many years regarding the sound ways to measure ingestive behavior. The development of the "rat hotel" would not have been possible without his contribution. Graduate students Laura Wilson Shaughnessy and Patrick Smith contributed to the early work with sugars. I had the pleasure of many conversations with two more recent graduate students in Professor Contreras' laboratory, Dave Pittman, and Jennifer Stratford.

My most sincere gratitude goes to the numerous undergraduate students who worked with me over the past decade. Julie Winchester, Elizabeth Fisher, Victoria Maleszewski, Erin Hawarah Brooks, Jodi R. Doty, Julie Schumm, Megan DenBleyker, Kim Ferencce, Barbara Thompson, Christina Riccardi, and Gwendolen B. OKeefe worked with us in the behavioral experiments. Thanks also go to the Technical Support Group: Stan Warmath, Ross Henderson, Paul Hendrick, Don Donaldson, and John Chalcraft.

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7 Fat Taste in Humans: Is It a Primary?

Richard D. Mattes

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The sense of taste is primitive (i.e., of early evolutionary origin), limited in repertoire and considered by most to be one of the minor senses. Along with olfaction and chemesthesis (chemical irritancy), it is a member of the chemical senses, reflecting the nature of the stimuli it transduces. In contrast to olfaction, which is capable of detecting stimuli emanating from near or far, taste is strictly a contact sense requiring effective stimuli to be in close proximity to its receptor cells. Despite

these humbling attributes, taste is a sense of particular importance to nutrition and health. This is increasingly apparent as evidence accumulates on the role of taste in the detection and metabolism of dietary fat, the topic of this chapter.

7.1 TASTE PRIMARIES

Historically, research on taste has focused on the number and nature of the primary qualities it comprises (Bartoshuk, 1978b). Dating back to Aristotle, there was considerable agreement on sweet, salty, sour, and bitter as primaries. Qualities such as alkaline, metallic, astringent, pungent, and harsh have been attributed to taste over the past 2 millennia, but are now viewed as being of chemesthetic or olfactory origin. Water or insipid has been suggested as well, but is currently discounted as the inclusion of a stimulus leading to the absence of sensation as a "primary" challenges logic. Some have argued that there are no primaries (Erickson and Covey, 1980; Schiffman et al., 1980; Erickson, 1982). This view is based on experimental observations such as the loss of quality identity in taste mixtures (Erickson, 1982) and discriminable differences among stimuli of a common quality (e.g., saltiness) (Schiffman et al., 1980). These data support a view that taste is synthetic, like vision, where primaries combine into qualities distinct from the component parts leading to the potential for an unlimited number of sensations. More recent studies have challenged these observations (Breslin et al., 1996; Keast and Breslin, 2002) and support is widespread that taste is comprised of a limited set of primaries.

Still, the number of primary qualities is in flux. A century ago the sensation elicited by glutamate was proposed as a taste primary (Ikeda, 2002). However, it was only with the recent identification of transduction mechanisms for glutamate (Chaudhari et al., 2002), and other amino acids (Nelson et al., 2002) that acceptance of "umami" as a taste primary was strengthened. Sodium glutamate remains the prototypical stimulus for this quality.

Analogous to the history with umami, "fatty" has been regarded as a primary taste quality at various times based on introspection. Aristotle included it in his list and viewed fatty as representing one end of a continuum bounded on the opposite end by saltiness (Paderborn, 1961). Interpretation of such a continuum is not clear, but may reflect more of a tactile or irritancy property. Thus, this scheme may reflect confusion between taste and somatosensory sensations. Indeed, Aristotle felt "…the sense of taste is an anomaly of touch" and "…has to be a type of touch, since taste is the realization of touchable food." Almost 2 millennia later, Jean Fernel, who authored the first major Western text on physiology, "De Naturali Parte Medicinae" (Fernel, 1581) also regarded fattiness as a primary. The claim was based on observation rather than experimental evidence, as was the approach of the time (Rothschuh, 1973). His view was not widely accepted then, or as recently as a decade ago. This is likely attributable to several points. First, it is readily apparent that the sensory impact of fat is predominantly due to its contribution to the mouthfeel of foods (e.g., viscosity, lubricity, moistness). Second, triglycerides, the predominant form of dietary fat, are of a size and structure that make binding to cell surface receptors or passage through channels of cell membranes unlikely. Thus,

there was no plausible transduction mechanism. Third, there are no taste-specific terms for fats or oils in common speech. Fourth, electrical stimulation of taste cells can elicit sensations of sour, salty, sweet, or bitter, as well as metallic, but does not lead to sensations attributed to fat exposure (Frank and Smith, 1991). Fifth, studies of water taste (i.e., taste quality reports to water or subadapting concentrations of taste compounds after exposures to higher concentrations of sapid substances), elicit sweet, sour, salty, and bitter sensations, but not fattiness (Bartoshuk, 1978a,b). However, identification of plausible transduction mechanisms, behavioral data from animal models, and data from controlled psychophysical as well as modified sham feeding (MSF) studies in humans are combining in support for a true taste component for dietary fat, or more specifically, fatty acids. Whether fat should be considered a taste primary in humans will be better evaluated following a review of this literature.

7.2 FAT TASTE TRANSDUCTION

Dietary fat is a substantive source of energy in humans and absorption efficiency is high, generally around 95% (Carey et al., 1983). Long-chain fatty acids are the predominant dietary form (Allison et al., 1999), but short-chain fatty acids can contribute small amounts of energy (Bergman, 1990). Fatty acids are also required for synthesis of a wide range of biologically active compounds involved with blood clotting, immune function, epithelial integrity, growth, fertility, erythrocyte structure, and neural function. Further, there is increasing recognition that fatty acids are important signaling molecules. They regulate gene expression through perixosome proliferator-activated receptors (PPARs) with resultant influences on processes ranging from atherosclerosis (Coburn and Abumrad, 2003) to insulin resistance (Ibrahimi and Abumrad, 2002; Coburn and Abumrad, 2003) to satiety (Hopman et al., 1984; Aponte et al., 1985; Xiong et al., 2004). Against such a backdrop, the existence of an oral chemosensory fatty acid detection system to aid in the identification of fat seems plausible.

The focus of recent work related to fat taste has been on free fatty acids (FFA), rather than triglycerides, oils, or solid dietary fats, because their structure makes them more likely signaling molecules. The source of these molecules in the oral cavity of humans has posed some concern. Esterified fatty acids are not effective ligands for presumptive receptors (e.g., Gilbertson et al., 1997) and although there is ample lingual lipase for generation of FFA in the oral cavity of rats (Kawai and Fushiki, 2003), existing evidence suggests the enzyme is below functional levels in adult humans (Spielman et al., 1993; Schiffman et al., 1998). Further, gradations of oral fat exposure are not accompanied by changes of salivary secretion, the presumptive carrier of hydrolytic enzymes (Hodson and Linden, 2004). However, enzymatic production of FFA to provide an adequate signal to taste receptor cells may not be required since low concentrations are present in dietary fats and these may be liberated during mastication (Weiss, 1970; Smith et al., 1986; Chow, 1992; Metzger et al., 1995; Ministry of Agriculture, Fisheries and Food, 1998; Mattes, 2001a). These molecules could serve as adequate stimuli through a number of candidate fatty acid transduction mechanisms (Table 7.1).

TABLE 7.1
Putative Taste Transduction Mechanisms for Fatty Acids; Location and Effective Stimuli

Mechanism	Site (Papillae)	Effective Stimuli
DRK	Fungiform, foliate, circumvallate	Fungiform: long-chain, *cis*-PUFA Foliate and circumvallate: long-chain PUFA and MUFA
CD36	Foliate, circumvallate	Long-chain SFA and UFA
GPR40	Circumvallate	C10–C16
GPR41	Foliate, circumvallate	SCFA
GPR43	Foliate, circumvallate	SCFA
GPR120	Fungiform, foliate, circumvallate	SFA and UFA C14–C20
Diffusion	Presumed all	All
Fatty acid transporters (FATP1, FATP2, FATP3, FATP4, FATP5)	FATP4 (lingual and palatal epithelium)	C10–C26
Modulation of receptors/ channels and/or membrane properties	Possibly fungiform	NA

PUFA, polyunsaturated fatty acids; MUFA, monounsaturated fatty acids; SFA, saturated fatty acids; UFA, unsaturated fatty acids; SCFA, short-chain fatty acids.

7.2.1 Delayed Rectifying Potassium (DRK) Channels

Electrophysiological studies show that isolated taste receptor cells from rat fungiform papillae depolarize when exposed to *cis*, long-chain, polyunsaturated fatty acids (PUFA) (Gilbertson et al., 1997). Linoleic (C18:2), linolenic (C18:3), arachidonic (C20:4), eicosapentaenoic (C20:5), and docosahexaenoic (C22:6) acids (DHA) all block outward potassium currents resulting in taste cell depolarization. The effects occur within tens of milliseconds and are reversible, but the time to reach maximal effect is considerably longer (10–30 min) than times observed with other basic taste qualities. Ten micromolar (μM) concentrations are known to be effective, but no studies of threshold effects for this mechanism have been reported. When esterified, *cis*, long-chain, PUFA are not effective channel blockers. With cells from fungiform papillae, 18 carbon monounsaturated, oleic (C18:1) and saturated, stearic (C18:0) fatty acids are neither effective, nor are shorter chain saturated fatty acids (e.g., caproic (C6:0), capric (C10:0), lauric (C12:0), myristic (C14:0), palmitic (C16:0)), or monounsaturated fatty acids (e.g., palmitoleic (C16:1) erucic (C22:1), nervonic (C24:1)). Fatty acids in the *trans* configuration (linolelaidic (C18:2), linolenelaidic (C18:3)) are also ineffective. This lack of effect for certain compounds holds for exposures ranging up to 100 μM. A less specific detection system for fatty acids

involving DRK in cells of circumvallate and foliate papillae has also been proposed (Hansen and Gilbertson, 2005).

7.2.2 CD36

The presence of a fatty acid transporter (FAT) in taste cells of rats was first reported in 1997 and was proposed to contribute to taste sensitivity (Fukuwatari et al., 1997). It shares 85% homology with human CD36, a ubiquitous fatty acid scavenger and, as such, is considered the rat ortholog of human CD36. In that early report, Northern blot analyses documented the presence of mRNA in the epithelial layer of circumvallate papillae and immunohistochemical staining localized it further to the apical region of cells in taste buds. The homolog was neither observed in nongustatory epithelium nor in the anterior tongue.

Subsequent studies corroborated and extended these findings. CD36 was confirmed in circumvallate papillae, as well as, to a lesser extent, in the foliate papillae of mice (Laugerette et al., 2005). Several groups have demonstrated that unlike wild-type mice, CD36 knock-out animals express no preference for fatty acids (Fushiki and Kawai, 2005; Laugerette et al., 2005), but they continue to exhibit a preference for sucrose and avoidance to quinine (Laugerette et al., 2005). Thus, the effects were lipid-specific.

These trials were short term to minimize documented (Suzuki et al., 2006) postingestive effects on preference. CD36 knock-out mice also consume equal quantities of 5% or 10% linoleic acid or nonnutritive paraffin oil in 1 h choice tests whereas wild-type mice prefer a PUFA stimulus. Recent studies in CD36 knock-out mice demonstrate CD36 is required for long-chain saturated and unsaturated fatty acid stimulated Ca^{2+} increases in taste bud cells (Gaillard et al., 2008).

CD36 has a binding affinity for long-chain fatty acids in the nanomolar range (Baillie et al., 1996; Ibrahimi et al., 1996) and binding is reversible (Baillie et al., 1996). Whether CD36 acts as a FAT or binding site for selected long-chain FFA has not been resolved. One hypothesis holds that CD36 acts as a docking site for *cis* long-chain PUFA and facilitates their blockage of DRK (Gilbertson, 1998). Its role in human taste has not been explored, but may be more complex because this model fails to account for observed sensitivities of humans for saturated fatty acids, stimuli that bind with CD36 (Baillie et al., 1996), but fail to alter DRK currents (Gilbertson et al., 1997). Fatty acid peroxidation products may also be able to bind to CD36 (Baillie and Abumrad, 1996). Such compounds are well recognized as effective olfactory stimuli, but there is limited knowledge of their efficacy as taste stimuli. In one human trial precluding olfactory stimulation, thresholds for oxidized FFA were of similar concentration to nonoxidized FFA (Chale-Rush et al., 2007).

7.2.3 G-Protein–Coupled Receptors

G-protein–coupled receptors (GPR) reportedly serve a nutrient sensing role in the human gastrointestinal (GI) tract (Fredriksson et al., 2003). They are also integral to transduction of sweet, bitter, and umami tastes (Hoon et al., 1999; Nelson et al.,

2001, 2002). The increasing evidence for common nutrient signaling systems in gustatory epithelium and the gut (Fushiki et al., 2002) provides a basis for exploring the role of GPR in FFA taste.

GPR120 has recently been identified in the apical region of rat taste bud cells from circumvallate, foliate, and fungiform papillae (Damak et al., 2007; Matsumura et al., 2007). It has not been detected in rat nongustatory oral epithelium. GPR120 is also expressed in human taste cells (Damak et al., 2007). GPR120 binds longer-chain saturated (C14–C18) and unsaturated (C16–C22) fatty acids (Hirasawa et al., 2005). It does not bind esterified fatty acids or medium-chain FFA (C8–C12) (Hirasawa et al., 2005; Damak et al., 2007). GPR120 is expressed in cells responding to sweet, bitter, and umami stimuli (Damak et al., 2007). This raises the question of whether fatty acids binding to GPR120 are direct taste stimuli or modulate responses of cells to other "basic" taste qualities.

GPR40 has been detected in taste cells from rat circumvallate papillae, but to a lesser degree than GPR120 (Damak et al., 2007). Another trial failed to identify this receptor in similar cells (Matsumura et al., 2007). Further support for a role of GPR40 in FFA taste comes from the observation that fat preference and intake are reduced in GPR40 knock-out mice compared to wild-type animals (Damak et al., 2007). Knock-outs show lower preference for 30% corn oil and 0.3% oleic acid and less ingestion of 30% corn oil, 3% lauric acid (C12:0), as well as 1% intralipid (Damak et al., 2006). Measurements of intracellular Ca^{2+} following stimulation of Chinese hamster ovary cells transfected with human GPR40 cDNA support these behavioral data from mice. They indicate saturated, medium-, and long-chain FFA (C8–C18), as well as long-chain unsaturated FFA (C18–C22), are ligands for GPR40 (Itoh et al., 2003). Further, GPR40 activation is dose dependent and directly related to chain length for saturated FFA, but not unsaturated FFA (Briscoe et al., 2003). Short-chain FFA (C2–C5) are not bound (Briscoe et al., 2003; Itoh et al., 2003). Importantly, while GPR40 knock-out animals have a lower preference for fat emulsions, they are still able to detect them and prefer them over vehicle. This suggests the presence of multiple fatty acid transduction systems.

Preliminary evidence indicates that GPR41 and GPR43 are expressed in taste cells from rat circumvallate and foliate papillae (Hansen et al., 2006). The proteins are also expressed in trigeminal neurons. Human GPR41 binds short-chain FFA (C1–C6), but not medium- or long-chain saturated or unsaturated FFA (Brown et al., 2003; LePoul et al., 2003; Xiong et al., 2004). The relative potency is C3–C5 greater than C2 which is greater than C1 (Brown et al., 2003). GPR43 also binds short-chain FFA (C1–C6), but not medium- or long-chain FFA (Brown et al., 2003; LePoul et al., 2003; Xiong et al., 2004). The relative potency generally diminishes with chain length, C2–C4 are greater than C5 which is greater than C6. However, formate (C1) has the weakest potency (Brown et al., 2003). Thus, the range of fatty acids bound by GPR43 is similar to that of GPR41, but with different rank order of strength. Evidence for a contribution of these receptors is only presumptive. However, recent psychophysical data (Mattes, 2008) indicate humans can detect short-chain fatty acids, possibly by taste, suggests further exploration of these transduction mechanisms is warranted.

7.2.4 Fatty Acid Transport Proteins

CD36 and GPRs may serve as nutrient detectors, but their role as FATs in taste cells is not established. Another family of proteins, fatty acid transport proteins (FATP) may serve this function (Stahl et al., 1999). FATP4 may be a particularly good candidate to explore as it is widely distributed in lingual and palatal mucosa (Laugerette et al., 2005) and, based on studies with enterocytes, binds saturated and unsaturated FFA between C10 and C26. It does not bind to short-chain or esterified FFA. Thus, it appears to compliment CD36. FATP5 is another attractive candidate since knock-outs reduce energy intake on a high-fat diet and this does not appear to be attributable to shifts of satiety hormones or digestive efficiency (Hubbard et al., 2006). Changes of taste have not been explored in this model. Members of the FATP family may also have enzymatic lipase activity which would trap FFA intracellularly and create a gradient to draw FFA into cells and activate downstream transduction mechanisms.

7.2.5 Passive Diffusion, Flip/Flop

While the evidence for fatty acid receptors on taste cells is compelling, this does not preclude nonprotein-mediated transduction mechanisms. Diffusion of lipophilic sweet and bitter compounds across taste cell membranes with activation of signaling pathways at intracellular sites (DeSimone, 2000; Peri et al., 2000; Zubare-Samuelov et al., 2005) demonstrates another potential mechanism for FFA taste transduction. The lipophilic, amphipathic (i.e., polar head group, hydrophobic tail) nature of FFA facilitates their migration to taste cell membranes and passive diffusion across them. The principal question about FFA passive diffusion is that the rate of movement across the membrane is unknown. Movement must be rapid to account for common taste experiences. There is evidence, in model lipid bilayers and nongustatory tissue, that FFA move rapidly (within milliseconds) and in high (millimolar) concentrations (Hamilton and Kamp, 1999). Some evidence from nongustatory tissue suggests that, when FFA availability is not rate limiting, diffusion is the predominant route of passage across membranes (Ibrahimi and Abumrad, 2002). There is little or no difference in long-chain FFA uptake by adipocytes from CD36 knock-out and wild-type mice (Febbraio et al., 1999). Further, uptake of short- and medium-chain FFA into cells is independent of CD36 (Ibrahimi and Abumrad, 2002). Other work supports a primary role for a saturable protein-mediated process (e.g., Baillie et al., 1996). To reconcile these data, it has been proposed that at low concentrations of FFA, receptor binding is linearly related to FFA availability. Above saturation, FFA translocation is predominantly determined by diffusion coefficients (Baillie et al., 1996) which vary directly with FFA chain length (Kamp and Hamilton, 2006). However, translocation is a multistep process requiring movement from the lipid source to the cell membrane, diffusion across the membrane, followed by desorption into the cytosol. Longer chain FFA are more hydrophobic so, when released from a carrier (e.g., food matrix), they will readily partition into lipid-rich membranes. Shorter chain FFA would require somewhat longer times to access the membrane, but once in contact, would quickly diffuse across (Ho, 1992). Permeability coefficients for octanoic

(C8) and capric (C10) acids are more than a 1000-fold higher than water (Kamp and Hamilton, 2006). Longer chain fatty acids would take longer, yet still move rapidly; approximately 1 s (Kamp and Hamilton, 2006). Desorption rates are less than 1 s and are more rapid for shorter chain and unsaturated fatty acids (Hamilton et al., 2002). Once within the cell, the FFA can be rapidly bound to intracellular proteins or trapped through metabolic processes (Mashek and Coleman, 2006). The role of diffusion of FFA across taste receptor cell membranes in transduction of fat taste has not been examined. However, in light of the ubiquity and speed of this process in multiple cell types in the body, assessment of this mechanism is warranted. Consistent with predicted diffusion rates, Ca^{2+} influx in mouse taste bud cells was greatest for palmitic acid followed by linoleic acid and then DHA (Gaillard et al., 2008).

7.2.6 Modulation of Taste Receptor Cell Responsiveness to Other Stimuli by FFA

FFA may also be detected by effects they exert on transduction mechanisms for other taste qualities. In one report (Gilbertson et al., 2005) using a 48 h two-bottle preference test, S5B rats had higher preference responses to a subthreshold 0.5 M saccharin solution containing 5 or 20 μM linoleic acid relative to the same concentrations of saccharin or linoleic acid alone. The addition of lauric acid to saccharin did not enhance responses, suggesting some specificity to the effect. Strain differences were also reported as heightened preferences were not observed with Osborne–Mendel rats. In a subsequent study (Pittman et al., 2006), 88 μM linoleic or oleic acid, or a combination of the two, were added to a suprathreshold range of sucrose, glucose, NaCl, quinine, and citric acid solutions. Preference was assessed by lick rates to 20 s stimulus exposures. Generally, the findings were consistent with a view that the addition of the FFA augmented the intensity of the nonfat stimuli. The addition of linoleic acid led to significantly increased responses for several concentrations of sucrose and glucose. In contrast, reductions of licking were observed with NaCl, citric acid, and quinine solutions. A comparable pattern was observed with oleic acid alone or a combination of the FFA suggesting a common transduction mechanism. The heightened rejection of sour and bitter taste stimuli may demonstrate signal enhancement effects as they are generally avoided tastes. However, the rejection of NaCl is difficult to interpret since it is generally regarded as inherently pleasant (Beauchamp, 1987). The most recent work by this group has not replicated the findings of FFA modulation of other taste qualities (McCormack et al., 2006).

Human trials exploring this phenomenon have failed to replicate these findings. In one, 17 male and 17 female adults were tested for taste thresholds and suprathreshold responsiveness to sucrose, NaCl, citric acid, and caffeine in the presence and absence of 1% (w/v) linoleic acid (Mattes, 2007). Contrary to predictions based on the animal studies, the addition of linoleic acid significantly raised threshold concentrations for NaCl, citric acid, and caffeine and, generally left unchanged or lowered intensity ratings for the four taste quality prototypes. In another trial, intensity ratings of sweet, salty, and sour taste compounds (species not described) were unaffected by the addition of oil where the triglycerides were enriched with DHA. The bitterness of quinine sulfate was diminished and umami (inosine monophosphate (IMP) + monosodium

glutamate (MSG)) taste was enhanced. These discrepant findings may be due to true species differences or methodological issues such as the higher concentration of FFA used in the human trials.

7.2.7 Whole Nerve Recordings and Transections of Centrally Projecting Neurons

While identification of transduction mechanisms for FFA by taste receptor cells is an important element in the argument that FFA are effective stimuli, evidence that transduction is coupled with neural activation is equally vital. To date, there has been only limited study of taste nerve involvement. One study reported male Sprague-Dawley rats were able to detect <3.0 μM linoleic acid and thresholds for females were about 11 μM (Stratford et al., 2006). Bilateral chorda tympani nerve transection increased linoleic acid taste thresholds to approximately 22 μM in both sexes. In another study with male and female Sprague-Dawley rats, chorda tympani cuts eliminated avoidance of 88 μM linoleic acid, to which an aversion had previously been established, whereas preferences were still noted for corn oil (Pittman et al., 2007). These findings indicate that the chorda tympani nerve is involved with FFA detection, but also that it is not solely responsible since taste responses to fats were not abolished by the cuts. The latter point is consistent with evidence indicating transduction may occur by taste cells in the foliate and circumvallate papillae which are subserved by the glossopharyngeal nerve. There may also be vagal input from taste cells in other regions of the oropharyngeal region. In a follow-up study by the same group, recordings were obtained from geniculate ganglion gustatory neurons (i.e., inclusive of chorda tympani fibers) of adult Sprague-Dawley rats exposed to 11, 22, 44, or 88 μM linoleic acid (Breza et al., 2007). No responses were observed while stimulation with sucrose and MSG remained effective. An explanation for these discrepant findings is not apparent. It was suggested that linoleic acid may modify taste cell responsivity to other qualities, but the thresholds measured in the earlier study were not for complex taste stimuli. Another possibility holds that only a small subset of chorda tympani fibers convey FFA information and their contribution to whole nerve recordings is masked by other input (Pittman et al., 2007). More recent work noted glossopharyngeal nerve cuts diminish the preference for 2% linoleic acid in mice in 30 min, two-bottle choice tests, and eliminates the preference in 48 h tests (Gaillard et al., 2008). Concurrent sectioning of the chorda tympani nerve eliminates differential responses in both tests, as it did for 4% sucrose and 0.1 mM denatonium. Further, sectioning of the glossopharyngeal alone or with the chorda tympani nerve reduced exocrine pancreatic secretions following oral linoleic fatty acid exposure (Gaillard et al., 2008). The data support a role played by both nerves in FFA detection, but do not establish whether the effect of the cut was to eliminate conveyance of signal by the nerve versus loss of viability of the taste cells they innervate. The nerve cut findings do indicate that tactile input is not required for FFA detection as discrimination of fat was abolished without elimination of trigeminal fibers.

Based on neural recordings from the orbitofrontal cortex of macaques, fat perception is predominantly attributable to textural cues, but odor is also effective (Verhagen et al., 2003). A contribution of taste was discounted, but could not be eliminated as a

subpopulation of cells responded to oral fat exposure but not to viscosity cues. There was a lack of response to 100 μM concentrations of linoleic and lauric acids, while oils led to activation in a small subset of neurons. An explanation of these findings is not presently clear, but may relate to the use of sodium salts of the fatty acids, species differences, or site of recording.

Overall, there are now presumptive transduction mechanisms for FFA varying in chain length and degree of saturation. Much of this work is based on model systems and animal tissue so the extent to which the data represent human physiology remains to be determined. Marked species differences in carbohydrate sensitivity and responsiveness have been well documented (Breslin and Huang, 2006). There is considerable consistency that only FFA are effective stimuli. However, low concentrations are present, a prerequisite in humans who lack the ability to hydrolyze triglycerides in the oral cavity.

7.3 FAT TASTE IN ANIMAL MODELS

Work to identify orosensory detection systems for FFA was stimulated, in part, by observations in animals that they detected and responded to such exposures behaviorally. Multiple approaches have now provided converging support.

7.3.1 One- and Two-Bottle Preference Tests

An early study contrasted responses of 6-week-old male Wistar rats to 1% oleic acid, linoleic acid, linolenic acid, methyl linoleate, triolein, and caprylic acid in 5 min, two-bottle preference tests (Tsuruta et al., 1999). A clear preference was observed for the oleic, linoleic, and linolenic acids over vehicle. There was also a hierarchy of preference among the long-chain FFA with linolenic > linoleic > oleic. A preference was also noted for methyl oleate, triolein, and methyl linoleate relative to vehicle. Caprylic acid was avoided. The short test period was intended to reduce the potential confounding effects of postingestive feedback on taste preference (e.g., Davis et al., 1995; Sclafani and Glendinning, 2005) and gustatory coding (Hajnal et al., 1999) from FFA ingestion. Duodenal infusion studies with labeled oleic acid and triolein, a triglyceride of oleate, indicate absorption does not occur for approximately 10 min (Tso, 1985; Greenberg et al., 1995). However, this does not account for oral exposure effects on mobilization of lipid stored in the GI tract from the previous meal (Mattes, 2002; Robertson et al., 2003; Parks, 2008). Xanthan gum (0.3%) was added to the stimuli to mask viscosity and lubricity cues the animals may have detected from the FFA. Such a control is vital for isolating potential taste effects because low concentrations of FFA (i.e., 3–100 μM) increase the intracellular Ca^{2+} concentration in selected lingual trigeminal neurons that presumably signal the presence of fat by a nongustatory route (Yu et al., 2007). These data are not fully consistent with the evidence on transduction mechanisms in that esterified fatty acids do not appear to be effective ligands for any of the putative receptors. However, this study did not control for a possible contribution of olfactory stimulation by the solutions. The preference for triolein and corn oil is attenuated in olfactory bulbectomized rats and rats rendered anosmic by irrigation with $ZnSO_4$ (Ramirez, 1993). Further, although

the evidence is mixed (Kimura et al., 2004), oxidation products may be detected and preferred by animals (Ramirez, 1992) and the sample preparation methods used in this study may not have adequately controlled the formation of such products. The adequacy of the xanthan gum to mask the lubricity properties of the fatty acids was also not established and may be a salient cue in rat preference tests (Ramirez, 1994). In 10 min choice tests, male ddY mice prefer 1% emulsions of corn oil, canola oil, and mixed vegetable oil (canola and soybean) with 0.3% xanthan gum to a fluid control (Takeda et al., 2000). The preference was comparable to that noted with palatable sucrose solutions (0.5%–20%). Following exposure to 100% corn oil for 32 days in a subgroup of the mice, no shift in preference was observed. Adherence to a high-fat diet also failed to alter fat preference in 6–8-week-old, male, Sprague-Dawley rats (Kimura et al., 2003). This is notable, given evidence of upregulation of CD36 with high-fat diets (see Ibrahimi and Abumrad, 2002; Poirier et al., 1996). Again, lack of control for olfactory stimulation precludes attribution of this preference to taste.

To address the contribution of olfaction, another trial tested 5-week-old, anosmic, Wistar rats (Fukuwatari et al., 2003). Stimuli contained 0.1%–2.0% oleate with 0.3% xanthan gum and were provided for only 5 min trials. A significant preference was observed for concentrations of 0.5% and above. Triolein was not preferred at any of the tested concentrations. These data provide stronger support for a true taste effect and are consistent with the receptor data indicating only FFA are effective taste stimuli. They also suggest that the odor of FFA may be contributing more to their detection at low concentrations. Evidence that animals exhibit clear preferences for fats of very similar fatty acid profile (e.g., extra light olive oil versus extra virgin olive oil) (Rice et al., 2000) suggests future research designs must account for subtle differences in test stimuli. Resolution of this may lie in better understanding of the specificity of oral fat taste transduction mechanisms.

Finally, in contrast to wild-type littermates, CD36 knock-out mice show no preference for dilute soybean oil, linoleic acid, or Sefa Soyate emulsions (Sclafani et al., 2007). However, at higher concentrations (2.5%–20%), knock-out mice do exhibit a preference. In this work, the two-bottle test was conducted over a 24 h period. These data confirm a role for CD36 in fat preference and demonstrate that other mechanisms, which cannot be narrowed to taste from this work, must be present for their detection.

7.3.2 Conditioned Place Preference

The conditioned place preference test approach has also yielded data consistent with a taste response to fat (Imaizumi et al., 2000). This technique assesses the rewarding properties of a stimulus after it has been paired with conditioning experiences in controlled environments. In one study, 6-week-old, male, ddY mice were provided corn oil from drinking bottles in a light box and water in a dark box. A control group was provided only water in both boxes. Thus, the treatment group received oral and postingestive signals from the fat and was hypothesized to develop a preference for the light box where they received the rewarding stimulation. Another group of rats had the fat or water infused intragastrically to eliminate oral fat exposure. If oral cues contribute to the detection and formation of a preference for fat, reduced or no preference for the light box would be predicted. Conditioning was conducted for

6 days followed by a test day where the amount of time spent in each box was monitored. Only the animals receiving oral exposure demonstrated a preference for the conditioning site supporting a view that the fat was detected by an orosensory signal. However, the lack of control over textural and olfactory cues precludes determination of an independent contribution of taste.

7.3.3 Sham Feeding

The sham-feeding technique provides an opportunity to isolate oral and postingestive influences on feeding. Animals can consume liquids normally, but a fistula in the stomach allows food to be drained out prior to absorption when open or to be processed normally when closed. Thus, data from this technique can complement the findings from the conditioned place preference data where orosensory factors are eliminated. In a one-bottle test, solutions of corn oil (nutritive) and mineral oil (nonnutritive) were avidly consumed, but when compared to a two-bottle choice test, the corn oil was unanimously preferred (Mindell et al., 1990). Testing of a range of corn oil concentrations revealed that the animals could discriminate and preferred 0.78% corn oil over a water vehicle that contained Tween-80, an emulsifier that would mimic some of the textural properties of the fat. Once again, textural and olfactory cues cannot be excluded as the basis for these findings, but they do demonstrate an independent contribution of preingestive signals.

7.3.4 Conditioned Aversions

Conditioned taste aversion paradigms have been used to explore fat taste as well. With this approach, oral exposure to fat is coupled with induced malaise or illness and the degree to which the sensory cues from the fat subsequently lead to rejection of fat, now predictive of illness, is measured. An early trial revealed adult, male Wistar rats generalized aversions induced with LiCl between butter, margarine, and lard, but not to nonnutritive Vaseline (Larue, 1978). A similar pattern was observed with anosmic rats suggesting the salient cue was either a texture or taste. This work was extended through another series of trials where male and female albino rats ingested sugar–corn oil or sugar–mineral oil solutions followed by aversion conditioning with LiCl or sham conditioning with NaCl administration (Smith et al., 2000). Subsequent two-bottle choice tests revealed aversions were targeted more to the corn oil than either sucrose or mineral oil. To minimize postingestive feedback, one trial was restricted to a 30 s exposure and similar results were obtained. Tests of generalization of effects between linoleic acid and corn oil supported a role for the FFA, but in conditioning trials of sucrose/linoleic acid mixtures, the fatty acid was not the predominant target. This work is suggestive of a taste cue, but cannot exclude a texture contribution. In a later study, 90-day-old Sprague-Dawley rats underwent aversion conditioning with 44, 66, or 88 μM unesterified oleic and linoleic fatty acids as the sensory stimuli (McCormack et al., 2006). Detection and avoidance of both fatty acids was observed at the 66 and 88 μM concentrations. The effects noted with one fatty acid generalized to the other. Further, comparable results were noted with the FFA forms and the less nutritionally relevant, but more experimentally expedient, sodium salts. It was argued

that texture was an unlikely explanation for the findings since viscosity measurements for the FFA stimuli differed from water by only 1.5%. However, viscosity is only one textural attribute contributed by fat. Olfaction was discounted because of the failure to note an aversion to the odor of ethanol, in which the FFA were dissolved, but it is not clear extrapolations across compounds are valid. Nevertheless, the data are consistent with a taste effect and document sensitivity at a low stimulus concentration.

7.3.5 Cephalic Phase Responses

Cephalic phase responses are vagally mediated physiological responses to sensory stimulation. They were first intensively studied by Pavlov (1910a,b). Examples include, but are not limited to, salivation, gastric acid secretion, pancreatic exocrine and endocrine secretions, shifts in blood flow, and renal function as well as thermogenesis in response to cognitive, visual, olfactory, gustatory, and tactile stimulation (Mattes, 1997; Zafra et al., 2006). These responses can serve as biomarkers for orosensory stimulation. A number of animal studies have monitored responses to oral exposures with dietary fats. A regulatory effect on gastric emptying has been demonstrated in rats provided corn oil emulsions orally or via gastric infusion (Kaplan et al., 1997). While delivery rate and volume directly influence emptying times with gastric infusion, these indices are unaffected when delivery includes oral exposure. One interpretation holds that the oral signal facilitates the expression of postgastric regulatory signals. Other work, demonstrates that oral exposure with linoleic, linolenic, and oleic fatty acids augments pancreatic exocrine secretion whereas no change occurs following oral exposure to the medium-chain fatty acid, caprylic acid (C8:0), nor to esterified forms of selected long-chain fatty acids (triolein) (Hiraoka et al., 2003; Laugarette et al., 2005). Moreover, treatment with atropine blocked the increase in protein output following FFA exposure, consistent with the signal being a neurally mediated, cephalic phase response.

Postabsorptive effects are also reported (Ramirez, 1985). When intubation of a load of corn oil into the stomach is accompanied by oral stimulation with water, sodium saccharin, or 0.1 mL of corn oil, plasma triglycerides (TG) are significantly elevated for hours with oral exposure to fat whereas the saccharin and water exposures have little effect. The attribute of fat exposure responsible for the effect was not addressed.

Collectively, studies with animal models provide consistent, albeit not definitive, evidence for an oral, likely taste, detection system for FFA. The applicability of these findings to humans remains uncertain. Species differences in sensitivity to the taste of different dietary compounds are well documented. Of particular concern for fat taste is the presence of lingual lipase to generate FFA in the oral cavity of mice and rats, while this appears not to be the case for humans.

7.4 FAT TASTE IN HUMANS

7.4.1 Psychophysical Studies

Accumulating evidence from psychophysical and fat challenge trials supports a role for fat detection in humans. However, few trials have been designed to isolate the taste component. One trial determined detection thresholds of 12 young and

12 elderly participants for oil-in-water emulsions of bleached, deodorized soybean oil, medium-chain triglyceride (MCT) oil, and light mineral oil with four different emulsifiers (Schiffman et al., 1998). A two-alternative forced choice staircase procedure was used where the last four of five reversals were averaged to estimate thresholds. Responses were made with and without nose clips. The young had lower thresholds averaged over all oils (5.3%, v/v) than the elderly (15.8%, v/v), but the key observation was that both groups had measurable thresholds. Among the young participants, thresholds tended to be lower with olfactory input, but this was not statistically significant. Thus, performance was solely based on olfaction. Thresholds were generally higher (i.e., less sensitive) for MCT than for soybean oil and emulsifier use led to two- to threefold differences. Acacia was associated with the lowest threshold and reportedly only suspends lipid in a water medium rather than interact with the stimulus. This suggests tactile cues may have contributed to performance, but are not fully responsible. The concentrations of FFA in the stimuli were not assessed.

More recently, taste responses to FFA varying in chain length and saturation have been reported. To explore the importance of saturation, detection thresholds were measured in 22 healthy adults who were PROP tasters (i.e., individuals with a heritable trait that enables them to detect low concentrations of propylthiouracil (see Prescott and Tepper, 2004)) (Chale-Rush, 2007a,b). The subjects sampled emulsions of linoleic (C18:2), oleic (C18:1), and stearic (C18:0) FFA, as well as a sample of oxidized linoleic acid. A masking approach was used to isolate the taste response. The vehicle contained gum acacia (5%, w/w) to mask viscosity effects, mineral oil (5%, w/w) to mask lubricity cues, nose clips were worn to eliminate olfactory input and testing was conducted under red light to exclude visual cues. To assess potential irritancy effects, testing was conducted with linoleic acid whether participants had undergone capsaicin desensitization or not (Lawless and Stevens, 1989). The fatty acids were stored under nitrogen and emulsions were prepared the day of testing by sonication while on ice to minimize the formation of oxidation products. Measurable thresholds were obtained from 21 to 22 participants for each FFA, as well as oxidized linoleic acid prior to and following desensitization. Threshold concentrations did not differ across the fatty acids suggesting they were detected by a common mechanism. This observation stands in contrast to findings from rats where sensitivity to linoleic acid is higher compared to oleic acid (Pittman et al., 2007). Given the various controls, it is posited that the thresholds were based on taste, however, this cannot be stated definitively. The desensitization procedure does not eliminate all trigeminal input and it is possible, albeit probably unlikely, that there are tactile properties of the fats not sufficiently masked that accounted for the responses. The threshold concentrations were generally in the range of 1000 μM (Chale-Rush et al., 2007a) which is higher than values reported for rats (Pittman et al., 2006), but this must be interpreted cautiously. Thresholds are probability functions with absolute responses determined, in part, by the medium in which the stimulus is carried. Thus, values can only be compared across studies when identical stimuli are used. In this set of studies, individuals were also tested for sensitivity to the FFA based on olfactory (ortho- and retronasal) and irritancy (oral and nasal) properties (Chale-Rush et al., 2007b). Thresholds were not significantly different for presumed taste-only and orthonasal olfactory-only thresholds, but were approximately twice as

high for retronasal thresholds and roughly 30% lower when taste and smell cues were concurrently available (i.e., tasting without nose clips). Irritancy did not appear to account for test performance. Correlations between the taste, olfactory, and irritancy thresholds were low and not significant, suggesting independence of the mechanisms subserving the different sensory modalities.

Identification of presumptive receptors for short-, medium-, and long-chain FFA suggests humans could be sensitive to each. We have recently begun to explore this using samples of linoleic (18:2), stearic (C18:0), lauric (C12:0), and caproic (C6:0) acids (Mattes, 2008). Each was prepared as described above to mask nongustatory cues. Measurable thresholds were obtained for each of these acids in nearly all of the 32 study participants. The detection threshold concentration for the short-chain caproic acid was about an order of magnitude lower than the medium and long-chain acids which did not differ from each other. The efficacy of lauric acid as a taste stimulus in rats is presently uncertain (Gilbertson, 2005; Pittman et al., 2007).

Taken together, the psychophysical data indicate humans can detect fatty acids varying in chain length and degree of saturation. None of the currently proposed receptor-mediated transduction mechanisms can account for this range of sensitivities as it exceeds the ligand specificity properties of each. Consequently, the data suggest multiple mechanisms are functional and/or that a nonspecific mechanism is involved (e.g., diffusion).

7.4.2 Individual Variability

Some data suggest there is marked interindividual variability in fat taste. This could be due to inherent or acquired physiological characteristics. The thresholds reported by Chale-Rush et al. (2007a,b) were obtained from PROP tasters. Some data suggests PROP tasters and nontasters differ in sensitivity to dietary fats. The first study to propose this reported that PROP medium and supertasters (determined by taste intensity ratings for graded PROP concentrations that matched or exceeded ratings for a graded concentration series of NaCl solutions) rated salad dressings that contained 40% fat as higher in fat than comparable samples with 10% fat. Nontasters did not discriminate between the two (Tepper and Nurse, 1997). However, the nontasters did rate the two samples as hedonically different whereas the medium and supertasters gave them comparable hedonic ratings (Tepper and Nurse, 1998). The basis of this distinction is not known, but may have been due to the fat's effect on a food attribute not directly linked to perceived fattiness. This work also noted that there was a direct association between taster status and fungiform papillae number and hypothesized that the more acute fat discrimination of tasters was attributed to the rich innervation of their more abundant papillae. Consistent with this, the tasters also reported heightened burn intensity for capsaicin. Others have reported mixed findings. A significant indirect association between perceived bitterness of PROP and liking of high-fat foods was noted in women, but there was a trend of a direct relationship in males (Duffy and Bartushuk, 2000). In free-choice profiling, PROP nontasters and supertasters gave similar overall impressions of the creaminess of dairy products, though used dissimilar properties to make their judgments (Kirkmeyer and Tepper, 2003). Attributing the greater sensitivity for fats to enhanced trigeminal innervation

in PROP tasters, suggests a tactile mechanism, rather than taste, however there is preliminary evidence for DRK and GPR120 on trigeminal neurons (Hansen et al., 2006; Yu et al., 2007).

There is also preliminary evidence that PROP tasters are more sensitive to FFA. Eighty percent (8/10) of PROP tasters were able to detect conjugated linoleic acid added to high-fat vanilla ice cream (final concentration of 0.05–0.06 mg/g); whereas only 17% (1/6) of nontasters could do so (Nasser et al., 2001). The ice cream vehicle presumably masked textural cues, but no control for olfactory stimulation was included. Other work suggests there may be subsets of FFA-tasters and nontasters, independent of PROP taster status. Based on the criterion of identifying nine or ten of ten 10 μM solutions of the sodium salt of linoleic acid, about 42% (14/24) of participants were classified as tasters (Kamphuis et al., 2003). Test–retest reliability in trials held 1 week apart was 95%. The lack of control for olfactory-based discrimination hampers definitive determination that the performance was taste-based. Indeed, in subsequent work, the superior performance by FFA-tasters was eliminated when olfactory cues were eliminated. The extrapolation of these data to dietary FAA exposure in free-living humans is uncertain due to use of the nonphysiological sodium salt, but there are data from rats suggesting the free form and sodium salt elicit common responses (McCormack et al., 2006). When challenged with detecting the sodium salts of linoleic and oleic fatty acids in ice cream, performance of the FFA-tasters and nontasters did not differ. This may indicate tactile cues were used for detection by tasters in the simple systems or, if taste was the basis of their performance, additional training could be required for discrimination of the fine distinctions required in a medium like ice cream.

The level of exposure to dietary fats may modify the preferred concentration of fat in foods (Mattes, 1993), appetitive sensations, and energy balance regulation (Cooling and Blundell, 1998). However, data relating to exposure effects on sensory discrimination of dietary fats have been mixed (Mattes, 1993; Cooling and Blundell, 1998; Guinard et al., 1999). Given evidence that some signaling pathways are inducible (e.g., CD36), further study of this issue is warranted.

7.4.3 Cephalic Phase Responses

Cephalic phase responses to oral fat exposure in humans also support the presence of a detection system for FFA in the oral cavity. This was first demonstrated in studies exploring the kinetics of vitamin A metabolism (Mendeloff, 1954). Vitamin A is a fat-soluble vitamin and is often used as a marker for intestinal lipid absorption. An initial indication that oral stimulation could augment lipid absorption was noted serendipitously when plasma vitamin A levels spiked in individuals allowed to eat foods that did not contain the vitamin. In a subsequent trial, it was shown that MSF (i.e., chewing and expectorating food) also promoted lipid absorption and that this effect was delayed by administration of atropine, a parasympathetic blocker, prior to modified sham feeding. This suggested the response was neurally mediated and that oral stimulation modified lipid processing. However, neither this line of study nor a number of subsequent trials (Robertson et al., 2002; Heath et al., 2004) attempted to identify the effective oral cue.

Studies with rats that received a fat load intragastrically (i.e., bypassing oral receptors) followed by oral stimulation with corn oil documented a unique role for fat as an oral stimulus. The oral lipid exposure led to a significantly prolonged serum TG elevation relative to stimulation with saccharin or water (Ramirez, 1985). These findings have now been replicated and extended in humans (Mattes, 1996, 2001a,b, 2002; Robertson et al., 2002; Crystal and Teff, 2006; Smeets and Westerterp-Plantenga, 2006; Parks, 2008). In an early trial, participants ingested 50 g of safflower oil in capsules to avoid oral exposure to the lipid. MSF with full-fat cream cheese led to an approximate doubling of plasma TG relative to no oral stimulation or exposure to fat-free cream cheese or the cracker alone and the concentration remained elevated for over 6 h (Mattes, 1996). Psychophysical testing indicated participants could not distinguish between the fat-free and full-fat stimuli and measurement of the expectorated samples revealed little had been ingested.

A subsequent study provided insights into the role of tactile cues and fat form (Mattes, 2001a). The stimuli included no oral stimulation (time control), mashed potatoes alone (vehicle), potatoes with Passelli (a carbohydrate-based fat replacer), potatoes with Simplesse (a protein-based fat replacer), potatoes with Olestra (a non-absorbable fat-based fat replacer), or potatoes with butter (an available fat source). Even though all tests were preceded by the same 50 g fat preload, oral exposure to the butter led to the greatest TG elevation. Sensory testing indicated that TG response could not be attributed to cognitive or palatability effects. Tactile properties were also discounted because all the fat replacers were designed to mimic this attribute of dietary fat. Analyses of the expectorated samples revealed the butter sample was richest in oleic acid (almost 1 mM) and tended to be highest for linoleic acid (almost 350 μM). Thus, relative to concentrations eliciting differential preferences in animal studies, ample stimuli concentrations were present to evoke a response.

Given evidence from electrophysiological recordings in macaques that the cue for dietary fat may be olfactory (Rolls et al., 1999), the next human trial was designed, in part, to document an olfactory effect on plasma TG. Stimulation included orthonasal exposure to full-fat cream cheese or oral (taste and olfactory) exposure to full-fat cream cheese immediately after lipid loading (Mattes, 2001b). The TG response was greater following oral exposure with lipid loading than with orthonasal stimulation. Given the hypothesized signal was olfactory, this unexpected observation prompted a recall of participants who were provided oral exposure with the nares blocked to eliminate the olfactory signal. The TG response was nearly identical to that observed with oral exposure suggesting taste was the effective cue.

Next, the efficacy of FFA varying in saturation was explored (Tittelbach and Mattes, 2001). At the time, electrophysiological and rat data indicated that only unsaturated fatty acids were effective taste stimuli. So, the postprandial TG responses were monitored following no oral exposure, or oral exposure to cracker alone or cracker containing unsaturated fatty acid plus margarine, cracker plus jelly, cracker with unsaturated margarine plus jelly or cracker plus butter. It was also hypothesized that greater TG responses may occur if the oral stimulus matched the nutrient profile of the load delivered to the gut. Thus, the capsules in this study contained butter, primarily a saturated fat source. Only stimulation with the unsaturated fat led to a significant TG elevation. However, the change of lipid load (i.e., butter instead

of safflower oil in prior work) complicates direct comparisons of this finding with previous evidence.

The ecological relevance of this response has begun to be explored. In particular, the early work used a 2 h stimulus intervention; an exposure far longer than common dietary eating events. To be of clinical relevance, the cephalic phase response should be apparent upon initial exposure to effective stimuli. One study explored cephalic phase responses to 3 min oral exposures of cognitively restrained and unrestrained eaters to no oral stimulation, stimulation with a nonfat cake or exposure to a high-fat cake (Crystal and Teff, 2006). Pancreatic polypeptide, an index of vagal efferent activity was significantly greater after oral exposure to the high-fat cake relative to the no stimulation condition and the response to the nonfat cake was intermediate. We have recently demonstrated that 10 s of oral exposure to linoleic acid is sufficient to modulate plasma TG (Mattes, 2008).

Another trial (Smeets and Westerterp-Plantenga, 2006) demonstrated the efficacy of MSF of a meal with linoleic acid or olive oil in elicitation of an augmented postprandial TG rise. Both led to TG elevations relative to oral exposure to water. The TG response to the actual ingestion of the food was greater than the response to MSF. Neither oral exposure nor ingestion of a meal containing oleic acid led to a differential response compared to water. However, there was a significant postprandial rise of plasma nonesterified fatty acids (NEFA) with eating or MSF with olive oil. MSF with linoleic acid also elicited a rise of NEFA. Thus, this study provides evidence for a detection system for linoleic and oleic fatty acids with the former leading to a more robust response. This is consistent with some animal work (Pittman et al., 2007; Yu et al., 2007). Human psychophysical studies do not reveal differences in thresholds for the two fatty acids, but this does not preclude differential effects on postprandial lipid metabolism.

Because of the rapidity of the TG rise following oral FFA stimulation, questions arose regarding the origin of this lipid. To explore this question, a feeding trial was conducted that provided a meal with one fatty acid profile followed by a second meal of a different profile (Fielding et al., 1996). The composition of chylomicron TG in the early postprandial period after the second meal revealed the early peak is rich in fatty acids from the previous meal. This was referred to as the "second meal effect." Evidence that oral fat exposure is particularly salient in eliciting the effect stems from a study where almonds (a rich source of monounsaturated fatty acids) were consumed as the last eating event of 1 day and capsules (thus bypassing oral detection) of safflower oil (a rich source of linoleic acid) were ingested the next morning (Mattes, 2002). The morning meal was accompanied by MSF of either a cracker alone or cracker with full-fat cream cheese. Oral fat exposure led to higher oleic/linoleic ratios in plasma relative to oral stimulation with the cracker alone or baseline. Another group failed to observe an effect of MSF on postprandial plasma TG concentration (Jackson et al., 2001), but the rapid TG rise has subsequently been replicated (Parks, 2008). Human jejunal biopsies 5 h following a high-fat meal revealed jejunal lipid was cleared after oral ingestion of a glucose solution, but not water (Robertson et al., 2003). This indicates a sapid solution is required and that lipid is stored in jejunal enterocytes between meals. The efficacy of oral fat exposure was not tested in this work. However, recent studies confirm fat's particular

efficacy for mobilization of stored lipid (Parks, 2008). Participants ingested a lipid load containing labeled triolein at 6:00 PM and another meal with triolein labeled at a different site the following morning of testing. After oral exposure to full-fat cream cheese the postprandial TG rise was 30% greater than the response to fat-free cream cheese and 48% of the first TG peak was comprised of the fat ingested the previous evening. Thus, lipid mobilization was specifically augmented by oral fat exposure.

Despite differences in lingual lipase secretion, the findings from these human trials are largely consistent with observations from animal models. Humans are able to detect low concentrations of FFA varying in chain length and saturation, apparently without the aid of olfactory and tactile cues. Further, this oral signaling augments postprandial TG concentrations, due, in part, to mobilization of TG presumably stored in jejunal enterocytes from the preceding meal.

7.5 SUMMARY AND FUTURE DIRECTIONS

The prevailing view is that taste is an analytical sense comprised of five primaries. However, the definition of a primary has not been clearly defined. Among the criteria commonly cited are (1) a unique transduction mechanism; (2) direct activation of taste receptor cells; (3) activation of neurons that convey taste information; (4) independence of the sensation evoked by prototypical effective stimuli from other taste qualities; and (5) a signal capable of supporting associative learning with specific physiological and/or behavioral responses. Based on these criteria, the question can be asked: Is "fat" a basic taste in humans?

With respect to the first criterion, multiple putative transduction mechanisms have been isolated (i.e., DRK, CD36, GPR40, GPR41, GPR43, GPR120, FATP, diffusion). They do not convey information about other sensory qualities and appear to cover the range of stimuli reported to evoke "fatty" sensations by FFA varying in chain length (C6–C22 thus far) and saturation (PUFA, MUFA, SFA). However, to date, the evidence is based on electrophysiological recordings from isolated rodent taste cells, behavioral responses of animals provided controlled sensory exposures as well as animals subjected to various forms of conditioning or gene knock-outs. A direct mechanism may be documented in humans in the future through targeted stimulation with specific agonists or antagonists. The data on this criterion are supportive, but not definitive.

To be considered a primary taste, it may be expected that responses to oral exposures are specific to the quality rather than a modulation of responsiveness to other primaries. Based on studies with rats, it has been proposed that the taste component of FFA stems from their modulation of responsiveness to the other basic tastes (Gilbertson et al., 2005; Pittman et al., 2006). However, subsequent work by this group has not replicated these findings (McCormack et al., 2006) nor has evidence from human studies (Mattes, 2007). The matter has not been fully addressed as questions remain about the concentrations of FFA tested in humans. The greatest problem with confirming a direct effect of FFA lies in the fact that they are effective multimodal stimuli. Unlike many of the prototypical taste stimuli for other basic taste qualities (e.g., sucrose, sodium chloride, caffeine, hydrochloric acid, MSG),

fatty acids clearly have somatosensory and olfactory properties, so isolation of the taste component is challenging. To date, masking has been used to minimize the contributions from the other properties, but additional work is required to verify the effectiveness of this approach. The evidence is preliminary, but suggests in animals and humans that FFA are independently effective taste stimuli.

Three nerves convey taste information from taste receptor cells to central sites. Sectioning the chorda tymani and/or glossopharyngeal nerves diminishes responsiveness of animals to oral exposures to FFA. No studies have been reported on vagus nerve involvement. Thus, FFA appear to activate taste neurons in various animal models (mice, rats, monkeys), but evidence is lacking in humans. This may be obtained through selective anesthesia or natural experiments where central projections are disrupted (e.g., chorda tympani nerve cuts). The data on this criterion are supportive, but not definitive for a taste component for FFA.

The debate over whether taste is analytical or synthetic has not been resolved, but the preponderance of evidence favors the former interpretation. Aside from reports that short-chain fatty acids may have a sour taste quality (Forss, 1972), there are no data indicating the sensations evoked by FFA can be produced by any combination of other basic qualities. However, no systematic study has been conducted on this issue. One hurdle is the development of a lexicon for the taste quality(ies) of FFA. Data indicate that fat, in taste mixtures, is a unique salient target for conditioning which suggests a lack of generalization, at least to sweetness (Smith et al., 2000). Aversions do generalize across FFA suggesting that fatty acids of different chain length and saturation share a common property (McCormick et al., 2006). Further work is needed to characterize the taste quality of FFA and whether they can be evoked by mixtures of other sensations in humans. There is insufficient evidence from humans to conclude that FFA have a unique taste quality.

The chemical senses are signaling systems that modulate ingestive responses as well as postingestive physiological processes that mimic those occurring during food ingestion (Mattes, 1997; Zafra et al., 2006). Many responses likely result from general neural activation while eating, but these responses can also be specific to the nature of the stimulus, particularly when they are sapid representatives of basic qualities with nutritional consequence. For example, oral stimulation with sweetness is particularly important for carbohydrate absorption from the GI tract (Giduck et al., 1987) and glycemic control (Teff, 2000). Nonnutritive salt exposure in the oral cavity modulates renal function and sodium excretion (Akaishi et al., 1991) and umami modifies endocrine secretions (Nijima et al., 1990). Strong evidence now exists in rats and humans of fat-specific effects (Ramirez, 1985; Mattes, 1996, 2001a, 2002; Crystal and Teff, 2006; Parks, 2008). Oral exposure to dietary fats mobilizes lipid stored in the GI tract from the previous meal, leading to a rapid and greater plasma TG rise. Fat taste, as opposed to tactile or odor properties, may be uniquely effective in this process (Mattes, 2001b). Thus, the nature of physiological responses to oral fat exposure is consistent with quality-specific effects for other nutritive taste primaries. Further work is needed to determine whether there is any specificity in response to selected FFA and to characterize the range and consequences of responses evoked through oral exposure. The data on FFA signaling effects from the oral cavity must be viewed as preliminary, but supportive.

Accepting that taste is analytical, there are no known constraints on the number of primary sensations that comprise the sense. Sweet, sour, salty, and bitter have been acknowledged primaries by most workers in the field and this fits with common experience. Umami has been added to the pantheon of primaries only more recently, based largely on elucidation of a unique transduction mechanism for glutamate and other amino acids. However, umami itself lacks a widely identified characteristic quality and is hypothesized to signal the presence of protein in foods. Fat taste may be more like umami than the other primary qualities in that it appears to have a unique mechanism for its detection, signals the presence of an essential macronutrient (i.e., fats), yet has a vague percept. If designation of primaries is now based on physiological criteria more than introspection, fat is a strong candidate for inclusion as a primary.

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Part III

Neural Representations of Dietary Fat Stimuli

8 Neural Representation of Fat Texture in the Mouth

Edmund T. Rolls

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8.1 SUMMARY

The brain areas that represent taste also provide a representation of oral texture. Fat texture is represented by neurons independently of viscosity: some neurons respond to fat independently of viscosity, and other neurons encode viscosity. The neurons that respond to fat also respond to silicone oil and paraffin oil, indicating that the sensing is not chemospecific, but is instead based on texture. This fat sensing is not related to free fatty acids (FFA), in that these neurons typically do not respond to FFA such as linoleic acid (LiA). Moreover, a few neurons with responses to FFA typically do not respond to fat in the mouth. Fat texture-sensitive neurons are found in the primary taste cortex in the rostral insula and adjoining frontal operculum, in the secondary taste cortex in the orbitofrontal cortex, and in the amygdala. In these regions, the fat texture responsiveness of these neurons may be combined with taste and/or oral temperature responses, and in the orbitofrontal cortex with olfactory responses. Different neurons respond to different combinations, providing a rich representation of the sensory properties of food. In the orbitofrontal cortex, feeding to satiety with one food decreases the responses of these neurons to that food, but not to other foods, showing that sensory-specific satiety and appetite modulation are represented in the orbitofrontal cortex. A complementary functional neuroimaging study in humans showed activation by fat in the mouth of the insula, orbitofrontal cortex, and a region to which it projects, the pregenual cingulate cortex. In summary, one way in which fat in the mouth is represented in the brain is by its texture, and an indication of what must be transduced has been provided by these neuroscience studies.

8.2 INTRODUCTION

The aim of this chapter is to describe how fat in the mouth is represented in the brain. This is an important issue, for it is not yet clear how oral fat is sensed, and evidence from neuroscience is providing indications about this. Moreover, fat in the diet may be pleasant, yet its intake must be controlled, and understanding the rules by which the pleasantness of fat is regulated is important. In addition, the brain's representation of oral fat is frequently in terms of particular combinations with other sensory aspects of food, including taste, texture, and olfactory inputs, and these combinations are important for understanding the full impact of fat in the mouth on the pleasantness of food.

Because the representation of fat in the mouth is closely linked to taste processing in the brain, we start with an overview of taste pathways and processing in the brain, before we consider how oral fat is represented in the same brain areas, and frequently but not always in combination with taste. To make the results relevant to understanding the control of human food intake, complementary evidence is provided by neurophysiological studies in nonhuman primates in which the taste and

related pathways are similar to those in humans, and by functional neuroimaging studies in humans. A broad perspective on brain processing involved in hedonic aspects of the control of food intake and in affective responses more generally is provided by Rolls (2005).

8.3 TASTE PROCESSING IN THE PRIMATE BRAIN

8.3.1 Pathways

A diagram of the taste and related olfactory, somatosensory, and visual pathways in primates is shown in Figure 8.1. The multimodal convergence that enables single neurons to respond to different combinations of taste, olfactory, texture, temperature, and visual inputs to represent different flavors produced often by new combinations of sensory input is afforded by the convergence of processing pathways evident in brain areas such as the orbitofrontal cortex (Rolls, 2007; Rolls and Grabenhorst, 2008).

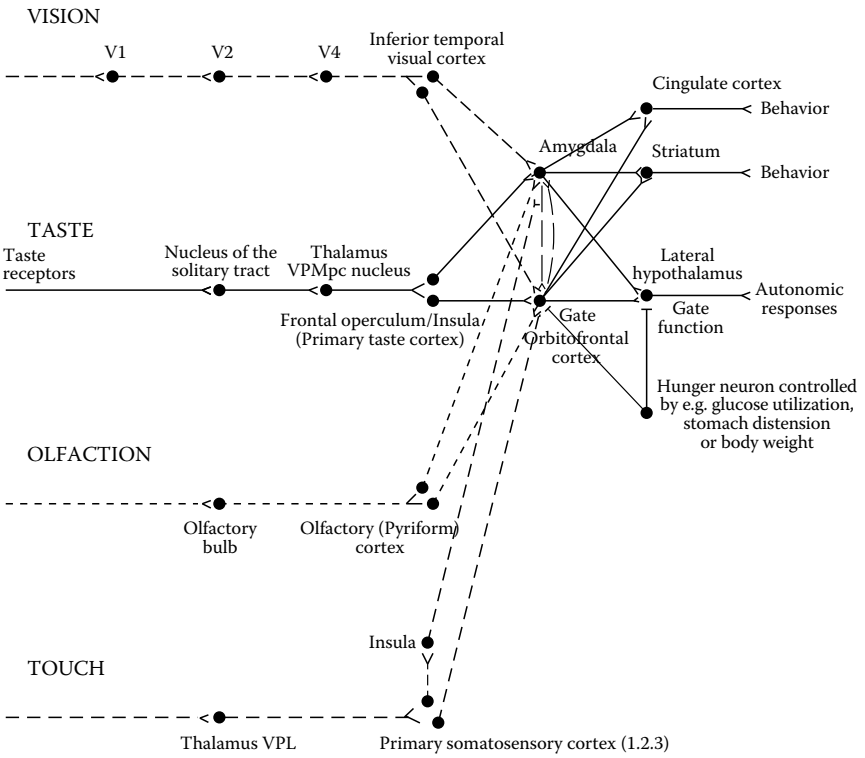


FIGURE 8.1 Schematic diagram of the taste and olfactory pathways in primates including humans showing how they converge with each other and with visual pathways. Hunger modulates the responsiveness of the representations in the orbitofrontal cortex of the taste, smell, texture, and sight of food (indicated by the gate function), and the orbitofrontal cortex is where the palatability and pleasantness of food is represented. VPMpc, ventralposteromedial thalamic nucleus; V1, V2, V4, visual cortical areas.

8.3.2 The Primary Taste Cortex

The primary taste cortex in the primate anterior insula and adjoining frontal operculum contains not only taste neurons tuned to sweet, salt, bitter, sour (Scott et al., 1986; Yaxley et al., 1990; Rolls and Scott, 2003), and umami as exemplified by monosodium glutamate (MSG) (Baylis and Rolls, 1991; Rolls et al., 1996b), but also other neurons that encode oral somatosensory stimuli including viscosity, fat texture, temperature, and capsaicin (Verhagen et al., 2004). Some neurons in the primary taste cortex respond to particular combinations of taste and oral texture stimuli, but do not respond to olfactory stimuli or visual stimuli such as the sight of food (Verhagen et al., 2004). Neurons in the primary taste cortex do not represent the reward value of taste, that is the appetite for a food, in that their firing is not decreased to zero by feeding the taste to satiety (Rolls et al., 1988; Yaxley et al., 1988).

8.3.3 The Secondary Taste Cortex

A secondary cortical taste area in primates was discovered by Rolls et al. (1990) in the caudolateral orbitofrontal cortex, extending several millimeters in front of the primary taste cortex. One principle of taste processing is that by the secondary taste cortex, the tuning of neurons can become quite specific, with some neurons responding, for example, only to sweet taste. This specific tuning (especially when combined with olfactory inputs) helps to provide a basis for changes in appetite for some but not other foods eaten during a meal.

8.3.4 Five Prototypical Tastes, Including Umami

In the primary and secondary taste cortices, there are many neurons that respond best to each of the four classical prototypical tastes: sweet, salt, bitter, and sour (Rolls, 1997; Rolls and Scott, 2003), but also there are many neurons that respond best to umami tastants such as glutamate (which is present in many natural foods such as tomatoes, mushrooms, and milk) (Baylis and Rolls, 1991) and inosine monophosphate (which is present in meat and some fish such as tuna) (Rolls et al., 1996b). This evidence, taken together with the identification of possible glutamate taste receptors (Zhao et al., 2003; Maruyama et al., 2006), leads to the view that there are five prototypical types of taste information channels, with umami contributing, often in combination with corresponding olfactory inputs (Rolls et al., 1998; McCabe and Rolls, 2007), to the flavor of protein. In addition, other neurons respond to water, and others to somatosensory stimuli including astringency as exemplified by tannic acid (Critchley and Rolls, 1996b), and capsaicin (Rolls et al., 2003b; Kadohisa et al., 2004).

8.3.5 The Pleasantness of the Taste of Food

The modulation of the reward value of a sensory stimulus such as the taste of food by motivational state, for example hunger, is one important way in which motivational behavior is controlled (Rolls, 1999, 2005). The subjective correlate of this modulation is that food tastes pleasant when hungry, and tastes hedonically neutral when it

has been eaten to satiety. We have found that the modulation of taste-evoked signals by motivation is not a property found in early stages of the primate gustatory system. The responsiveness of taste neurons in the nucleus of the solitary tract (Yaxley et al., 1985) and in the primary taste cortex (frontal opercular, Rolls et al., 1988; insular, Yaxley et al., 1988) is not attenuated by feeding to satiety. In contrast, in the secondary taste cortex, in the caudolateral part of the orbitofrontal cortex, it has been shown that the responses of the neurons to the taste of glucose decreased to zero while the monkey ate it to satiety, during the course of which the behavior turned from avid acceptance to active rejection (Rolls et al., 1989). This modulation of responsiveness of the gustatory responses of the orbitofrontal cortex neurons by satiety could not have been due to peripheral adaptation in the gustatory system or to altered efficacy of gustatory stimulation after satiety was reached, because modulation of neuronal responsiveness by satiety was not seen at the earlier stages of the gustatory system, including the nucleus of the solitary tract, the frontal opercular taste cortex, and the insular taste cortex.

8.3.6 Sensory-Specific Satiety

In the secondary taste cortex, it was also found that the decreases in the responsiveness of the neurons were relatively specific to the food with which the monkey had been fed to satiety (Rolls et al., 1989; Critchley and Rolls, 1996c).

This evidence shows that the reduced acceptance of food which occurs when food is eaten to satiety, and the reduction in the pleasantness of its taste (Cabanac, 1971; Rolls and Rolls, 1977, 1982; Rolls et al., 1981a,b, 1982, 1983a), are not produced by a reduction in the responses of neurons in the nucleus of the solitary tract or frontal opercular or insular gustatory cortices to gustatory stimuli. Indeed, after feeding to satiety, humans reported that the taste of the food on which they had been satiated tasted almost as intense as when they were hungry, though much less pleasant (Rolls et al., 1983b). This comparison is consistent with the possibility that activity in the frontal opercular and insular taste cortices as well as the nucleus of the solitary tract does not reflect the pleasantness of the taste of a food, but rather its sensory qualities independently of motivational state. On the other hand, the responses of the neurons in the caudolateral orbitofrontal cortex taste area and in the lateral hypothalamus (Rolls et al., 1986) are modulated by satiety, and it is presumably in areas such as these that neuronal activity may be related to whether a food tastes pleasant, and to whether the food should be eaten (see further Scott et al., 1995; Critchley and Rolls, 1996a; Rolls, 1996, 1999, 2000a,b; Rolls and Scott, 2003). In addition to providing an implementation of sensory-specific satiety (probably by habituation of the synaptic afferents to orbitofrontal neurons with a time course of the order of the length of a course of a meal), it is likely that visceral and other satiety-related signals reach the orbitofrontal cortex (as indicated in Figure 8.1) (from the nucleus of the solitary tract, via thalamic nuclei) and there modulate the representation of food, resulting in an output that reflects the reward (or appetitive) value of each food (Rolls, 2005).

It is an important principle that the identity of a taste, and its intensity, are represented separately from its pleasantness (Grabenhorst and Rolls, 2008; Grabenhorst

et al., 2008). Thus it is possible to represent what a taste is, and to learn about it, even when we are not hungry.

8.4 THE REPRESENTATION OF FLAVOR: CONVERGENCE OF OLFACTORY AND TASTE INPUTS

At some stage in taste processing, it is likely that taste representations are brought together with inputs from different modalities, for example, with olfactory inputs to form a representation of flavor (see Figure 8.1). We found (Rolls and Baylis, 1994) that in the orbitofrontal cortex taste areas, of 112 single neurons which responded to any of these modalities, many were unimodal (taste 34%, olfactory 13%, and visual 21%), but were found in close proximity to each other. Some single neurons showed convergence, responding, for example, to taste and visual inputs (13%), taste and olfactory inputs (13%), and olfactory and visual inputs (5%). Some of these multimodal single neurons had corresponding sensitivities in the two modalities, in that they responded best to sweet tastes (e.g., 1 M glucose), and responded more in a visual discrimination task to the visual stimulus which signified sweet fruit juice than to that which signified saline; or responded to sweet taste, and in an olfactory discrimination task to fruit odor. The different types of neurons (unimodal in different modalities, and multimodal) were frequently found close to one another in tracks made into this region, consistent with the hypothesis that the multimodal representations are actually being formed from unimodal inputs to this region.

It thus appears to be in these orbitofrontal cortex areas that flavor representations are built, where flavor is taken to mean a representation which is evoked best by a combination of gustatory and olfactory input. This orbitofrontal region does appear to be an important region for convergence, for there is only a low proportion of bimodal taste and olfactory neurons in the primary taste cortex (Rolls and Baylis, 1994; Verhagen et al., 2004).

The bimodal neurons appear to be built by learning. Critchley and Rolls (1996a) showed that 35% of orbitofrontal cortex olfactory neurons categorized odors based on their taste association in an olfactory-to-taste discrimination task. Rolls et al. (1996b) found that 68% of orbitofrontal cortex odor-responsive neurons modified their responses in some way following changes in the taste reward associations of the odorants during olfactory taste discrimination learning and its reversal. (In an olfactory discrimination experiment, if a lick response to one odor, the S+, is made a drop of glucose taste reward is obtained; if incorrectly a lick response is made to another odor, the S−, a drop of aversive saline is obtained. At some time in the experiment, the contingency between the odor and the taste is reversed, and when the "meaning" of the two odors alters, so does the behavior. It is of interest to investigate in which parts of the olfactory system the neurons show reversal, for where they do, it can be concluded that the neuronal response to the odor depends on the taste with which it is associated, and does not depend primarily on the physicochemical structure of the odor.) These findings demonstrate directly a coding principle in primate olfaction whereby the responses of some orbitofrontal cortex olfactory neurons are modified by, and depend upon, the taste with which the odor is associated (Rolls, 2001, 2002a,b).

8.5 THE REPRESENTATION OF THE PLEASANTNESS OF ODOR IN THE BRAIN: OLFACTORY AND VISUAL SENSORY-SPECIFIC SATIETY, THEIR REPRESENTATION IN THE PRIMATE ORBITOFRONTAL CORTEX, AND THE ROLE OF SENSORY-SPECIFIC SATIETY IN APPETITE

It has also been possible to investigate whether the olfactory representation in the orbitofrontal cortex is affected by hunger, and thus whether the pleasantness of odor is represented in the orbitofrontal cortex. In satiety experiments, Critchley and Rolls (1996c) showed that the responses of some olfactory neurons to a food odor are decreased during feeding to satiety with a food (e.g., fruit juice, or cream) containing that odor. In particular, seven of nine olfactory neurons that were responsive to the odors of foods, such as blackcurrant juice, were found to decrease their responses to the odor of the satiating food. The decrease was typically at least partly specific to the odor of the food that had been eaten to satiety, potentially providing part of the basis for sensory-specific satiety. It was also found for eight of nine neurons that had selective responses to the sight of food that they demonstrated a sensory-specific reduction in their visual responses to foods following satiation. These findings show that the olfactory and visual representations of food, as well as the taste representation of food, in the primate orbitofrontal cortex are modulated by hunger. Usually a component related to sensory-specific satiety can be demonstrated.

8.6 THE RESPONSES OF ORBITOFRONTAL CORTEX TASTE AND OLFACTORY NEURONS TO THE SIGHT, TEXTURE, AND TEMPERATURE OF FOOD

The orbitofrontal cortex of primates is also important as an area of convergence for somatosensory inputs, related, for example, to the texture of food including fat in the mouth. We have shown, for example, that single neurons influenced by taste in this region can in some cases have their responses modulated by the texture of the food. This was shown in experiments in which the texture of food was manipulated by the addition of methyl cellulose or gelatine, or by puréeing a semisolid food (Rolls, 1998, 1999).

It has been shown that some of these neurons with texture-related responses encode parametrically the viscosity of food in the mouth (using a methyl cellulose series in the range 1–10,000 cP), and that others independently encode the particulate quality of food in the mouth, produced quantitatively, for example, by adding 20–100 μm microspheres to 1000 cP methyl cellulose ("Gritty") (Rolls et al., 2003b). The two neurons shown as examples in Figure 8.2 illustrate some of these properties. Neuron bk244 had a graded increase of firing rate to viscosity in the range 10–1000 cP, had no taste responses, did respond to oils, and did not respond to capsaicin. Neuron bo34 also had a graded increase of firing rate to viscosity in the range 10–10,000 cP; did respond to some tastes (glucose, sweet; HCl, sour; and quinine, bitter) but not to others (NaCl and MSG), did not respond to oils, and did respond to capsaicin. These neurons illustrate that taste, viscosity, fatty oils, and capsaicin can

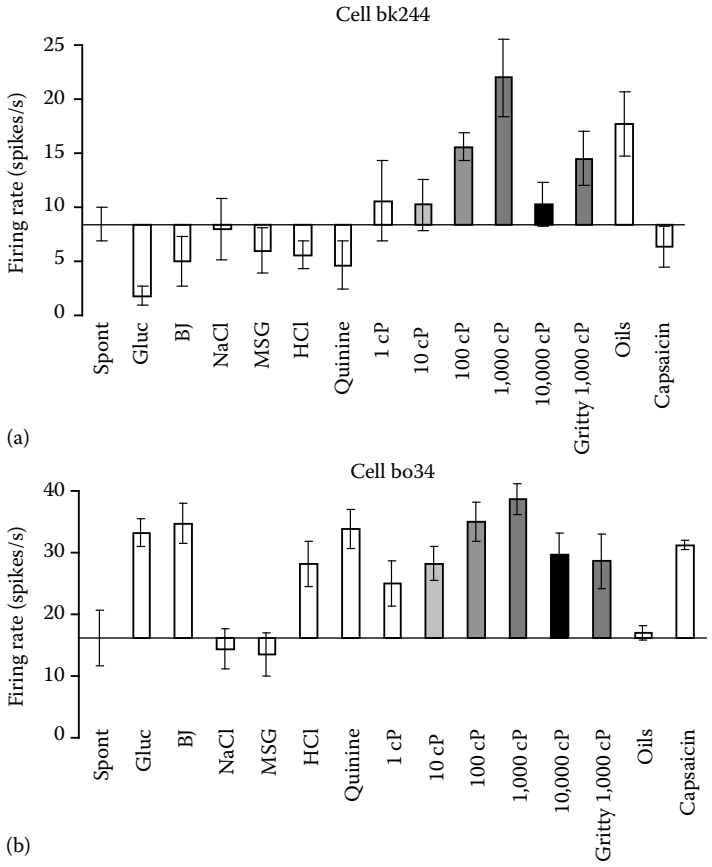


FIGURE 8.2 Oral somatosensory and taste inputs to orbitofrontal cortex neurons. (a) Firing rates (mean ± sem) of viscosity-sensitive neuron bk244 which did not have taste responses, in that it did not respond differentially to the different taste stimuli. The firing rates are shown to the viscosity series (CMC 1–10,000 cP, to the gritty stimulus (1000 cP CMC with Fillite microspheres), to the taste stimuli 1 M glucose (Gluc), 0.1 M NaCl, 0.1 M MSG, 0.01 M HCl and 0.001 M QuinineHCl, and to fruit juice (BJ). Spont = spontaneous firing rate. (b) Firing rates (mean ± sem) of viscosity-sensitive neuron bo34 which had no response to the oils (mineral oil, vegetable oil, safflower oil, and coconut oil, which have viscosities that are all close to 50 cP). The neuron did not respond to the gritty stimulus in a way that was unexpected given the viscosity of the stimulus, was taste tuned, and did respond to capsaicin. (After Rolls, E.T. et al., *J. Neurophysiol*., 90, 3711, 2003. With permission.)

be coded for independently by a population of neurons of which these are examples. The oils used in this study included mineral oil, silicone oil, vegetable oil, coconut oil, and safflower oil (see Table 8.1) (Rolls et al., 2003b).

In addition, recent findings (Kadohisa et al., 2004) have revealed that some neurons in the orbitofrontal cortex reflect the temperature of substances in the mouth, and that this temperature information is represented independently of other sensory inputs by some neurons, and in combination with taste or texture by other neurons.

TABLE 8.1
Stimuli

Stimulus	Abbreviation	Concentration	MW	App. Viscosity (cP)	Chemical Group
Glucose	G	1 M	180	1	Monosaccharide aldohexose
Black currant juice	BJ	20%		1	Mixture
Monosodium glutamate	M	0.1 M	187	1	Amino acid salt
NaCl	N	0.1 M	58	1	Inorganic salt
HCl	H	0.01 M	36	1	Inorganic acid
Quinine HCl	Q	0.001 M	387	1	Alkaloid
Water	V1 or 1 cP	5 mM NaCl		1	
CMC[a]	V10 or 10 cP	0.2 g + 1l V1	70,000	10	Polysaccharide
CMC	V100 or 100 cP	4.0 g + 1l V1	70,000	100	Polysaccharide
CMC	V1000 or 1000 cP	11.0 g + 1l V1	70,000	1000	Polysaccharide
CMC	V10,000 or 10,000 cP	24.0 g + 1l V1	70,000	10,000	Polysaccharide
Mineral oil	MO	100%		25	Hydrocarbon mixture
Silicone oil	SiO or SilO	100%		100 or 280	Silicon–oxygen polymer
Vegetable oil	VOo or VOf	100%		55	Fat
Coconut oil	CO	100%		40	Fat
Safflower oil	SaO or SafO	100%		50	Fat
Single cream	SC	100%		12	Emulsion
Lauric acid C12:0	LaA	100 μM		1	FFA
Linoleic acid C18:2	LiA	100 μM		1	FFA

[a] CMC, carboxy-methyl-cellulose.

8.7 THE MOUTH FEEL OF FAT: ORBITOFRONTAL CORTEX, PRIMARY TASTE CORTEX, AND AMYGDALA

Texture in the mouth is an important indicator of whether "fat" is present in a food, which is important not only as a high-value energy source, but also as a potential source of essential fatty acids. In the orbitofrontal cortex, Rolls et al. (1999) have found a population of neurons that responds to the texture of fat in the mouth. Figure 8.3 shows an example of one of these neurons. The neuron increased its firing rate to cream (double and single cream, with the fat proportions shown), and responded

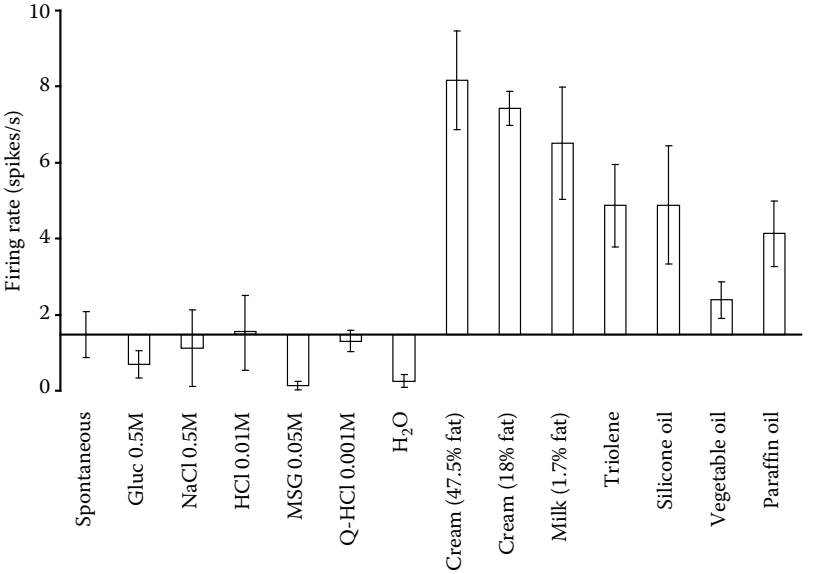


FIGURE 8.3 A neuron in the primate orbitofrontal cortex responding to the texture of fat in the mouth. The neuron increased its firing rate to cream (double and single cream, with the fat proportions shown), and responded to texture rather than the chemical structure of the fat in that it also responded to 0.5 mL of silicone oil ($Si(CH_3)_2O)_n$) or paraffin oil (hydrocarbon). The neuron did not have a taste input. Gluc, glucose; NaCl, salt; HCl, sour; Q-HCl, quinine, bitter. The spontaneous firing rate of the cell is also shown. (After Rolls, E.T. et al., *J. Neurosci*., 19, 1532, 1999. With permission.)

to texture rather than the chemical structure of the fat in that it also responded to 0.5 mL of silicone oil ($Si(CH_3)_2O)_n$) or paraffin oil (hydrocarbon). The neuron did not have a taste input. The firing rate responses are shown against the baseline spontaneous firing rate of the neuron.

Figure 8.4 shows an example of a fat-responsive neuron in the orbitofrontal cortex from the study of Verhagen et al. (2003a) where the evoked neuronal activity to the indicated stimuli is plotted as a function of viscosity. The cell showed strong and similar responses to all the oils tested, but did not respond to any of the carboxymethyl-cellulose (CMC) viscosity series below 10,000 cP, and to none of the CMC viscosity series with viscosities in the range of the oils. (The stimuli used, and their abbreviations, are in Table 8.1.) The neuronal responses were significantly different between the oils and the spontaneous firing rate and V10–V100 (both $p < .001$). Further, in contrast to the robust excitatory responses to safflower oil (45 ± spikes/s) and coconut oil (50 ± spikes/s), which are rich in LiA and lauric acid (LaA) bound into triglycerides, the responses to LiA and LaA were slightly below spontaneous rate, providing evidence that the neurons did not respond to fats based on gustatory sensitivity to the fatty acids. Figure 8.5 shows another example of a fat-sensitive neuron in the orbitofrontal cortex.

A different type of neuron is now described, to make it clear how selective the type of neuron shown in Figures 8.4 and 8.5 is for fat texture. In this comparison

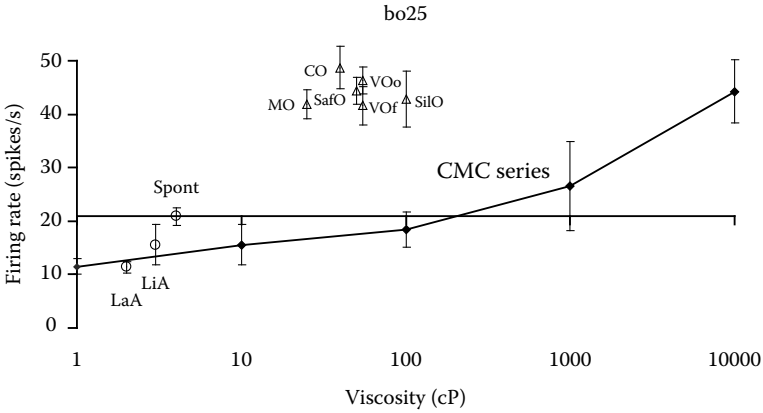


FIGURE 8.4 Evoked activity graphed against apparent stimulus viscosity for fat-responsive neuron bo25. The line indicates the responses to the CMC viscosity series. The mean and the standard error of the mean responses calculated in a 1 s period over four to six trials are shown here and elsewhere unless otherwise indicated. The oils evoked significantly higher activity than either the spontaneous activity or CMC at corresponding apparent viscosities. The oils were vegetable oil (VOo, VOf), safflower oil (SO), coconut oil (CO), silicone oil (SilO), and mineral oil (MO). Linoleic acid (Lin) and lauric acid (Lau) were also tested. Details of the stimuli are in Table 8.1. The information that reaches this type of neuron is independent of a viscosity-sensing channel, in that the neuron did not respond to the methyl cellulose (CMC) viscosity series. (After Verhagen, J.V. et al., *J. Neurophysiol*., 90, 1514, 2003a. With permission.)

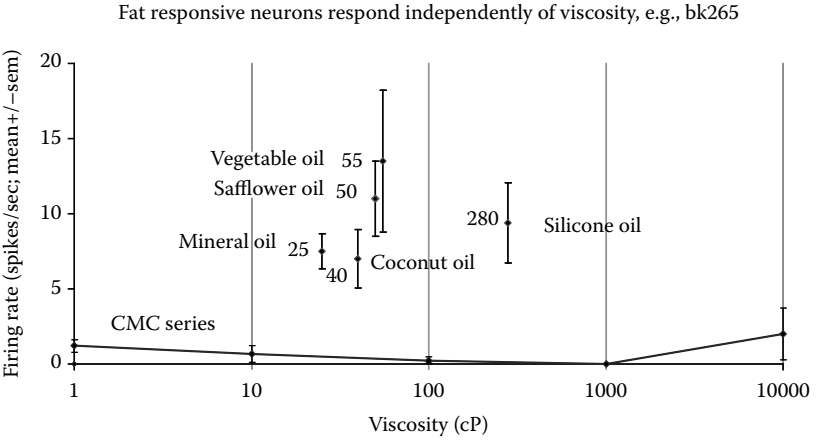


FIGURE 8.5 A neuron in the primate orbitofrontal cortex responding to the texture of fat in the mouth independently of viscosity. The cell (bk265) increased its firing rate to a range of fats and oils (the viscosity of which is shown in cP). The information that reaches this type of neuron is independent of a viscosity-sensing channel, in that the neuron did not respond to the methyl cellulose (CMC) viscosity series. The neuron responded to the texture rather than the chemical structure of the fat in that it also responded to silicone oil ($Si(CH_3)_2O)_n$) and paraffin (mineral) oil (hydrocarbon). Some of these neurons have taste inputs. For abbreviations see Table 8.1. (After Verhagen, J.V., Rolls, E.T., and Kadohisa, M., *J. Neurophysiol*., 90, 1514, 2003a. With permission.)

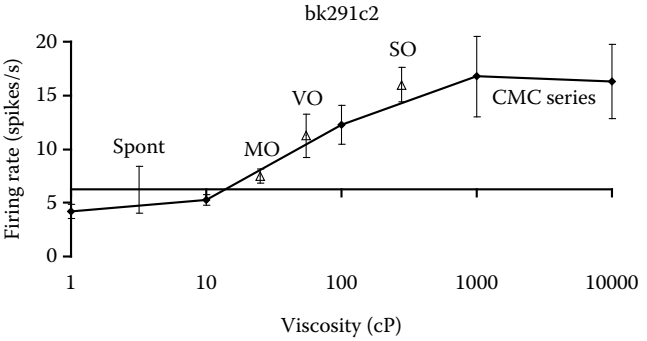


FIGURE 8.6 Evoked activity graphed against apparent stimulus viscosity for viscosity-sensitive neuron bk291c2. The line indicates the responses to the CMC series. The oil-evoked activity follows that evoked by the CMC viscosity stimuli at corresponding apparent viscosities. For abbreviations see Table 8.1. (After Verhagen, J.V. et al., *J. Neurophysiol*., 90, 1514, 2003a. With permission.)

type of neuron, the responses to oils were apparently determined by the viscosity of the oils, and the neurons were effectively viscosity sensitive rather than fat sensitive. For example, the neuron illustrated in Figure 8.6 showed an increasing neuronal response as the viscosity of the CMC series increased from V10–V1000; and the responses to mineral, vegetable, and silicone oil, plotted at their viscosities, follow this viscosity–sensitivity curve closely.

We also observed neurons that did respond to the CMC viscosity series but not to the oils (Verhagen et al., 2003a). This type of neuronal response, which also illustrates how neurons can be tuned to a range of viscosities, is exemplified by the neuron shown in Figure 8.7. The neuronal responses show an upward trend with increasing viscosity (V1–V1000), and a reduction at V10,000. However, none of the oils evoked significant activity. Thus the responses of this neuron show another way in which orbitofrontal cortex neurons can discriminate between fat texture and viscosity, by in this case responding to information conveyed through a viscosity information channel but not through a fat-sensitive information channel. Part of the interest of this type of neuron is that although the oils had viscosities that were in the range 25–100 cP (see Table 8.1), the neuron did not respond to this level of viscosity when it was expressed through the presence of an oil. This provides evidence that oils must therefore have other textural properties (reflected for example in their slickness) that prevent this type of neuron from responding. This neuron had firing rates to LiA and LaA (4, 3 spikes/s, respectively) that were predictable by their viscosity, as shown in Figure 8.7.

In this study, 5.4% of the orbitofrontal cortex neurons responded to fat, viscosity and/or taste (Verhagen et al., 2003a). Of the 14 neurons with responses to fat in the mouth where the response to fat was independent of viscosity (i.e., could not be predicted from viscosity) (1.6% of all screened neurons), 9 responded to both taste and to fat, 4 to taste and to viscosity, and 1 did not respond to taste or to viscosity. Thirteen of the 18 neurons responsive to viscosity also responded to taste, 4 of those also to fat where the response to the fat could be predicted from the viscosity, and

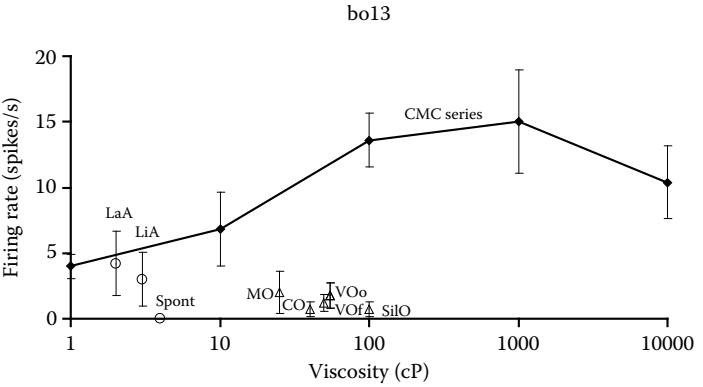


FIGURE 8.7 Evoked activity graphed against apparent stimulus viscosity for neuron bo13. The line indicates the responses to the CMC series. This neuron did respond to viscosity (of the CMC series) but no activity was evoked by the oils. For abbreviations see Table 8.1. (After Verhagen, J.V. et al., *J. Neurophysiol*., 90, 1514, 2003a. With permission.)

5 responded to only viscosity. In addition, the number of neurons that was tuned to the CMC viscosity series and which did not respond to fat in the way that would be predicted by the CMC viscosity tuning (see example in Figure 8.7) was 4 of the 50 neurons analyzed.

The results of these studies on orbitofrontal cortex neurons (Rolls et al., 1999; Verhagen et al., 2003a) show that fat-sensitive neurons respond not only to fats such as vegetable oil and other fatty oils in the mouth, and to substances rich in fat such as cream and chocolate, but also to chemically different substances which have a similar slick or oily texture such as mineral oil (pure hydrocarbon), and silicone oil ($Si(CH_3)_2O)_n$). This evidence thus indicates that the mechanisms that sense fat and to which these neurons respond are sensing a physical rather than a chemical property of the stimuli. The results also provide evidence that the responses of fat-sensitive neurons are not based on a texture information channel that is tuned to viscosity. In particular, although some neurons in the orbitofrontal cortex are tuned to viscosity (see examples in Figures 8.6 and 8.7), many (10/14) of the fat-sensitive neurons did not respond to the viscosity series of stimuli (see examples in Figures 8.4 and 8.5); or had responses to the fats and some of the CMC viscosity series, where the response to fat could not be accounted for by viscosity (4/14); and 4 neurons were responsive to viscosity but did not respond to fat (see example in Figure 8.7). The latter type of neuron (Figure 8.7) is rather interesting, for the implication is that although there is a viscosity-sensing channel that responds to stimuli including fats based on their viscosity (Figure 8.6), there is also a mechanism for representing viscosity when it is not produced by a fatty oily stimulus, as in Figure 8.7.

Important conclusions then about the representation of oral texture in the brain (and illustrated by the types of neuron shown in Figures 8.4 through 8.7) are (1) There is an information channel that represents fat independently of viscosity. (2) There is an information channel that represents viscosity and also responds to fat based on the viscosity of the fat. This channel thus responds to viscosity independently of whether the

eliciting stimulus is a nonfat or a fat. (3) There is an information channel that encodes viscosity provided that it is not associated with an oily substance such as a fat. The third type of channel could reflect a separate sensing mechanism in the mouth; or it could reflect competitive and expansion recoding processes (Rolls and Deco, 2002; Rolls, 2008b) produced in the cortex by inputs reflecting, for example, fat sensitivity without viscosity in a first channel, and viscosity with fat sensitivity in another channel.

Gustatory mechanisms have been revealed in rat oral taste cells that may mediate a possible fat taste: the slow modulation of K-channels by polyunsaturated FFA such as LiA (Gilbertson et al., 1997; Gilbertson, 1998). However, salivary lipase which could release fatty acid from fat in rats to activate such a mechanism, is hardly present in humans (Gilbertson et al., 1997; Gilbertson, 1998), so that this mechanism may not be important in humans. Further evidence that this chemical sensing mechanism may not be important in primates including humans is that the time course of the activation of the K-channel mechanism is very slow (Gilbertson et al., 1997; Gilbertson, 1998), and does not match the rapidly developing subjective sensation of fat in the mouth. However, to test this possibility, responses by the population of orbitofrontal cortex neurons to the FFA, LiA, and LaA were measured, and for most neurons responses were not found, that is for most neurons the activity evoked by these stimuli was indistinguishable to that evoked by water (Verhagen et al., 2003a). In particular, of 37 neurons tested with LaA and LiA, 34 had no significant responses compared to water. Of the three neurons that had statistically significant responses in this comparison, all three consisted of a smaller response than was obtained to water, and in two cases the statistical significance was marginal, i.e., $p \approx .05$. To assess whether the firing rates obtained to LaA and LiA could predict the responses of the neurons to coconut oil (high in lauric conjugated to glycerol) and to safflower (high in LiA conjugated to glycerol), linear regression analysis was performed across the sample of 14 fat-sensitive neurons in the orbitofrontal cortex (Verhagen et al., 2003a). There was no significant correlation between the responses to the fatty acids and these two fat stimuli. (For LaA, $r = .45$, $p = .20$; for LiA, $r = .61$, $p = .06$.) Thus, the responses to fats by this population of neurons cannot be accounted for by sensitivity to LaA and LiA. By contrast, the responses to fats could be predicted by their response to the texture of silicone oil. (For silicone oil vs. coconut oil $r = .99$, $p < .001$; while for silicone oil vs. safflower oil $r = .99$, $p < .001$.) Together, these points of evidence (Verhagen et al., 2003a) suggest that fat in the mouth can be sensed in primates at least independently of any oral gustatory mechanism for FFA (a mechanism suggested by Gilbertson (1998) in rodents). These data suggest that different sensing mechanisms and percepts are evoked by FFA as compared to fatty oils. Perceptual responses to FFA, if large enough not to also taste sour (Forss, 1972), depend at least partly on the trigeminal nociceptive pathway and may be associated with the percept of oral irritation. To the extent that fatty acid taste may occur in humans, it may tend to make food unpleasant, with a rancid flavor, and consistent with this, food manufacturers minimize the content of free fatty acids in foods (Mattes, 2009). The oils, whether triglyceride-based or not, are sensed by a somatosensory-textural pathway and may be associated with the mouth feel of fatty/slickness. It is the fat texture component that may impart pleasant sensory attributes to fat, as shown by the evidence that orbitofrontal cortex fat texture neurons in macaques respond less to fat texture after feeding to satiety with a high fat food (Rolls et al., 1999),

with the pleasantness of oral fat represented in humans in the orbitofrontal and pregenual cingulate cortex (Grabenhorst et al., 2009).

Some of the fat-related neurons do though receive convergent inputs from the chemical senses, in that in addition to taste inputs, some of these neurons respond to the odor associated with a fat, such as the odor of cream (Rolls et al., 1999). Feeding to satiety with fat (e.g., cream) decreases the responses of these orbitofrontal cortex neurons to zero on the food eaten to satiety [including its odor (Critchley and Rolls, 1996c)], but if the neuron receives a taste input from, for example, glucose taste, that is not decreased by feeding to satiety with cream (Rolls et al., 1999). Thus there is a representation of the macronutrient fat in this brain area, and the activation produced by fat is reduced by eating fat to satiety. It is thus the reward, affective, or hedonic value of fat that is represented in the orbitofrontal cortex.

The common perceptual quality among the oils is the slick/fatty mouthfeel. The function of the fat texture-sensitive neurons (Rolls et al., 1999; Verhagen et al., 2003a) may be to allow recognition of fatty substances in the mouth, based on texture information received through the somatosensory system. Consistent with this, Mela (1988) reported that humans rate the fat content of dairy products based on their textural properties. We note that the types of neuron described here, responding to fat independently of viscosity, to viscosity whether it is produced by nonfat or by fat, and to viscosity when it is not being produced by a fatty/oily texture, provide an excellent representation of many textural properties of food, including creaminess, for which ratings provided by humans depend on a variety of textural properties (Bourne, 2002). Further breadth to the representation is provided by the taste and olfactory inputs to some fat-sensitive neurons (Critchley and Rolls, 1996c; Rolls et al., 1999; Verhagen et al., 2003a). This is consistent with the hypothesis that a food's flavor (and appearance) is represented in the orbitofrontal cortex by integration of somatosensory, gustatory, temperature, olfactory, visual, and cognitive information in such multimodal neurons (Rolls and Baylis, 1994; Critchley and Rolls, 1996a,c; Rolls et al., 1996a, 2008a; Kadohisa et al., 2004; Guest et al., 2007; Rolls, 2007; Grabenhorst and Rolls, 2008; Grabenhorst et al., 2008).

Fat texture, oral viscosity, and temperature, for some neurons in combination with taste, are represented in the macaque primary taste cortex in the rostral insula and adjoining frontal operculum (Verhagen et al., 2004). This could reflect convergence of taste and texture inputs in the insular cortex, or the convergence could be present already at earlier stages of taste processing. It is known that some neurons in the taste thalamus (nucleus VPMpc) have thermal responsiveness in monkeys (Pritchard et al., 1989) and rats (Verhagen et al., 2003b). In the periphery, it is known that chorda tympani fibers in the monkey (Sato et al., 1975) and hamster (Ogawa et al., 1968) show significant correlations between the responses to HCl and those to cooling (20°C), and between the responses to sucrose and warming (to 40°C). Some lingual nerve fibers in monkeys were activated by cooling to 15°C but not by taste (Danilova and Hellekant, 2002). There may be no studies in the periphery of the effects of food-relevant oral stimuli such as viscosity and fat texture. It is also possible that oral somatosensory information reaches the anterior insular/frontal opercular primary taste cortex via cortico-cortical connections, perhaps from areas 3b which contain oral somatosensory representations of, for example, touch of the tongue, teeth, and palate (Manger et al., 1996; Jain et al., 2001) and which might

send afferents to the anterior insular/frontal opercular primary taste cortex (Mufson and Mesulam, 1982; Friedman et al., 1986).

Given that an important input to the orbitofrontal cortex is from the primary taste cortex (Baylis et al., 1995), the responses of orbitofrontal cortex neurons to fat texture, and also oral viscosity, temperature, and taste, are likely to be produced at least in a large part via the primary taste cortex.

These oral sensory properties of food, including viscosity and fat texture, and also the sight and smell of food, are also represented in the primate amygdala (Rolls, 2000b; Rolls and Scott, 2003; Kadohisa et al., 2005a,b), which also receives inputs from the primary taste cortex. Interestingly, the responses of these amygdala neurons do not correlate well with the preferences of the macaques for the oral stimuli (Kadohisa et al., 2005a), and feeding to satiety does not produce the large reduction in the responses of amygdala neurons to food (Rolls, 2000b; Rolls and Scott, 2003) that is typical of orbitofrontal cortex neurons.

8.8 ACTIVATION OF THE HUMAN BRAIN BY ORAL SIGNALS, INCLUDING FAT TEXTURE

8.8.1 Taste

In humans it has been shown in neuroimaging studies using functional Magnetic Resonance Imaging (fMRI) that taste activates an area of the anterior insular/frontal opercular cortex, which is probably the primary taste cortex, and part of the orbitofrontal cortex, which is probably the secondary taste cortex (Francis et al., 1999; Small et al., 1999; O'Doherty et al., 2001; de Araujo et al., 2003b). It has been shown that within individual subjects separate areas of the orbitofrontal cortex are activated by sweet (pleasant) and by salt (unpleasant) tastes (O'Doherty et al., 2001).

Francis et al. (1999) also found activation of the human amygdala by the taste of glucose. Extending this study, O'Doherty et al. (2001) showed that the human amygdala was as much activated by the affectively pleasant taste of glucose as by the affectively negative taste of NaCl, and thus provided evidence that the human amygdala is not especially involved in processing aversive as compared to rewarding stimuli. Zald et al. (1998) had shown earlier that the amygdala, as well as the orbitofrontal cortex, respond to aversive (saline) taste stimuli. The study above (O'Doherty et al., 2001), however, shows that there is nothing special about aversive taste stimuli in relation to the brain areas activated, for pleasant stimuli also activate the amygdala and orbitofrontal cortex.

Another study has shown that umami taste stimuli, of which an exemplar is MSG and which capture what is described as the taste of protein, activate similar cortical regions of the human taste system to those activated by a prototypical taste stimulus, glucose (de Araujo et al., 2003a). A part of the rostral anterior cingulate cortex (ACC) was also activated. When the nucleotide 0.005 M inosine 5′-monophosphate (IMP) was added to MSG (0.05 M), the blood oxygenation-level dependent (BOLD) signal in an anterior part of the orbitofrontal cortex showed supralinear additivity, and this may reflect the subjective enhancement of umami taste that has been described when IMP is added to MSG. Overall, these results illustrate that the responses of

the brain can reflect inputs produced by particular combinations of sensory stimuli with supralinear activations, and that the combination of sensory stimuli may be especially represented in particular brain regions.

8.8.2 Odor

In humans, in addition to activation of the pyriform (olfactory) cortex (Zald and Pardo, 1997; Sobel et al., 2000; Poellinger et al., 2001), there is strong and consistent activation of the orbitofrontal cortex by olfactory stimuli (Zatorre et al., 1992; Francis et al., 1999). In an investigation of where the pleasantness of olfactory stimuli might be represented in humans, O'Doherty et al. (2000) showed that the activation of an area of the orbitofrontal cortex to banana odor was decreased (relative to a control vanilla odor) after bananas were eaten to satiety. Thus activity in a part of the human orbitofrontal cortex olfactory area is related to sensory-specific satiety, and this is one brain region where the pleasantness of odor is represented.

An important issue is whether there are separate regions of the brain discriminable with fMRI that represent pleasant and unpleasant odors. To investigate this, we measured the brain activations produced by three pleasant and three unpleasant odors. The pleasant odors chosen were linalyl acetate (floral, sweet), geranyl acetate (floral), and alpha-ionone (woody, slightly food related). The unpleasant odors chosen were hexanoic acid, octanol, and isovaleric acid. We found that they activated dissociable parts of the human brain (Rolls et al., 2003a). Pleasant but not unpleasant odors were found to activate a medial region of the rostral orbitofrontal cortex. Further, there was a correlation between the subjective pleasantness ratings of the six odors given during the investigation with activation of a medial region of the rostral orbitofrontal cortex. In contrast, a correlation between the subjective unpleasantness ratings of the six odors was found in regions of the left and more lateral orbitofrontal cortex. Activation was also found in the ACC, with a middle part of the anterior cingulate activated by both pleasant and unpleasant odors, and a more anterior part of the ACC showing a correlation with the subjective pleasantness ratings of the odors (Rolls et al., 2003a). These results provide evidence that there is a hedonic map of the sense of smell in brain regions such as the orbitofrontal cortex and cingulate cortex.

8.8.3 Olfactory-Taste Convergence to Represent Flavor, and the Influence of Satiety

To investigate where in the human brain interactions between taste and odor stimuli may be realized to implement flavor, we performed an event-related fMRI study with sucrose and MSG taste, and strawberry and methional (chicken) odors, delivered unimodally or in different combinations (de Araujo et al., 2003c). The brain regions that were activated by both taste and smell included parts of the caudal orbitofrontal cortex, amygdala, insular cortex and adjoining areas, and ACC. It was shown that a small part of the anterior (putatively agranular) insula responds to unimodal taste and to unimodal olfactory stimuli; and that a part of the anterior frontal operculum is a unimodal taste area (putatively primary taste cortex) not activated by olfactory stimuli. Activations to combined olfactory and taste stimuli where there was little or

no activation to either alone (providing positive evidence for interactions between the olfactory and taste inputs) were found in a lateral anterior part of the orbitofrontal cortex. Correlations with consonance ratings for the smell and taste combinations, and for their pleasantness, were found in a medial anterior part of the orbitofrontal cortex. Similarly, Small et al. (2004) also found supra-additive interactions between congruent taste and smell stimuli in areas including the caudal orbitofrontal cortex, and ACC (see also Small and Prescott, 2005). These results provide evidence on the neural substrate for the convergence of taste and olfactory stimuli to produce flavor in humans, and where the pleasantness of flavor is represented in the human brain.

McCabe and Rolls (2007) have shown that the convergence of taste and olfactory information appears to be important for the delicious flavor of umami. They showed that when glutamate is given in combination with a consonant, savory, odor (vegetable), the resulting flavor can be much more pleasant than the glutamate taste or vegetable odor alone. Moreover, they found using functional brain imaging with fMRI that the glutamate and savory odor combination produced much greater activation of the pregenual cingulate cortex and medial orbitofrontal cortex than the sum of the activations by the taste and olfactory components presented separately. Further, activations in these brain regions were correlated with the pleasantness, consonance of the taste and olfactory components, and the fullness of the flavor, of the stimuli. Similar nonlinear effects were not found for sodium chloride and vegetable odor. Rolls and McCabe thus proposed that glutamate acts by the nonlinear effects it can produce when combined with a consonant odor. They further proposed the concept that umami can be thought of as a rich and delicious flavor that is produced by a combination of glutamate taste and a consonant savory odor. Glutamate is thus a flavor enhancer because of the way that it can combine nonlinearly with consonant odors.

8.8.4 Oral Viscosity and Fat Texture

To investigate the representation of oral texture including fat texture in the human brain, de Araujo and Rolls (2004) used event-related fMRI while stimuli of three viscosities (1 cP and CMC 50, and 1000 cP), a fatty oil, or 1 M sucrose used to localize taste areas, were delivered intra-orally in volumes of 0.75 mL. The fat stimulus was vegetable oil (rapeseed oil consisting of 6.1 g of saturate fat, 54.4 g of mono-unsaturated fat, and 26.9 g of polyunsaturated fat per 100 mL, Sainsbury's Supermarkets, U.K.) with a measured viscosity of 50 cP. This oil was chosen as it was the most odorless and tasteless of those that could be obtained. A tasteless solution [containing the main ionic components of saliva, 25 mM KCl + 2.5 mM $NaHCO_3$ in distilled water (de Araujo et al., 2003b)] was used as a control which was subtracted from the activations to the test stimuli.

First, we found activation of the anterior insular (putative primary) taste cortex of humans by oral viscosity stimuli (Figure 8.8 middle), in a region that was shown to be taste-related by its activations to oral sucrose. Indeed, the BOLD activation here was proportional to the log of the viscosity of the oral stimuli. Fat also activated this region (Figure 8.8 middle), though not in a way that was identified with the fMRI method as being qualitatively different from the activation produced by a viscosity stimulus made to the same viscosity value with CMC. We hypothesized therefore that

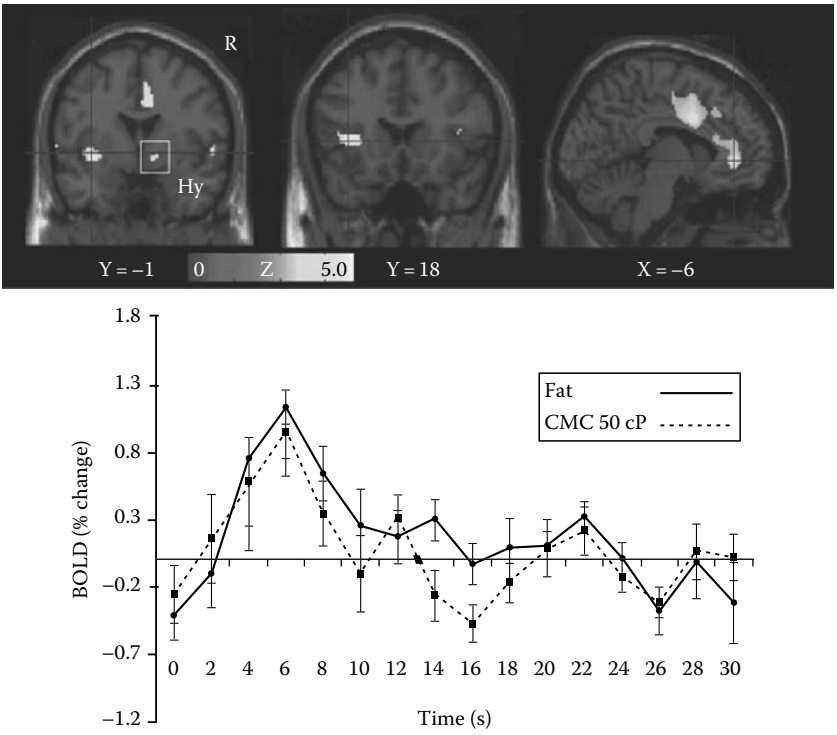


FIGURE 8.8 (See color insert following page 166.) fMRI study of the responses to the oral delivery of fat as assessed by the comparison (fat-control). Activations were observed in the mid-insula and hypothalamus (Hy) (top row left), anterior insula (top row middle), and ACC (top row right). The average time-course data (across trials and subjects) from the mid-insular cortex (from the voxels marked by the crosshairs in the top row left) are shown in the bottom row for the conditions Fat and CMC 50 cP. (After de Araujo I.E.T. and Rolls E.T., *J. Neurosci*., 24, 3086, 2004. With permission.)

the activation of this region in humans corresponds to the details revealed by single neuron recording in macaques, namely that some neurons in the primary taste cortex are activated by taste unimodally, some by viscosity unimodally, some by both taste and viscosity, and some by fat texture (Verhagen et al., 2004). The fMRI findings on the human anterior insular cortex are consistent with the hypothesis that the same processing takes place in the human anterior insular cortex (de Araujo and Rolls, 2004).

Second, we found activation of a midinsular region behind the primary taste cortex that was activated by viscosity and by fat but not by taste (Figure 8.8 left). This may be a purely somatosensory part of the insula that is a higher order somatosensory cortical area, this part of which is devoted to intraoral somatosensory inputs. The somatosensory representation of the oral cavity is located in this part of the insula extending anteriorly to the orbitofrontal cortex (Manger et al., 1996; Jain et al., 2001). This midinsular cortex may represent a range of somatosensory properties of the oral activity, for in a study of the effects of intraoral water, we found that activation in the same midinsular region was produced by water when thirsty but

not after thirst was quenched. We interpreted this as a somatosensory effect related to relief of a dry mouth by water, in that this region was again not activated by taste stimuli (de Araujo et al., 2003b).

Third, we found activations produced by fat in the mouth in the orbitofrontal cortex, where some neurons in macaques specifically encode oral fat independently of viscosity (Rolls et al., 2003b; Verhagen et al., 2003a), in a region to which this projects, the pregenual cingulate cortex (Figure 8.8 right, at the location shown by the crosshairs), and also more dorsally in the ACC (Figure 8.8 right). The activation by fat in the pregenual cingulate cortex was especially interesting, in that the activation here to fat was independent of viscosity (produced by CMC) (see Figure 8.9). This pregenual cingulate region was also activated by sucrose taste, and is a strong candidate for a brain region activated by the hedonic properties of fat. This pregenual cingulate region has been shown to contain taste-responsive neurons (Rolls, 2008a). Further evidence linking this pregenual cingulate region to pleasant affective properties (Bush et al., 2000) of sensory stimuli is that the same region is activated by water when it tastes pleasant during thirst (de Araujo et al., 2003b), by pleasant but not unpleasant odors (Rolls et al., 2003a), and by pleasant but not by painful touch (Rolls et al., 2003c). Further, this pregenual cingulate region is also implicated in the control of autonomic function (Critchley et al., 2004). Further, the ACC can be activated by hedonically relevant stimuli, including chemosensory, and somatosensory stimuli (Zald et al., 1998; Small et al., 1999; Zatorre et al., 2000; Rolls, 2005, 2009; McCabe and Rolls, 2007; Rolls and McCabe, 2007; Grabenhorst and Rolls, 2008; Grabenhorst et al., 2008; McCabe et al., 2008; Rolls and Grabenhorst, 2008; Rolls et al., 2008a,b).

The findings show that the details of the representation of fat and oral texture that has been uncovered by single neuron analyses in the macaque insula, orbitofrontal cortex, and connected areas is likely to also apply in humans in the corresponding areas in which activations to similar stimuli have been found (de Araujo and Rolls, 2004).

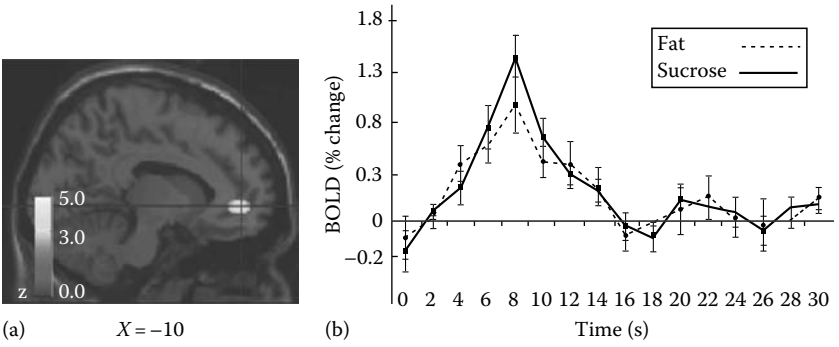
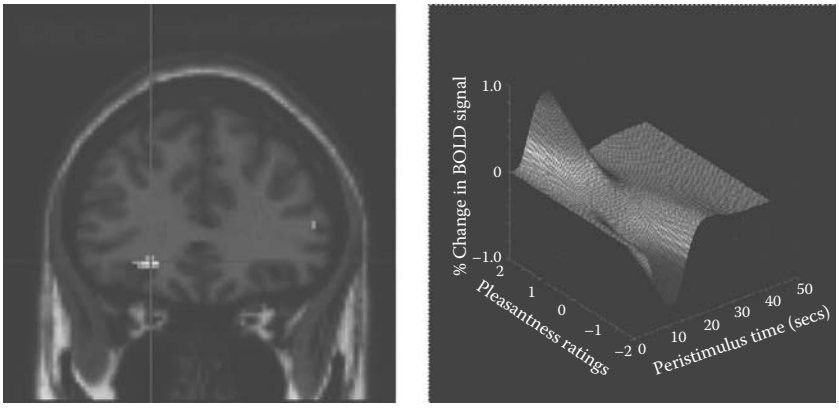


FIGURE 8.9 (See color insert following page 166.) (a) Rostral ACC activation by fat-control and sucrose-control, as revealed by conjunction analysis. (b) The corresponding average time-course data (across trials and subjects) from the voxel marked by the crosshairs are shown. (After de Araujo I.E.T. and Rolls E.T., *J. Neurosci*., 24, 3086, 2004. With permission.)

8.8.5 The Pleasantness of the Flavor of Food

To assess how satiety influences the brain activations to a whole food which produces taste, olfactory, and texture stimulation, we measured brain activation by whole foods before and after the food was eaten to satiety (de Araujo et al., 2003a). The aim was to show using a food that has olfactory, taste, and texture components the extent of the region that shows decreases when the food becomes less pleasant, in order to identify the different brain areas where the pleasantness of the odor, taste, and texture of food are represented. The foods eaten to satiety were either chocolate milk (which had a fat texture component), or tomato juice (which did not have a fat texture component). A decrease in activation by the food eaten to satiety relative to the other food was found in the orbitofrontal cortex (Kringelbach et al., 2003) but not in the primary taste cortex (see Figure 8.10). This study provided evidence that the pleasantness of the flavor of food, and sensory-specific satiety, are represented in the human orbitofrontal cortex.

Given that there are individual differences in the palatability of food, can these individual differences be related to the functioning of brain systems such as the



(a) (b)

FIGURE 8.10 (See color insert following page 166.) Areas of the human orbitofrontal cortex with activations correlating with pleasantness ratings for food in the mouth. (a) Coronal section through the region of the orbitofrontal cortex from the random effects group analysis showing the peak in the left orbitofrontal cortex (Talairach coordinates $x, y, z = -22, 34, -8$, Z-score = 4.06), in which the BOLD signal in the voxels shown in yellow was significantly correlated with the subjects' subjective pleasantness ratings of the foods throughout an experiment in which the subjects were hungry and found the food pleasant, and were then fed to satiety with the food, after which the pleasantness of the food decreased to neutral or slightly unpleasant. The design was a sensory-specific satiety design, and the pleasantness of the food not eaten in the meal, and the BOLD activation in the orbitofrontal cortex, were not altered by eating the other food to satiety. The two foods were tomato juice and chocolate milk. (b) Plot of the magnitude of the fitted hemodynamic response from a representative single subject against the subjective pleasantness ratings (on a scale from –2 to +2) and peristimulus time in seconds. (After Kringelbach, M.L. et al., *Cereb. Cortex*, 13, 1064, 2003. With permission.)

orbitofrontal and pregenual cingulate cortex involved in the affective (hedonic) representations of food?

Some individuals, chocolate cravers, report that they crave chocolate more than noncravers, and this is associated with increased liking of chocolate, increased wanting of chocolate, and eating chocolate more frequently than noncravers (Rodriguez et al., 2007). In a test of whether these individual differences are reflected in the affective systems in the orbitofrontal cortex and pregenual cingulate cortex, Rolls and McCabe (2007) used fMRI to measure the response to the flavor of chocolate, to the sight of chocolate, and to their combination, in chocolate cravers vs. noncravers. It was shown that the sight of chocolate produced more activation in chocolate cravers than noncravers in the medial orbitofrontal cortex and ventral striatum. For cravers vs. noncravers, a combination of a picture of chocolate with chocolate in the mouth produced a greater effect than the sum of the components (i.e., supralinearity) in the medial orbitofrontal cortex and pregenual cingulate cortex. Furthermore, the pleasantness ratings of the chocolate and chocolate-related stimuli had higher positive correlations with the fMRI BOLD signals in the pregenual cingulate cortex and medial orbitofrontal cortex in the cravers than in the noncravers. Thus there were differences between cravers and noncravers in their responses to the sensory components of a craved food in the orbitofrontal cortex, pregenual cingulate cortex, and ventral striatum, and in some of these regions the differences are related to the subjective pleasantness of the craved foods. Differences in the insular taste cortex were not found. An implication is that individual differences in brain responses to very pleasant foods help to understand the mechanisms that drive the liking for specific foods by indicating that some (but not other brain systems such as the insular taste cortex) respond more to the rewarding aspects of some foods, and thus influence and indeed even predict the intake of those foods (which was much higher in chocolate cravers than noncravers) (Rolls and McCabe, 2007). Although texture is of course not the only contributor to the effects of chocolate, it is one important aspect of the sensory properties of chocolate.

8.9 CONCLUSIONS

Fat in the mouth is represented by its texture in the primary taste cortex in the insula, in the orbitofrontal cortex, in the amygdala, and in the ACC. Fat texture is represented by neurons independently of viscosity: some neurons respond to fat independently of viscosity, and other neurons encode viscosity. The neurons that respond to fat also respond to silicone oil and paraffin oil, indicating that the sensing is not chemospecific, but is instead based on texture. This fat sensing is not related to FFA, in that these neurons typically do not respond to FFA such as LiA. Moreover, a few neurons with responses to FFA typically do not respond to fat in the mouth. The fat texture representations by neurons may be combined with taste and/or oral temperature responses, and in the orbitofrontal cortex with olfactory responses. Different neurons respond to different combinations, providing a rich representation of the sensory properties of food. In the orbitofrontal cortex, feeding to satiety with one food decreases the responses of these neurons to that food, but not to other foods, showing that sensory-specific satiety and appetite modulation are represented in the

orbitofrontal cortex. In humans, individual differences in activations in areas such as the orbitofrontal cortex and pregenual cingulate cortex to a complex food such as chocolate are related to the affective value of the foods, and how much is eaten. In summary, one way in which fat in the mouth is represented in the brain is by its texture, and an indication of what must be transduced has been provided by these neuroscience studies.

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9 Advantageous Object Recognition for High-Fat Food Images

Ulrike Toepel, Jean-François Knebel, Julie Hudry, Johannes le Coutre, and Micah M. Murray

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9.1 INTRODUCTION

How the human brain recognizes and differentiates objects from one another and likewise groups them into categories according to common features/actions has been a topic of neuropsychological and neuroscientific investigation for several decades. For example, ventral posterior temporal cortices have been found to play a major role not only in object processing in general (see Tanaka, 1997; Ungerleider and Haxby, 1994 for reviews), but also in category-selective processing (Moore and Price, 1999; Peissig and Tarr, 2007; Perani et al., 1995, 1999). One axis along which objects appear to be differentiated is whether their referent is something living or an artifact (i.e., manufactured). This distinction appears to hold true for visually presented stimuli (e.g., Caramazza and Shelton, 1998; Gerlach, 2007; Martin, 2007) as well as their auditory counterparts (Lewis et al., 2005; Murray et al., 2006). In addition to this categorical distinction, there is abundant evidence that the processing of faces

(and places) may recruit highly specialized neural circuitry (e.g., Bentin et al., 2007; Kanwisher and Yovel, 2006). One perspective is that such specialized responsiveness follows from the social importance of faces in human interactions. More recently, investigations have revealed that objects and words can also be differentially processed according to their associated actions by regions of the ventral premotor cortex and posterior parietal areas (see Culham and Valyear, 2006; Johnson-Frey, 2004; Lewis et al., 2005; Pulvermuller, 2005 for reviews).

Applying a similar line of reasoning, other socially or biologically important object categories would also be predicted to engage specialized neural circuitry. Foods and their proper discrimination, for example, are of utmost importance for an organism's survival. While this object category has been the subject of comparatively few functional neuroimaging studies, there is evidence to suggest that food images may activate brain regions distinct from those activated by other object categories (Killgore et al., 2003; LaBar et al., 2001; Rothemund et al., 2007; Santel et al., 2006; Simmons et al., 2005). In particular, viewing pictures of foods as opposed to nonfoods has been shown to activate frontal cortices as well as the frontal operculum and the insula (Killgore et al., 2003; LaBar et al., 2001; Simmons et al., 2005), the latter thought to be primary gustatory cortex regions (O'Doherty et al., 2001). Viewing foods can also modulate the activity in secondary gustatory regions within the orbitofrontal cortex (OFC; Beaver et al., 2006; Simmons et al., 2005). In addition to this, wider network of brain regions, striate, and extrastriate visual areas also exhibit modulated activity during the categorization of foods from tools (Killgore et al., 2003; LaBar et al., 2001; Simmons et al., 2005).

However, from this evidence alone the basis upon which foods are discriminated from other objects and categorized among each other cannot be determined. For example, it may be either the reward and/or sensory features of the stimuli that drive the observed differential responses. Also, from these data it cannot be discerned which area(s) among the network of regions exhibiting differential responses is first involved and in what sequence other regions contribute to the discrimination of foods. Rather, temporal information is essential for constructing accurate models of food discrimination and evaluation. Electrical neuroimaging based on electroencephalographic recordings has been proven to be a powerful tool to identify the spatiotemporal dynamics during the categorization of other object categories than food (e.g., tools, animals, vehicles, and faces). It has been shown that categorization and in some cases within-category discrimination occurs within the initial 100–200 ms following stimulus presentation (Bentin et al., 2007; Eger et al., 2003; Fabre-Thorpe et al., 2001; Ji et al., 1998; Johnson and Olshausen, 2003; Kanwisher and Yovel, 2006; Michel et al., 2004a; Pizzagalli et al., 1999; Proverbio et al., 2007; Thorpe et al., 1996; VanRullen and Thorpe, 2001).

Given this evidence, we hypothesized that the discrimination of food from nonfood images would occur within a similar time frame as that of other object categories. Moreover, although food is quintessential for survival, inherent differences exist with respect to nutritional and energetic value as well as hedonic attributes. As such, even though energetic value and palatability are critical factors influencing eating behavior, decisions regarding food intake are often guided by factors that can be detrimental for an individual's health. Often, high-fat foods are consumed with more pleasure, and

in larger quantities, than low-fat foods. One possibility is that the drive for hedonic experiences may lead to inappropriate food consumption behavior. In turn, overeating of detrimental foods can ultimately result in obesity, diabetes, and hypertension, which are increasingly prevalent among industrialized societies. Understanding the decision processes leading to food selection and consumption, and in particular how the brain appraises the presentation of foods before they are ingested, is likely to prove essential for learning how to control and correct inappropriate eating behavior.

For these reasons, the study presented here examined the influence of foods' energetic content (i.e., dietary fat) on object recognition. We recorded visual evoked potentials (VEPs) while healthy participants viewed photographs of high-fat and low-fat foods as well as food-related kitchen utensils. Their task was to classify whether the photographs contained food or not, making fat content orthogonal to task demands. Electrical neuroimaging analyses of the VEPs allowed us to identify the spatiotemporal mechanisms mediating the discrimination of foods from non-foods and revealed advantageous object recognition processes for high-fat foods.

9.2 EXPERIMENTAL PROCEDURES

9.2.1 Participants

Fifteen remunerated volunteers (eight males), aged 19–36 years (mean ± SEM = 27.7 ± 1.2 years), participated in the electroencephalography (EEG) portion of the study. Eleven of these participants were right-handed, and four ambidextrous according to the Edinburgh Handedness Inventory (Oldfield, 1971). Their BMIs were within the normal range (mean ± SEM = 21.6 ± 0.6 kg/m^2). None of the participants had current or prior neurological or psychiatric illnesses or self-reported eating disorders. All participants had normal or corrected-to-normal vision and were naive with regard to the food evaluation character of the experiment. All of the EEG recording sessions started between 13:00 and 14:00 h to control for circadian modulations of hunger. Further, participants were instructed (and also themselves reported) to have eaten lunch before the recording sessions. All participants provided written informed consent to the procedures, which were approved by the Ethics Committee of the Faculty of Biology and Medicine of the University of Lausanne.

9.2.2 Stimuli and Procedure

Participants were presented with four blocks of trials that each included 150 color photographs in pseudorandomized orders. In each block there were 50 high-fat food, 50 low-fat food, and 50 nonfood (No-Food) photographs, the latter being kitchen utensils (Figure 9.1). The food images had been subdivided into high-fat vs. low-fat classes (Hi-Fat vs. Lo-Fat), using the nutrition database of the United States Department of Agriculture (www.nal.usda.gov/fnic) and the Swiss nutritional database (released by the Swiss Federal Office of Health and the Swiss Federal Institute of Technology, Zurich). The fat content of the Lo-Fat class ranged from 0 to 1.6 g/100 g and that of the Hi-Fat group from 3.0 to 81.1 g/100 g. Our prior work demonstrates that healthy individuals can reliably rate the fat content of these images (Toepel et al., 2009).

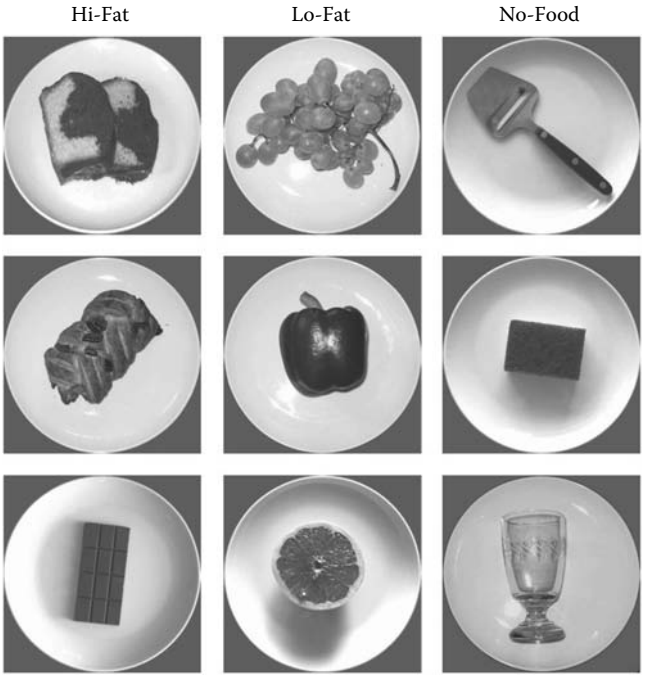


FIGURE 9.1 Exemplar photographs of Hi-Fat foods, Lo-Fat foods, and No-Food kitchen tools used in this study. Note that the photographs have been reproduced here in grayscale, but that color versions were used during the experiment.

All photographs measured 300 × 300 pixels, which corresponded to ~6° visual angle on the computer monitor and were taken using an identical background and from an identical top-view angle. The luminance of each image was adjusted to a standard value calculated from the entire image database. Full details of these procedures have been reported elsewhere (Knebel et al., 2008). Images were centrally presented for 500 ms on a 21″ CRT monitor that participants viewed within an electrically shielded and sound-attenuated booth. Immediately after each image a question mark was presented, indicating that participants should now decide via button-press if the preceding image had been a food or a nonfood item. To minimize eye movements, a central fixation cross was present whenever no image or question mark was present. Following response execution, the intertrial interval (ITI) varied randomly between 250 and 750 ms. Stimulus presentation and response recordings were controlled by E-Prime (Psychology Software Tools Inc., Pittsburgh, USA; www.pstnet.com/eprime).

9.2.3 Electroencephalography Acquisition and Analyses

Continuous EEG was acquired at 512 Hz through a 160-channel Biosemi ActiveTwo system (Biosemi, Amsterdam, the Netherlands) referenced to the CMS-DRL ground (which functions as a feedback loop driving the average potential across the

montage as close as possible to the amplifier zero). Details of this circuitry, including a diagram can be found on the Biosemi Web site (http://www.biosemi.com/pics/zero_ref1_big.gif). All analyses were conducted using CarTool software (http://brainmapping.unige.ch/Cartool.htm). To calculate VEPs, epochs of EEG from 98 ms pre- to 488 ms poststimulus onset (i.e., 50 data points before and 250 data points after stimulus onset) were averaged for each condition (i.e., Hi-Fat and Lo-Fat images) and each participant, separately. In addition to a ±80 μV artifact rejection criterion, EEG epochs containing eye blinks or other noise transients were removed by trial-to-trial inspection of the data. Prior to group averaging, data from artifact electrodes of each participant were interpolated (Perrin et al., 1987). Data were also baseline corrected using the 98 ms prestimulus period, band-pass filtered (0.59–40 Hz), and recalculated against the average reference.

The main objective of this study was to examine the spatiotemporal mechanism contributing to the implicit discrimination of Hi-Fat and Lo-Fat foods from No-Food objects. To this end, electrophysiological analyses were applied that use both local and global measures of the electric field at the scalp. These so-called electrical neuroimaging analyses allowed us to differentiate effects following modulations in the strength of responses of statistically indistinguishable brain generators from alterations in the configuration of these generators (viz. the topography of the electric field at the scalp). As these methods have been extensively detailed elsewhere (Michel et al., 2004a,b; Murray et al., 2004, 2006, 2008), we provide only the essential details here.

As a first level of analysis and in order to minimize the possibility of missed effects (type II errors), we analyzed waveform data from all electrodes as a function of time poststimulus onset in a series of pair-wise comparisons (*t*-tests). Temporal autocorrelation at individual electrodes was corrected through the application of an 11 contiguous data-point temporal criterion (~20 ms) for the persistence of differential effects [this criterion is also applied to the analysis of Global Dissimilarity and Global Field Power (GFP) described below].

The global measures of the VEP orthogonally analyzed response strength and response topography. Modulations in the strength of the electric field at the scalp were assessed using Global Field Power (GFP; Lehmann and Skrandies, 1980) from each stimulus condition and each participant, which is the root mean square across the electrode montage at each instant in time of the VEP. GFP waveforms were analyzed as described above using a millisecond-by-millisecond paired *t*-test (with correction for temporal autocorrelation, as above) for the Hi-Fat vs. No-Food and Lo-Fat vs. No-Food contrasts, separately. Modulations in response topography were first analyzed in a pair-wise manner using Global Dissimilarity (Lehmann and Skrandies, 1980), which is the root mean square of the difference between two GFP-normalized electric fields. This metric is then statistically analyzed millisecond-by-millisecond using a nonparametric randomization procedure that has been dubbed "TANOVA" (Murray et al., 2008).

The topography of the VEP was also subjected to a hierarchical clustering analysis to determine the sequence of maps describing the VEP from each experimental condition (Murray et al., 2008). This is first conducted at the group-average level and then statistically evaluated at the single-subject level. The dependent measure output is relative map presence in milliseconds, which indicates the amount of time over

a given interval that each map that was identified in the group-averaged data best accounted for the response from a given individual participant and condition. This is based on the spatial correlation between maps identified at the group-average level and single-subject data. These values were then submitted to a repeated measure ANOVA using within-subject factors of condition and map. Importantly, this labeling procedure is not exclusive such that a given time period of the VEP for a given participant and stimulus condition is often labeled with multiple template maps.

The topographic pattern analysis at the group level (and subsequent fitting procedure at the single-participant level) in conjunction with the Global Dissimilarity test is a method for determining if and when different generator configurations underlie each experimental condition. One neurophysiologic utility of these analyses is that topographic changes indicate differences in the brain's underlying active generators (Lehmann, 1987). In addition to testing for modulations in the electric field topography across conditions, this analysis also provides a more objective means of defining VEP components. That is, we here defined a VEP component as a time period of stable electric field topography, which we then used as a basis for determining time periods to submit to source estimation procedures.

9.2.4 Electroencephalography Source Estimations

We estimated the sources in the brain underlying the VEPs from each stimulus type using a distributed linear inverse solution applying the local autoregressive average (LAURA) regularization approach (Grave de Peralta et al., 2001, 2004; see Michel et al., 2004b for a comparison of inverse solution methods). LAURA selects the source configuration that better mimics the biophysical behavior of electric vector fields (i.e., activity at one point depends on the activity at neighboring points according to electromagnetic laws described in the Maxwell equations). In our study, homogenous regression coefficients in all directions and within the whole solution space were used. LAURA uses a realistic head model, and the solution space included 4024 nodes, selected from a $6 \times 6 \times 6$ mm grid equally distributed within the gray matter of the Montreal Neurological Institute's average brain. The results of the above topographic pattern analysis defined time periods for which intracranial sources were estimated and statistically compared between conditions. Statistical analyses of source estimations were performed by first averaging the VEP data across time to generate a single data point for each participant and condition. This procedure increases the signal-to-noise ratio of the data from each participant. Scalar values of source estimations (15 participants × 2 conditions) were then calculated for each of the 4024 nodes. Paired *t*-tests were performed at each node, and only nodes with *p*-values ≤ 0.005 ($t_{(14)} \geq 3.33$) were considered significant. The results of the source estimations were rendered on the MNI brain with the Talairach and Tournoux (1988) coordinates of the largest statistical differences indicated.

9.3 DIFFERENTIAL RESPONSES TO FOOD IMAGES OCCUR IN TWO PHASES

Task performance was near-perfect for all experimental conditions. The 15 participants correctly categorized on average (±SEM) 98.8% ± 0.6% of the No-Food images,

99.4% ± 0.3% of Hi-Fat food images, and 99.7% ± 0.3% of Lo-Fat food images. There was no performance difference between high-fat and low-fat food images, though a one-way ANOVA revealed a main effect experimental condition due to the slightly poorer performance with nonfood images ($F_{(2,13)} = 4.43$; $p = 0.034$).

Analyses of the VEP first focused on identifying the timing of response differences between Hi-Fat, Lo-Fat, and No-Food images. Figure 9.2a displays statistical scatter plots (SCPs) from the Hi-Fat vs. No-Food contrast and Lo-Fat vs. No-Food contrast. Differential responses to Hi-Fat food images vs. No-Food images were first evident over the 90–120 ms and 180–400 ms poststimulus periods. By contrast, differential responses to Lo-Fat food images vs. No-Food images were postponed and first evident at only 180–280 ms poststimulus. This pattern of results provides initial hints to an advantage for Hi-Fat images during the discrimination of food from nonfood images.

In order to determine the neurophysiologic basis of this advantage, electrical neuroimaging analyses on the global electric field modulations between Hi-Fat vs. No-Food viewing and Lo-Fat vs. No-Food viewing were conducted (see Materials and Methods; Murray et al., 2008). Modulations in response strength (vis-à-vis GFP; Figure 9.2b) arise at similar latencies for the Hi-Fat vs.

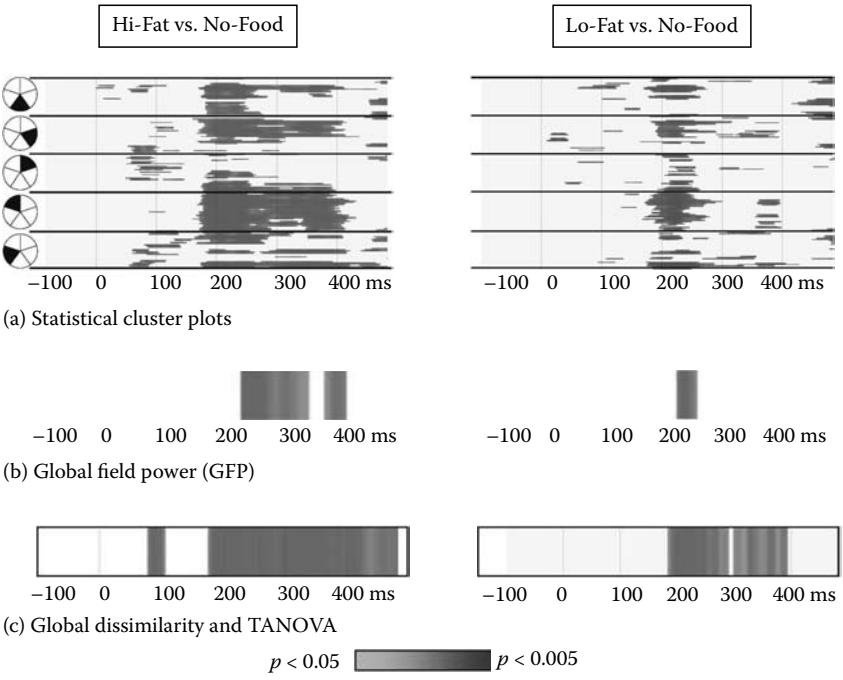


FIGURE 9.2 Electrophysiological results when contrasting the Hi-Fat with the No-Food condition and the Lo-Fat with the No-Food condition (left and right columns, respectively). (a) SCPs display electrode-wise and millisecond-wise paired *t*-tests. (b) Analysis of GFP identified response strength modulations. (c) Analysis of Global Dissimilarity via TANOVA identified modulations in response topography.

No-Food contrast (227–334 ms and 357–400 ms poststimulus periods) and also for the Lo-Fat vs. No-Food contrast (226–261 ms poststimulus). Modulations in response topography (vis-é-vis Global Dissimilarity and TANOVA; Figure 9.2c) set off earlier for the Hi-Fat vs. No-Food contrast (82–111 ms and 180–480 ms poststimulus periods) than for the Lo-Fat vs. No-Food contrast (187–297 ms and 303–398 ms poststimulus periods). These results provide an indication that there are two phases of differential responses to food images based on the analysis of the global electric field on the scalp. The early phase involves the activity of distinct configurations of brain areas for Hi-Fat food images. The later phase involves the activity of distinct configurations of brain areas for both Hi-Fat and Lo-Fat food images.

To determine the sequence and commonality of these differential responses, we performed a hierarchical cluster analysis on the concatenated dataset (see Section 9.2 for details). This analysis identified a sequence of ten different topographies whose global explained variance across the whole dataset was 97.92%. This sequence was generally identical for all three experimental conditions, with the exception of the 88–140 ms and 217–316 ms poststimulus periods when different maps were identified across conditions (Figure 9.3). The fitting analysis in turn revealed significant condition × map interactions for both the 88–140 ms ($F_{(2,28)}$ = 3.75; p = 0.036; left bar graph) and the 217–316 ms poststimulus periods ($F_{(2,28)}$ = 3.69; p = 0.038; right bar graph). Post hoc contrasts for the 88–140 ms period

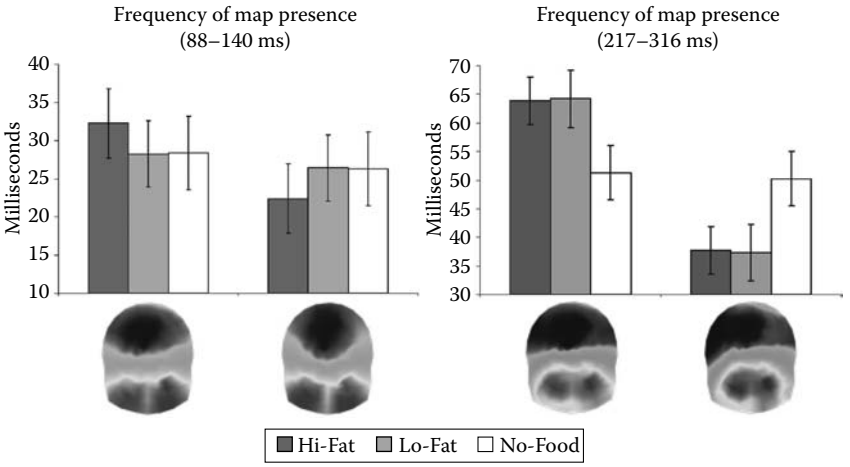


FIGURE 9.3 Outcome of the topographic pattern analysis across all three experimental conditions. Different maps were identified in the group-average responses over two time windows: 88–140 ms poststimulus onset and 217–316 ms poststimulus onset. The bar graphs illustrate the relative presence of each map in the single-subject data from each condition (i.e., fitting procedure; see Section 9.2 for details). Over the 88–140 ms window, one map better accounted for responses to Hi-Fat food images than either other condition. Over the 217–316 ms window, one map better accounted for responses to foods, irrespective of their fat content, than the No-Food condition.

confirmed that one map generally better accounted for responses to Hi-Fat than Lo-Fat images ($t_{(14)} = 2.16$; $p < 0.05$) as well as No-Food images ($t_{(14)} = 2.09$; $p = 0.055$). No differences in map presence were apparent between responses to Lo-Fat and No-Food conditions ($p > 0.90$). During the period between 217 and 316 ms, post hoc contrasts confirmed that one particular map topography better accounted for the observed VEP responses to Hi-Fat as opposed to No-Food viewing ($t_{(14)} = 2.29$; $p = 0.038$) as well as the response topography for the contrast Lo-Fat vs. No-Food viewing ($t_{(14)} = 2.43$; $p = 0.029$). No statistical differences were apparent within this time period between the response topographies to Hi-Fat and Lo-Fat image viewing ($p > 0.90$).

The present study provides evidence that the brain's discrimination of food from nonfood images varies according to the energetic value (i.e., fat content) of the referent foods. The latency at which differential responses from No-Food images were first observed was approximately 100 ms earlier for Hi-Fat than for Lo-Fat food images. The high energetic (and reward) value of Hi-Fat foods induces a benefit for their discrimination from nonedible objects.

The timing of the early categorization effect (88–140 ms) is in general agreement with previous findings on the categorization of natural vs. artificial objects (Kiefer, 2001; Proverbio et al., 2007) and the discrimination of animals appearing within complex visual scenes (Thorpe et al., 1996; VanRullen and Thorpe, 2001). Similarly, pictures with varying emotional content (Gianotti et al., 2008) and biologically relevant aspects of face perception were found to be effective at similar latencies as the present effects (e.g., Braeutigam et al., 2001; Michel et al., 2004a; Mouchetant-Rostaing et al., 2000; Pizzagalli et al., 1999). Because early differential processing in our study was particular to Hi-Fat images (which were otherwise controlled for perceptual features; see Knebel et al., 2008 for details), we postulate that the temporal advantage for these foods is likely linked to the greater salience—either in terms of energetic content or associated reward—of Hi-Fat edibles. A similar functional architecture has been proposed for the case of emotional face processing (e.g., Pourtois and Vuilleumier, 2006).

The categorization effects for food from nonfood images extended over two temporally distinct phases. While during the early phase (88–140 ms), only Hi-Fat foods were differentially processed (in terms of VEP topography) from No-Food images, during the later phase (217–316 ms) Hi-Fat and Lo-Fat foods both yielded differential effects vs. No-Food images. Hi-Fat and Lo-Fat classes of food images were discriminated in a topographically similar manner to those of No-Food images. Effects at similar latencies during object categorization have been interpreted previously in terms of visual semantics and/or access to conceptual information, i.e., reflecting the processing of object knowledge and associations stored in the brain (e.g., Doniger et al., 2000, 2001; Hamm et al., 2002; Hauk et al., 2007; Henson, 2003; Johnson and Olshausen, 2003; McPherson and Holcomb, 1999; Murray et al., 2006; Sehatpour et al., 2006; Sitnikova et al., 2006; Viggiano and Kutas, 1998). This later period of differential responses might therefore reflect more detailed conceptual analysis of the particular foods and nonfood items and/or access to additional (semantic/action) associations linked to the images.

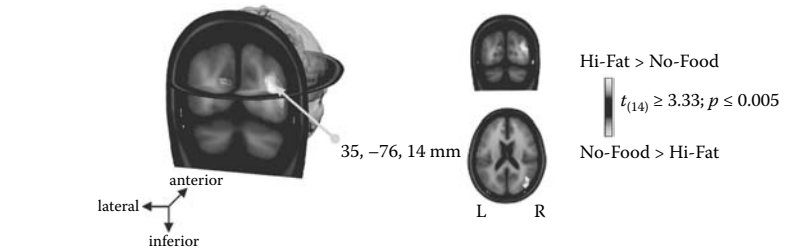
9.4 A REGION WITHIN THE RIGHT EXTRASTRIATE VISUAL CORTEX IS MORE RESPONSIVE TO HI-FAT FOOD IMAGES DURING INITIAL CATEGORIZATION

To this point, analyses of the surface-recorded VEPs revealed two time periods of differential responses to food vs. nonfood images. The earlier time period (88–140 ms) was characterized by a distinct VEP topography for responses to Hi-Fat food images, whereas the later time period (217–316 ms) was characterized by a general difference between food and nonfood images irrespective of their fat content. This pattern is consistent with a processing advantage for the discrimination of Hi-Fat food images and appears to be mediated by the engagement of a distinct configuration of intracranial brain generators.

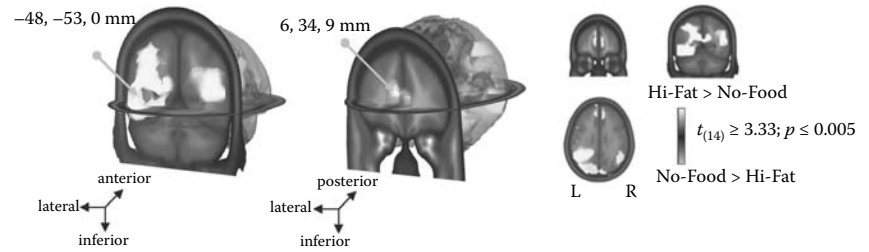
Source estimations from both time periods were then statistically analyzed to identify brain regions contributing to these effects. During the early period of topographic differences (88–140 ms), all three conditions (Hi-Fat, Lo-Fat, and No-Food) included prominent sources within occipital, posterior temporal, and parietal areas. Statistical contrasts of the Hi-Fat and No-Food source estimations, using a threshold of $t_{(14)} \geq 3.33$ ($p \leq 0.005$), identified a region within the right extrastriate visual cortex that was more responsive to Hi-Fat food images (BA19; coordinates of maximal *t*-value = 35, −76, 14 mm; Figure 9.4a). By contrast, there were no reliable differences between Lo-Fat and No-Food source estimations over this time window.

During the later period of topographic differences (217–316 ms; Figure 9.4b), there were significantly stronger responses to Hi-Fat than No-Food stimuli within a set of parietal and inferior occipitotemporal regions. A medial prefrontal region (BA10/32; maximal *t*-value = −6, 34, 9 mm) was more responsive to the No-Food than to the Hi-Fat condition. The contrast of Lo-Fat vs. No-Food stimuli revealed much the same set of regions (Figure 9.4c), with stronger sources within parietal and occipitotemporal regions in response to Lo-Fat food images. Likewise, medial prefrontal regions were more active in response to No-Food viewing (BA10/32; maximal *t*-value = 6, 45, 26 mm).

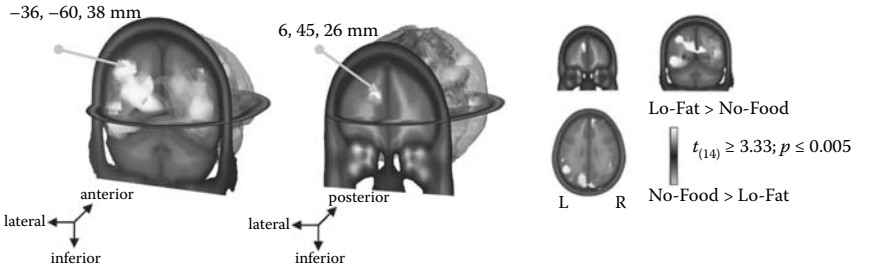
During the early phase (88–140 ms), responses to Hi-Fat foods resulted in suprathreshold activity in the right extrastriate visual cortex (BA19). Numerous prior studies implicate such regions in (categorical) object processes (e.g., Delorme et al., 2004; Hauk et al., 2007; Khateb et al., 2002; Sehatpour et al., 2006) as well as the discrimination of food from nonfood images (Killgore et al., 2003; Santel et al., 2006; Simmons et al., 2005). In particular, Killgore et al. (2003) observed stronger activity within extrastriate visual areas when contrasting the viewing of high-calorie foods and nonfood images, but not when contrasting the viewing of low-calorie foods and nonfood images. Visual cortices have also been ascribed a role in abstract reward processing (Hollander et al., 2005; Krawczyk et al., 2007; Small et al., 2005) as well as more generally in processing highly salient stimuli resulting from focused attention (Bradley et al., 2003; Gazzaley et al., 2005; Moratti et al., 2004). The advantageous processing of Hi-Fat food images observed here lends further support to the proposition that saliency (here derived from energetic value and potential reward) can modulate early responses in extrastriate visual cortices.



(a) Hi-Fat vs. No-Food (88–140 ms)



(b) Hi-Fat vs. No-Food (217–316 ms)



(c) Lo-Fat vs. No-Food (217–316 ms)

FIGURE 9.4 (See color insert following page 166.) Statistical differences between the group-averaged LAURA source estimations (N = 15) displayed on a three-dimensional rendering of the MNI template brain with maxima indicated by arrows and Talairach coordinates. (a) Statistical difference between the source estimations for Hi-Fat vs. No-Food viewing over the 80–140 ms poststimulus period. (b) Statistical difference between the source estimations for Hi-Fat vs. No-Food viewing over the 217–316 ms poststimulus period. (c) Statistical difference between the source estimations for Lo-Fat vs. No-Food viewing over the 217–316 ms poststimulus period.

A distributed bilateral network extending from occipitotemporal to parietal brain areas was more strongly active in response to both Hi-Fat and Lo-Fat food images during the latter phase of differential processing (217–316 ms). In addition to their role in object processing, similar networks have been previously reported in hemodynamic neuroimaging studies of food viewing in healthy (Killgore et al., 2003) as

well as obese individuals (Karhunen et al., 1997; Rothemund et al., 2007; Wang et al., 2002). Neural source estimations over the second phase of differential effects also included higher activity in the medial prefrontal cortex (mPFC) in response to nonfood images. These and nearby regions have been proposed to be involved in evaluation and decision-making processes (Volz et al., 2006) and found to be active when participants discriminated kitchen utensils from a range of control objects (Santel et al., 2006).

9.5 SUMMARY AND CONCLUSION

The discrimination of edible from nonedible objects is mandatory for survival. To date, the speed and brain regions by which these object categories can be differentiated remain unknown. This study identified the spatiotemporal brain dynamics of the visual discrimination of food images with high as well as low energetic values (in terms of dietary fat) from nonfood images. Electrical neuroimaging analyses of VEPs in humans revealed a processing advantage for high-energy foods. While high-energy foods were discriminated within the first 100 ms by visual cortices, effects with low-energy foods occurred ~100 ms later in temporal alignment with a second categorization phase for high-energy foods. This later processing stage was subserved by posterior temporoparietal cortices and likely reflects a more general evaluation of foods as a class of objects and the association with their nutritional values (i.e., edibility and satiety).

We propose that these early effects are indicative of the ability of the human visual system to perform evaluations of complex images not only in terms of the objects they represent, but also in terms of the likely energy and reward that the referent object can comport. This line of reasoning parallels and extends what has already been described in the case of emotional expressions of faces (e.g., Kanwisher and Yovel, 2006; Pourtois and Vuilleumier, 2006). The present results likewise provide temporal information that will be needed to inform, curtail, and complement models of food discrimination and evaluation that have hitherto been based on results of hemodynamic imaging; highlighting the role that electrical neuroimaging can play in cognitive neuroscience research.

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Part IV

Sensory Appeal of the Fat-Rich Diet

10 Preference for High-Fat Food in Animals

Yasuko Manabe, Shigenobu Matsumura, and Tohru Fushiki

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10.1 INTRODUCTION

The phenomenon of animals preferring high-fat foods has been accepted as natural behavior. Animals are equipped with fat not only for energy storage, but also for regulation of body temperature and as a source of many hormones. It is reasonable that animals eat and store fat based on physiological demands. On the other hand, eating an excessive amount of fat causes many metabolic diseases such as type II diabetes,

atherosclerosis, and cardiovascular disease. Reflecting the current health situation in industrialized nations, fat studies are focused on why we overeat high-fat foods and how we can cope with accumulating body fat. Ironically, many tasty and palatable foods such as snack foods, ice cream, donuts, and so on, contain large amounts of fat. The high palatability of fatty foods has been reported in many articles. Animals, including humans, show a hedonic preference for fat that increases with fat concentration (Drewnowski and Greenwood, 1983; Imaizumi et al., 2000a). When it comes to dietary fat, we cannot regulate proper calorie intake, and so we consume more calories than we physiologically need. In a long-term drinking test for corn oil in mice, the mice continued to prefer corn oil and ingested excess calories beyond their physiological needs (Takeda et al., 2001a).

Why are fatty foods so tasty? Why do we lose our desire to balance calorie intake when ingesting fat? Researchers are increasingly interested in studying the palatable features of fat to address these simple questions. Accumulating data suggest that the high palatability of fat can be attributed to many factors, including its texture (Rolls et al., 2003; De Araujo and Rolls, 2004; Kadohisa et al., 2005), flavor (Ramirez, 1993; Kinney and Antill, 1996), taste (Gilbertson et al., 1997; Gilbertson, 1998; Abumrad, 2005; Laugerette et al., 2005; Matsumura et al., 2007), and postingestive effect (Sclafani and Vigorito, 1987; Suzuki et al., 2003).

In this chapter, we discuss a wide range of physiological responses to fat, from fat recognition on the tongue to laboratory animal behavior in response to fat.

10.2 POSSIBLE FACTORS INVOLVED IN THE HIGH PALATABILITY OF FAT

10.2.1 Taste

10.2.1.1 Receptors for Fatty Acids on the Tongue

Fatty foods are tasty and preferable. When mice were offered both fried potatoes and boiled potatoes at the same time, they significantly preferred fried potatoes (Imaizumi et al., 2001b). Mice also preferred a corn oil solution to vehicle during a 10 min two-bottle choice test paradigm (Takeda et al., 2000). How did mice recognize fat in the oral cavity in such a short period? In the past, researchers believed that the preference for dietary fat came mostly from its texture and flavor. However, the sensation of fat spreads throughout the oral cavity when we eat high-fat foods such as butter or fresh cream. Does fat have a recognizable taste? At the moment we do not have an appropriate word to describe the fatty taste, and we are still not sure that the fatty sensation in the oral cavity is one of taste. The authors of recent studies suggest that there may be fatty acid (FA) receptors on the tongue that play an important role in the recognition of FAs (Table 10.1).

CD36/FAT is an 88 kDa glycoprotein, originally discovered as an FA-binding protein (FABP) in adipocytes (Abumrad et al., 1993). Its role as a possible FAs recognition receptor on the tongue was first reported in rats (Fukuwatari et al., 1997). Northern blotting and immunohistochemical study showed the expression of CD36 in the circumvallate papillae, specifically localized in the apical parts of taste bud cells (Fukuwatari et al., 1997). In support of these data, CD36-null mice showed an attenuated preference for a linoleic acid solution. Furthermore, CD36-null mice

TABLE 10.1
Fatty Acids Receptors on the Tongue

Receptors	Taste Cell Expression	Methods	References
CD36	Fungi., Foli., CV.,	RT-PCR immunohistochemistry	Fukuwatari et al. (1997)
GPR120	CV.	RT-PCR immunohistochemistry	Matsumura et al. (2007)
GPR40	Fungi., Foli., CV.,	Immunohistochemistry	Cartoni et al. (2007)
FABP	Fungi., Foli., CV., Epi.	RT-PCR	Laugerette et al. (2005)
ACBP	Fungi., Foli., CV., Epi.	RT-PCR	Laugerette et al. (2005)
FATP4	Fungi., Foli., CV., Epi.	RT-PCR	Laugerette et al. (2005)

Fungi, fungiform papillae; Foli., foliate papillae; CV, circumvallate papillae; Epi., epithelium.
Note: ACBP, Acyl-CoA binding protein; FATP, Fatty acid transporter protein.

with esophageal ligations displayed abolished pancreatic secretions in response to FAs (Laugerette et al., 2005). Finally, activation of c-fos in neurons of the nucleus of the solitary tract stimulated by FA deposition on the tongue of wild-type mice was abolished in CD36-null mice (Gaillard et al., 2007). Those data strongly suggest that CD36 on the tongue acts as an FA receptor.

In addition to CD36, the G protein–coupled receptor 120 (GPR120) was found in circumvallate, fungiform, and foliate papillae by real-time polymerase chain reaction (RT-PCR) and immunohistochemistry (Matsumura et al., 2007). GPR120 was first found in the colon as a long-chain FA recognition receptor (Hirasawa et al., 2005). GPR120 has a seven-transmembrane structure, which is different from the two-transmembrane structure of CD36 (Abumrad, 2005), but similar to the seven-transmembrane bitter, sweet, and umami receptors, which are also G protein–coupled receptors (Chandrashekar et al., 2006).

Polyunsaturated long-chain fatty acids (PUFAs), which are preferred by mice, are strong ligands for GPR120 (Hirasawa et al., 2005), suggesting that GPR120 on the tongue is also a possible fat recognition receptor. Recently, GPR40 was also found on the tongue in circumvallate, foliate, and a small number of fungiform papillae; therefore, it too, might be involved with the FA recognition on the tongue (Cartoni et al., 2007). Glossopharyngeal whole nerve recordings in GPR40 knockout (KO) mice showed a diminished response to oleic acid, linoleic acid, linolenic acid, and docosahexaenoic acid. Although GPR40 KO mice showed an attenuated preference for intake of corn oil, the mice's electrophysiological as well as behavioral responses to FA solutions were normal, suggesting that other FA receptors besides GPR40 are important in the recognition of FAs. Considering these facts, there seem to be various kinds of FAs recognition receptors on the tongue that might have distinct roles.

TRPM5, a member of the transient receptor potential (TRP) family, has been reported as a possible downstream component of the FA receptors, signaling cascade. TRPM5 is a calcium-activated cation channel expressed in the taste receptor cells

important for the detection of many tastants (Zhang et al., 2003; Damak et al., 2006). G protein–coupled taste receptors such as T1Rs, T2Rs, and mGluR4 signal through a common pathway involving the activation of phospholipase C (PLC), leading to the hydrolysis of phosphatidylinositol 4,5-biphosphate (PIP_2) into inositol triphosphate (IP_3) and diacylglycerol (DAG), which in turn stimulate the release of Ca^{2+}. TRPM5 is coexpressed with the IP_3 receptor and PLC beta 2, suggesting that TRPM5 is a part of the PLC-IP_3 signaling pathway (Liman, 2007). TRPM5-null mice showed no licking response to a sweet tastant, a diminished preference ratio for sweet and umami tastants, and a reduced response to bitter taste (Damak et al., 2006). Sclafani et al. reported that TRPM5 KO mice showed no preference for soybean oil emulsion in the initial two-bottle choice test, while gustducin (one of the important signaling molecules in the taste cells) KO mice showed a normal response to the soybean oil (Sclafani, 2007). GPR40 and GPR120 were reported to be coexpressed with the TRPM5 (Cartoni et al., 2007). These findings suggest that TRPM5 but not gustducin is one of the signaling components that play a role in fat recognition on the tongue. Signaling pathways involved in fat recognition on the tongue are beginning to be uncovered, but much remains unknown and more research is needed to help us understand the oral perception of FAs.

10.2.1.2 How Do Rodents Recognize Fat in the Oral Cavity?

Dietary oil consists of >90% triacylglycerols, and a small percentage of mono- or diacylglycerols and FAs. This fact raises the question of whether we recognize triacylglycerol, monoacylglycerol, diacylglycerol, or FAs when we perceive the taste of fat. So far no receptor for triacylglycerols has been identified, whereas several FA receptors, such as CD36 (Abumrad et al., 1993), FABP (Stremmel et al., 1985), fatty acid transporters (FATPs), GPR40 (Briscoe et al., 2003), and GPR120 (Hirasawa et al., 2005) have been found expressed in various organs. As described above, CD36, GPR40, and GPR120 are expressed in the circumvallate papilla. In behavioral studies, rats prefered 1% oleic acid, linoleic acid, and linolenic acid to 0.3% xanthan gum (Tsuruta et al., 1999). A similar preference for FAs is also observed in mice (Yoneda et al., 2007a), suggesting that rodents can recognize FAs on the tongue. Interestingly, rats also display a preference for pure triacylglycerol (Kawai and Fushiki, 2003). When offered a choice between a triolein solution and a vehicle, rats showed a significant preference for the triolein solution. How do rodents recognize triolein on the tongue? Kawai et al. answered this simple question by showing that when rats were offered a triolein solution with the lipase inhibitor orlistat, their preference for triolein was abolished. Therefore, lingual lipase released from Ebner's glands cleaves triacylglycerols on the tongue to release free FAs (Kawai and Fushiki, 2003) (Figure 10.1). The small percentage of free FAs found in dietary oil or those released from triacylglycerols are recognized on the tongue, possibly through an FA receptor such as CD36 or GPR120, which might be important to evoke the fat sensation in the oral cavity of rodents. However, it seems difficult to extrapolate the results from the animal experiments to a model for humans, since humans have a lower level of lingual lipase than rodents. Humans have an orosensory mechanism to detect FAs and perceive them as attractive ingredients. Free FAs released voluntarily from triacylglycerols in foods might be important for humans as a signal of fat.

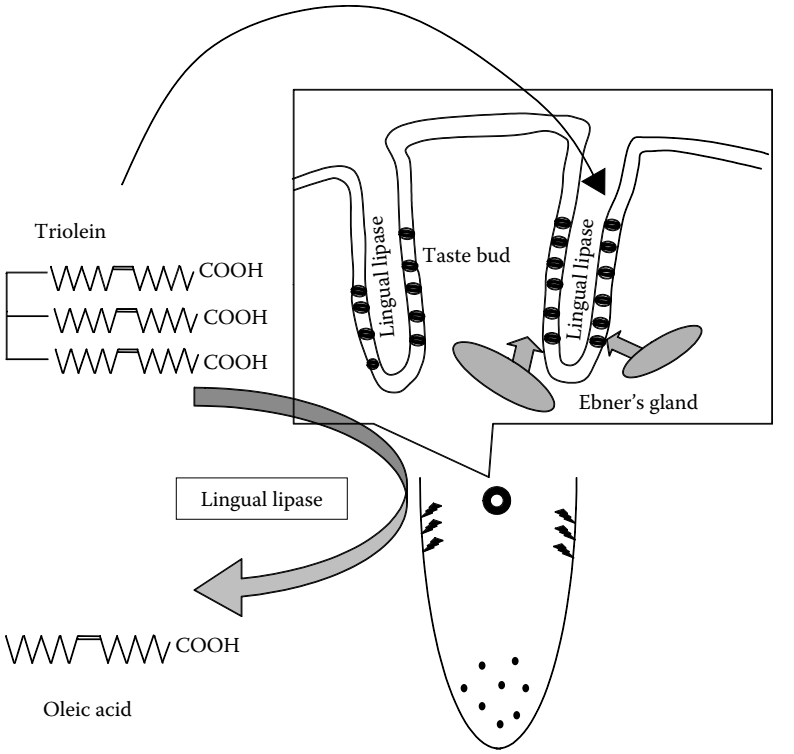

FIGURE 10.1 Schematic representing the mechanisms of fat recognition on the tongue. Dietary fats consist of mostly triacylglycerols. Triacylglycerols (triolein in this figure) are digested by the lingual lipase secreted from Ebner's glands. In a few seconds, triacylglycerol is cleaved into FAs and mono- or diacylglycerols. These released FAs are recognized by FA receptors such as CD36 or GPR 120 in the circumvallate papilla. (Modified from Kawai, T. and Fushiki, T., *Am. J. Physiol. Regul. Integr. Comp. Physiol*., 285, R447, 2003.)

The fact that rodents recognize FAs on the tongue has also been supported by patch clamp experiments. In isolated rat taste receptor cells, extracellular application of FAs inhibited the delayed rectifying K^+ channel (Gilbertson et al., 1997, 1998). This effect was limited to polyunsaturated fats with double bonds in the *cis* configuration (linoleic acid C18:2, linolenic acid C18:3, arachidonic acid C20:4, eicosapentaenoic acid C20:5, docosahexaenoic acid C22:6), suggesting that fat was recognized in the oral cavity and inhibition of the delayed rectifying K^+ channel is involved in the signal transduction mechanism for FAs (Gilbertson et al., 1997). As described above, TRPM5 is expressed in taste receptor cells and may be one of the downstream signaling mechanisms of GPCRs in the taste cells.

10.2.1.3 Oral Stimulation by FA Evokes a Physiological Response

Sensory stimulation induces many metabolic responses within a few minutes. This nerve-mediated phenomenon is known as the cephalic phase response. An example is provided by the induction of pancreatic enzyme secretion after stimulation of

the tongue by dietary oils or FAs. In esophagotomized rats, oral stimulation by corn oil or long-chain fatty acids (oleic acid, linoleic acid, linolenic acid) increased pancreatic enzyme secretion, but stimulation with the middle-chain fatty acid, caprylic acid, or the FA derivative, methyl linoleate did not have this effect (Hiraoka et al., 2003). These data imply that the carboxylated groups and the length of the FA chain are also important in the recognition of FAs on the tongue. In support of these data, CD36-null mice were reported to be devoid of cephalic phase response when linoleic acid was applied to the tongue (Laugerette et al., 2005), which suggests that some long-chain FAs with double bonds are potentially recognized through the CD36 receptor on the tongue, thereby sending a signal to the brain that leads to the initiation of the cephalic phase response.

10.2.2 Odor

The odor of food is an important signal that tells us whether we can eat and swallow certain foods. Fatty foods have a distinct odor. While the flavors in fatty foods/dietary oils come mostly from the free FAs or flavor components from various ingredients, the responsible signal in dietary oils is not yet well understood. Since many studies about odor in dietary oils have been devoted to the negative effect of oil flavor due to oxidation, polymerization, hydrolyzation, and so on, our understanding of the stimulation of appetite by the odor in dietary oil is limited at present. Within the limited reports of studies related to the FAs in dietary oil, short- to middle-chain FAs were recognized in a special area of the olfactory bulb (Mori et al., 1992), while long-chain FAs were considered to be odorless.

Deep-fried foods have a distinctive and attractive odor. Seven volatile compounds (2,4-decadienal, 2-heptenal, 2-octenal, 2,4-decadienal, 2,4-octadienal, 2,4-nonadienal, and (*E*,*Z*)2,4-nonadienal) were found to produce the deep-fried odor in trilinolein (Warner et al., 2001). Also, 2-alcans (aldehydes) were detected during the heating of triolein, which may contribute to its distinctive odor. This indicates that the odor in fatty foods might come in part from by-products formed during cooking.

How important is odor when selecting dietary fat? Olfactory-blocked mice either treated with $ZnSO_4$ to destroy olfactory sensory neurons or for which the olfactory nerve was cut were used to study whether oil flavor is the primary factor leading to the selection of fatty foods. In mice with a sectioned olfactory nerve, no preference for high-fat foods was observed (lab chow to which was added 9% vegetable oil), while sham operated or normal mice showed a preference for high-fat foods (Kinney and Antill, 1996), thus highlighting the importance of the olfaction system when selecting dietary oil. On the other hand, Ramirez reported that rats with anosmia induced by an olfactory bulbectomy showed reduced but not abolished preference for 1% corn oil (Ramirez, 1993). To support these data, Takeda et al. reported that $ZnSO_4$- treated mice showed a preference for corn oil at concentrations greater than 5% but could not discriminate between the vehicle and a 1% solution of corn oil (Takeda et al., 2001b), suggesting that, at low concentration, corn oil is chosen based on olfactory cues.

Thus, odor cues play a role in oil detection at low concentrations; however, olfactory-blocked rodents can still detect oil at higher concentrations.

10.2.3 Texture

Another specific feature of oil is its oily texture, which was thought to come mainly from its viscosity. However, Ramirez showed that rats conditioned to avoid the oil viscosity (67 cp) by injection of LiCl avoided oils (triolein, mineral oil, silicon oil) with a wide range of viscosities (5–203 cp), but did not avoid an oil-free solution of similar viscosity (Ramirez, 1994). Similarly, there was no direct relation between the perceived fat content and viscosity in sensory assessments by humans (Mela et al., 1994), suggesting that viscosity is not a main component of fat perception.

How much does the oily texture contribute to fat preference? Some neurons in the amygdala responded not only to fat, but also to nonfat oils such as silicone oil and mineral oil (Kadohisa et al., 2005). Interestingly, those neurons did not respond to FAs and lauric acid, suggesting that some neurons in the amygdala are tuned to the texture itself independently of FA recognition, a modality important in recognizing fat-like foods in the mouth (Kadohisa et al., 2005). In another experiment, a one-bottle test in nondeprived rats showed that 30% corn oil and 30% mineral oil were equally acceptable. However, when rats are fasted, the preference for corn oil emulsion is increased in two-bottle tests, suggesting that emulsified oil is attractive to the rat, but the postingestive effects of corn oil likely enhanced the preference for corn oil (Ackroff et al., 1990). In summary, these results indicate that oily texture is one of the important signals for oily foods recognition but it is not the main factor guiding fatty foods selection.

10.2.4 Postingestive Effect of Dietary Oil

A specific feature of dietary oil is that it is high in calories. Triacylglycerol has 9 kcal/g, which is more than two times greater than the amount of calories in carbohydrates (4 kcal/g) and proteins (4 kcal/g). The high calorie count in fat might contribute to the palatability of fatty foods. When rats are offered a high-fat (HF) diet their food intake and preference for oil are increased compared with rats fed a high-carbohydrate diet (Reed et al., 1990; Lucas et al., 1998). Rats fed the HF diet with sham feeding increased their intake on the first try but not on a subsequent try, suggesting that sensory stimulation is important on the first try, before postingestive effects are in place. This also points out the important role of the postingestive effect in the high palatability of fat (Reed et al., 1990). Experiments regarding the postingestive effect of dietary oil are discussed further in the next section.

10.3 BEHAVIORS DRIVEN BY THE HIGH PALATABILITY OF DIETARY FAT AND FATTY ACIDS

10.3.1 Behavioral Assays for the Measurement of Food Palatability

There are various methods for assaying the high palatability of foods (Table 10.2). The two-bottle choice test (Figure 10.2a) is a conventional method for determining preference. In this test, rodents are offered a pair of bottles simultaneously, and the

TABLE 10.2
Available Behavioral Assays to Test Fat Preference

Methods	Purpose	Time Required	Pros	Cons
Two-bottle choice test	Preference linked to orosensory stimulation	10 min	Easy to perform, relative value	Comparison between only two samples
Licking test	Preference linked to orosensory stimulation	60 s	Short-term test, absolute evaluation	Less sensitive among similar solutions
CPP	Preference for the sample including oral, postingestive, and rewarding effects	10 days	Easy to perform	Less sensitive for detecting the reinforcing effect
Operant task	Preference for the sample including oral, postingestive, and reinforcing effects	About 1 month	Absolute value among samples	Long test term

consumed volume is measured for a defined time. If the test period is short (under 10 min), the preference is considered to be an indication of orosensory stimulation. If the test period is longer (>30 min), the preference is thought to reflect orosensory stimulation as well as postingestive effects. The two-bottle choice test is simple and easy to perform, but it allows one to assess only the relative preference between the two bottles.

Another way to assess the preference for food is the licking test (Figure 10.2b), which was applied based on the phenomenon that animals show a high initial licking rate for a palatable solution. The intensity of food palatability was shown as an absolute value (the initial licking rate). The procedure, an animal is placed in the test chamber and allowed access to a stainless steel drinking spout from which the test solution is offered. The licking rate is recorded by a computer. In this procedure, the postingestive effects are completely excluded, since the initial licking rate is usually measured within a minute (30–60 s). Therefore, the initial licking rate is considered to reflect the preference to the solution in the oral sensation. For a detailed description of the licking test, refer to Yoneda et al. (2007a).

When we eat fatty foods, we find them tasty partly because of sensory stimulation, which, along with postingestive effects, makes us eager to eat more. The conditioned place preference (CPP) test (Figure 10.2c) and the operant task (Figure 10.2d) have also been used recently to measure the intense palatability of food, including oral stimulation and the postingestive effect of a test sample. Both methods were developed to study the reinforcing or rewarding effect of addictive drugs such as morphine and opioids, and therefore measure more than just the palatability of foods.

The CPP test measures reinforcing effects based on the preferences for a specific environment associated with a preferable stimulus. A detailed description of the CPP test for foods is given in Guyon et al. (1993) and Imaizumi et al. (2000b). In short, the CPP test chamber consists of two boxes, a box with lighting (light box)

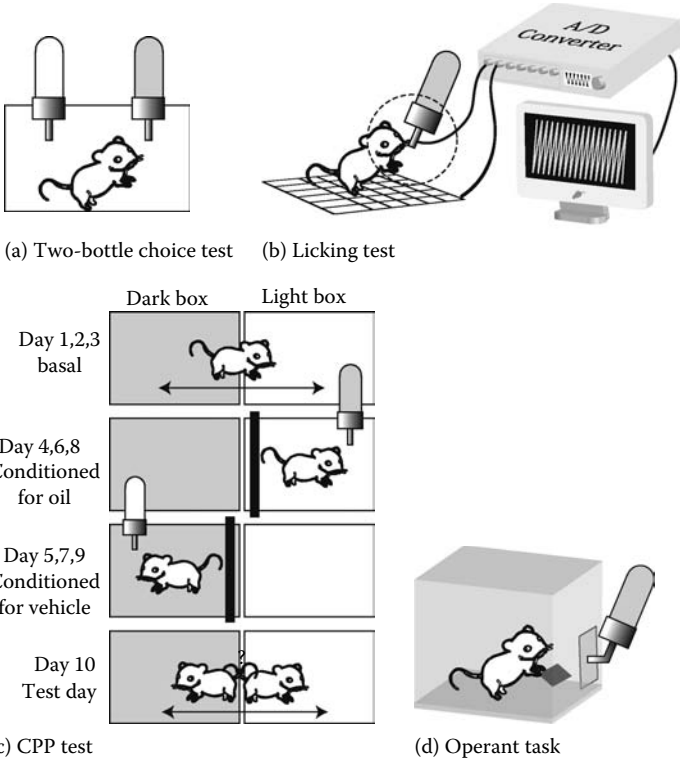

FIGURE 10.2 Behavioral assays available to assess food palatability. (a) The two-bottle choice test is used to understand which solution is preferable between two samples. The preference value (one-bottle intake divided by the total intake) or intake volume for a certain time is used for the evaluation of the preference. (b) The licking test is used to assess for the intensity of food palatability using the initial licking rates for the first 60 s. Licking rates are converted from the positive voltage peak. (c) The CPP test is used for the assessment of food reward. The test chamber consists of two boxes, a box with lighting (light box) and a box without lighting (dark box), which were separated by a guillotine door in the middle. Mice are acclimated in the box (day 1 to day 3). The time spent on day 3 is used as the basal spent time (baseline). Day 4 to day 9 is used as a conditioning period. Mice are alternately conditioned for a test sample in the light box or for water/vehicle in the dark box. On the test day (day 10), mice are freely moved to both boxes without any samples, and the time spent in the light box is measured. The CPP index represents the difference between the time spent on day 3 (baseline) and that on day 10 (test day). If the time spent on day 10 is significantly longer, it can be concluded that the test sample elicited a rewarding effect. (d) The operant test is used to assess the reinforcing effect of foods by measuring the effort mice will produce to receive the test sample. The operant task chamber is equipped with a lever, a liquid dipper, a stimulus light, and a buzzer on the wall. Mice are trained to press the lever at a defined number to get a test sample (reinforcer). When mice complete a defined task (lever press), the stimulus light and the buzzer are operated for 1 s, followed by the delivery of 10 μL of the reinforcer. The PR schedule, which shows the progressive rate of lever pressing to get the reinforcer, has been widely used to quantify the reinforcing effect. The break point in the PR test, which was defined as the maximum number of lever presses within a limited time, is used for the evaluation of rewarding effect.

and a box without lighting (dark box). The boxes are connected through a guillotine door in the middle. The CPP test runs over 10 days. From day 1 to day 3, mice are acclimated to the boxes and baseline behaviors in both boxes are measured. Time spent on day 3 in the light box is used as the baseline preference. Day 4 to day 9 is used as a conditioning period, during time the which mice are alternately conditioned for a food test sample in the light box or for water in the dark box. Day 10 is the test day. Mice are freely moved to both boxes without any food samples, and the time spent in the light box is measured. The CPP index is used as the difference between the time spent on day 3 (baseline) and that on day 10 (test day). If the time spent on day 10 is significantly increased, it is assumed that the test sample elicited a rewarding effect.

The operant task is another well-known method for studying drug addiction; that can also be used to assess the reinforcing effects of foods (Elmer et al., 2002; Hayward et al., 2002; Ward and Dykstra, 2005). Progressive ratio (PR) schedules in the operant task provide the reinforcing properties of gustatory stimuli as the absolute value for each tastant (Reilly, 1999). The break point in the PR test, which is defined as the last number of lever presses to get the reinforcer, is taken as an index of the reinforcer value. Using this method, sucrose was shown to be a positive reinforcer in rats (Reilly, 1999). Both the CPP test and the operant task are important tools for evaluating the rewarding effects of foods.

10.3.2 Rodent Preferences Point Out the High Palatability of Dietary Fat

All assays point to a preference for dietary oil by rodents (Figure 10.3). Ten-minute two-bottle choice tests show that mice prefer corn, canola, or mixed vegetable oil over vehicle (Figure 10.3a) (Takeda et al., 2000). In a test of various concentrations of corn oil, mice consistently preferred the higher concentration of corn oil to the lower concentration (Yoneda et al., 2007a) with 100% corn oil being the most preferable solution, suggesting that mice can discriminate oil concentration in the oral cavity. The postingestive effect cannot be ignored during this test since 10 min is enough time for the oil to reach the digestive organs. To evaluate oral stimulation caused by dietary oil, the licking test is useful as it evaluates the reaction to a sample solution within 60 s. In accordance with the two-bottle choice test results, the initial licking rate for corn oil was significantly higher than for the vehicle (Figure 10.3b), and the licking count increased in a concentration-dependent manner (Yoneda et al., 2007a). In addition to dietary oil, mice prefer low concentrations of FAs solutions. Solutions of 0.25%–1% linoleic acid (LA), which is a main FA in corn oil, are preferred over the vehicle in a two-bottle choice test, while solutions at concentrations greater than 1% are not preferred by mice (Yoneda et al., 2007a). Considering the finding that the lingual lipase digested the triacylglycerol (Kawai and Fushiki, 2003) and released small amounts of FA on the tongue, it is reasonable to conclude that mice prefer low concentrations of FA. The fact that the mice preferred not only dietary oil but also a low concentration of FA solution during both a two-bottle choice test and a licking test suggests that FAs in fatty foods have a key role in the detection of fat in the oral cavity.

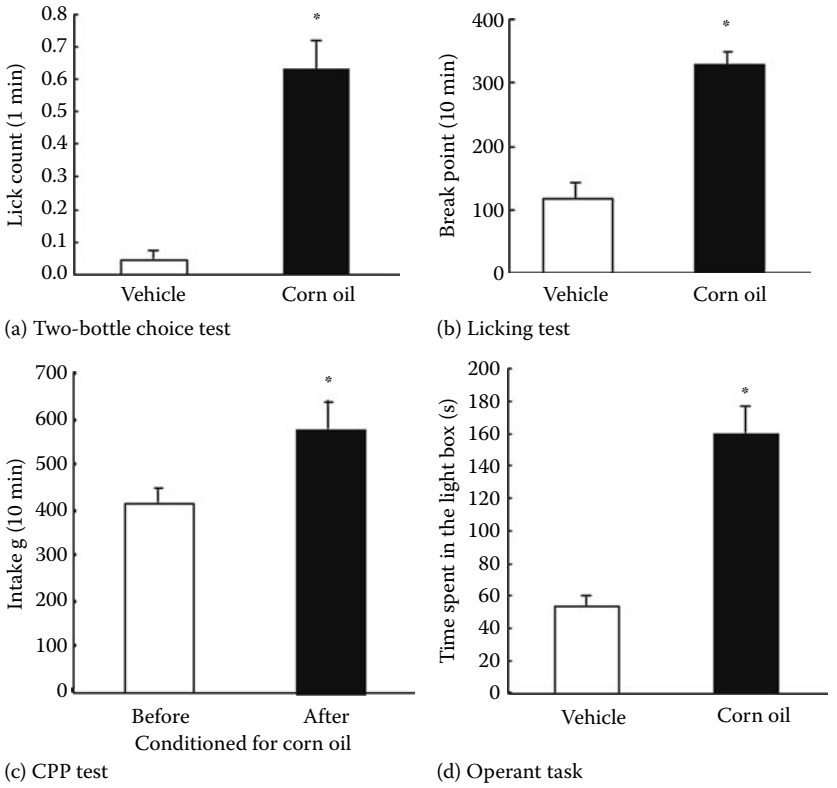

FIGURE 10.3 The preference or rewarding effect for corn oil as evaluated by several behavioral assays. (a) Two-bottle choice test between vehicle (mineral oil) and 100% corn oil. Mice were given a choice between a bottle of pure mineral oil and one of pure corn oil, for 10 min. The intake volume of each solution is shown. Mice significantly preferred the corn oil. (Data are from Yoneda, T. et al., *Physiol. Behav.*, 91, 304, 2007a.) (b) Licking test for mineral oil and corn oil. The licking count for the first 60 s was recorded as the initial licking rate. The licking rate for corn oil was significantly higher than that for mineral oil. (Data are from Yoneda, T. et al., *Physiol. Behav.*, 91, 304, 2007a.) (c) CPP test for corn oil. Mice were conditioned for corn oil in the light box and for water in the dark box. Time spent in the light box on the test day was measured. Time spent in the corn oil–conditioned light box was significantly increased. The method for the CPP test was from Suzuki et al. (2003). (d) Operant task for corn oil. Mice were trained to press a lever under an FR schedule, followed by the PR schedule. The break point was defined as the last ratio level completed before 10 min elapsed without the mouse receiving a reinforcer. The break point for corn oil was significantly higher than that for the vehicle. (Data from Yoneda, T. et al., *Life Sci.*, 81, 1585, 2007b.)

10.3.3 Postingestive Effect and Rewarding Effect of Dietary Fat

The postingestive effect has long been considered an important factor in food selection and preference. During an experiment in which rats were given a choice between a sucrose octaacetate (SOA)-polycose solution and pure sucrose powder, they initially

preferred the sucrose powder, but during the latter part of the test session (5–8 days), the rats developed a preference for the SOA-polycose solution over the sucrose powder, suggesting that a carbohydrate-mediated postingestive effect affected their preference (Sclafani, 1987). In the CPP test, rats showed a place preference for 18% sucrose but not for 0.1% saccharin, a sweetener with no calories, although the licking response for the saccharin solution was similar to that for the sucrose (Agmo and Marroquin, 1997). Interestingly, the rats showed a place preference when a glucose injection was combined with saccharin drinking. These results suggest that a postingestive effect contributes to the food selection behavior. This phenomenon was also applied to fat preference and its associated behavior. The rodent appetite for fat seems to be stronger than that for carbohydrate.

When mice were offered fried potatoes and boiled potatoes at the same time, they significantly preferred the fried potatoes, whether the oil used for frying had been lard or corn oil (Imaizumi et al., 2001a,b). When fed isocaloric diets of high-fat (HF) or high-carbohydrate content, rats consumed more of the HF diet, suggesting that there is a postingestive action of the HF diet-stimulating food intake (Lucas et al., 1998). Taken together, these data suggest that the postingestive effect of dietary fat also contributes to increased fat intake.

When given a mixture of glucose, saccharin, and corn oil solution, rats have trouble regulating calorie intake while this ability is not affected when given only water and food or glucose solution (Takeda et al., 2001a).

Thus, dietary fat ingestion seems to have an impact on the regulation of energy intake in rats. This phenomenon might be explained by the rewarding and reinforcing effects of dietary oil as revealed by CPP tests for corn oil (Figure 10.3c) (Imaizumi et al., 2000b). Time spent in the light box after being conditioned for corn oil increased significantly, suggesting that corn oil stimuli elicited a rewarding effect. A reinforcing effect following stimulation by corn oil was also observed in the operant task (Figure 10.3d) (Ward et al., 2007; Yoneda et al., 2007b), where the break point for the corn oil was significantly increased, thus pointing to a reinforcing effect. Furthermore, this reinforcing effect of corn oil was increased in a concentration-dependent manner since the reinforcing effects of 50% and 100% corn oil were significantly higher compared with that of 0% corn oil (Yoneda et al., 2007b).

These results show that the high palatability of dietary fat derives not only from its orosensory recognition but also from postingestive factors. The rewarding or reinforcing effect of dietary fat might be one reason why animals lose the ability to appropriately regulate calorie intake and proceed to overeat fatty foods.

10.3.4 Fat Substitutes and the Postingestive Effect

Compared to proteins and carbohydrates, FAs are high in calories (9 kcal/g), which might be one reason, together with high intake, for the increased incidence of obesity worldwide. To help people avoid overeating fatty foods, researchers have developed fat substitutes with a similar texture but fewer calories in the hope that they might be helpful.

A wide variety of fat substitutes have been developed to reduce fat intake from dietary foods (Wylie-Rosett, 2002). Most of these fat substitutes, which were derived

from a carbohydrate or protein base, were designed to mimic the texture of fat. In addition, a few fat-based fat substitutes have been used, including sugar–fatty acid esters or mono- or diacylglycerol instead of triacylglycerol.

In the United States, Olestra™, which consists of FAs esterified to sucrose, was permitted for use in the preparation of snacks. In a 2-week trial with human subjects using Olestra, subjects had 8% lower total energy intake and 11% lower fat intake when using Olestra compared with subjects not using it (Hill et al., 1998). In a 1-year study of heavy use of Olestra, energy intake from fat decreased 2.7%, although the total energy intake was not different from that in those who did not use Olestra (Patterson et al., 2000). Therefore, a low calorie fat substitute can be used as an alternative source of fat; however, the low calorie count does not help to reduce total energy intake, thus highlighting the large contribution of the postingestive effect in fat consumption. In a supportive study investigating the effect of the postingestive phenomenon on food intake, subjects were preloaded with yogurt adulterated with either saccharin, glucose, or starch. The results show that during the following meal, intake was increased in the saccharin-preloaded group, suggesting that noncalorie sweeteners do not have a reducing effect on total calorie intake (Rogers and Blundell, 1989). Therefore, people trying to lose weight by using fat or sugar substitutes should be careful in monitoring their total calorie intake.

To understand the postingestive effect of fat, we used one of the fat substitutes, sorbitol FA ester (SOR), in an animal study. SOR consists of FAs esterified onto a sorbitol molecule, which is nondigestible and contains few calories (1.5 kcal/g). When mice were offered both 2% sorbitol FA esters and its vehicle at the same time in a two-bottle choice test for 10 min, the mice significantly preferred SOR. In a long-term two-bottle choice test between 100% corn oil and 100% SOR, mice drank equal amounts of the two solutions during the first 30 min. However, the mice showed a preference for corn oil after 30 min and up to 24 h, suggesting that mice considered that SOR was similar to corn oil for the first 30 min but not thereafter. In terms of texture in SOR, mice with a conditioned taste aversion to corn oil avoided SOR, and vice versa, suggesting that oral cues did not allow them to discriminate between the two solutions. Therefore, the preference of corn oil over SOR observed after 30 min might be related to the postingestive effect of corn oil.

In another series of experiments, mice were intragastrically administered corn oil before CPP for SOR. Interestingly, SOR paired with calories elicited a rewarding effect. Neither an intragastric injection of corn oil alone nor oral stimulation of SOR alone elicited the rewarding effect, suggesting that both oral stimulation and caloric content are important in eliciting the rewarding effect in the CPP test (Suzuki et al., 2003). To understand the mechanism of the reinforcing effect of dietary oil, a beta-oxidation blocker (mercaptoacetate: MA) was used since beta-oxidation is a pathway toward using FAs as an energy source. Intraperitoneal injection of MA before conditioning for corn oil in the CPP test attenuated the reinforcing effect of corn oil, while it did not affect the reinforcing effect of sucrose (Figure 10.4) (Suzuki et al., 2006), suggesting that the process of beta-oxidation after ingesting the corn oil is important for supplying energy information. In terms of the high palatability of dietary oil, not only the sensory stimulus, but also the postingestive effect seem to be important.

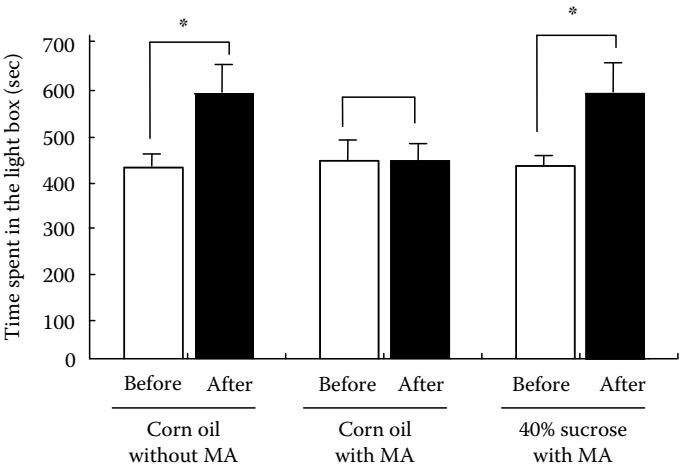

FIGURE 10.4 Postingestive effects are important for the rewarding effect in the CPP test. Mice were conditioned for the test sample in the light box and for water in the dark box. Thirty minutes before beginning the conditioning trials, mice were injected with a beta-oxidation blocker, MA (400 μmol/kg), or saline. Time spent in the test sample conditioned light box on the test day was measured. The reinforcing effect of corn oil was attenuated by the injection of MA. (Data from Suzuki, A. et al., *Nutrition*, 22, 401, 2006.)

If the postingestive effect of corn oil is important for its rewarding effect, then one can ask whether oil-like oral stimulation needs to be paired with energy from fat through the postingestive effect or energy from carbohydrates or proteins to elicit the same rewarding effect. To address this question, we used a CPP test with glucose calorie loading in the stomach for SOR. Mice were intragastrically injected with glucose into the stomach and experienced oral stimulation with the oil-like texture of SOR. Interestingly, even though glucose as a source of calories was intragastrically injected before conditioning for SOR, the rewarding effect was elicited (Suzuki et al., 2006). These results suggest that an energy-sensing mechanism is important in eliciting the rewarding effect. At the moment, it remains unclear which energy-sensing mechanism is involved in the rewarding effect of corn oil.

These studies imply that using a fat substitute might be helpful in reducing calorie intake from fat but not in decreasing total calorie intake. To overcome the high palatability of fatty foods, we should consider not only the sensory similarities of these substitutes but also the contribution of postingestive effects on their palatability.

10.4 REWARDING EFFECT OF DIETARY FAT: CENTRAL MECHANISMS

10.4.1 Possible Link between Taste and Peripheral Information

How are taste information and peripheral information linked? Interestingly, taste cells from the tongue and enteroendocrine cells from the gut are paraneurons (Fujita, 1991), which share common signaling mechanisms. These cells receive chemical stimuli on the apical side and then transmit the information by releasing neurotransmitters

on their basolateral side. Studies of the response to the same stimulus elicited by taste cells and enteroendocrine cells support the evidence that they share signaling mechanisms and cooperate to elicit an appropriate behavioral response. Taste markers such as alpha-gustducin, T2Rs, and T1R3 are expressed in the gastrointestinal tract (Rozengurt, 2006; Margolskee et al., 2007; Sclafani, 2007), and it was reported recently that the T1R3 + T1R2 receptor complex serves as a sugar sensor in the gut. Detection of sugar in the lumen of the intestine regulates glucose transporter and is followed by the secretion of hormones such as GLP-1 and GIP, suggesting that taste receptors in the intestine play an important role in glucose homeostasis (Margolskee et al., 2007). Interestingly, CD36 and GPR120, two FA receptors expressed in the tongue, were first found in the intestine (Chen et al., 2001; Hirasawa et al., 2005). It is possible that food selection and preference come from a complex combination of tongue detection and gut detection.

10.4.2 Brain Mechanism Underlying the Rewarding Effect

The brain mechanisms involved in dietary fat overconsumption have been studied. It is well known that the reinforcing effects of many addictive drugs are mediated via the dopaminergic system. Recent data suggest that food reward is also under the control of the dopaminergic system in the brain; more precisely, D1 and D2 dopamine receptors are candidate receptors for this effect. Which receptor type is involved in the rewarding effect or the reinforcing effect of dietary oil? Pretreatment with D1 antagonists, SCH23390 (0.03 mg/kg) and haloperidol (0.1 mg/kg), antagonized the rewarding effect in the CPP test. On the other hand, (±)-sulpiride, a D2 antagonist, did not affect reward in the CPP test, suggesting that the rewarding effect elicited by the dietary oil might be mediated via D1 receptors in the brain (Imaizumi et al., 2000b). However, the break point for corn oil was attenuated by pretreatment with (–)-sulpiride, a D2 receptor antagonist, in the operant PR test, while SCH23390, a D1 receptor antagonist, did not influence the break point (Yoneda et al., 2007b). It seems that the reinforcing effect of corn oil in the operant task is mediated through D2 receptors in the operant task. This discrepancy between the CPP test and the operant test comes from the detecting system in the test. Recent studies suggest that D1 receptors in particular contribute to the instrumental and the reward-related incentive learning process (Kelley et al., 1997; Dalley et al., 2005). D1 antagonists might affect the learning process in the CPP test, since subjects were pretreated with those drugs before they were conditioned for corn oil.

Using microdialysis methods in rats implanted with gastric fistulae, Liang et al. reported that oral stimulation by corn oil released dopamine in the nucleus accumbens (Liang et al., 2006). This result also implies that taste stimulation by corn oil directly affects certain brain mechanisms.

The opioidergic system is related to food reinforcement (Solinas and Goldberg, 2005; Smith and Berridge, 2007) and is involved in the reinforcing effect of corn oil. Naloxone, an opioid receptor antagonist, was reported to reduce the preference for high-fat foods in human subjects. In other studies, opioid agonists influenced the intake of high-fat diets (Ookuma et al., 1998; Zhang et al., 1998). In the CPP test, a corn oil–induced CPP was diminished by treatment with naloxone, the μ opioid receptor antagonist 7-benzylidenenaltrexone (BNTX), or the δ opioid receptor

antagonist naltriben. U-50488H, an opioid agonist of the κ receptor, which was reported to exert the opposite actions of μ receptor, also blocked corn oil–induced CPP, although it increased the corn oil intake in mice (Imaizumi et al., 2001b). These data imply that not only the dopaminergic system but also the opioidergic system could be involved in the reinforcing effect of fat.

How and when are opioids and dopamine released and put to action in the brain for the intake of dietary oil? Mizushige et al. reported that the mRNA level of pro-opiomeranocortin (POMC), a beta-endorphin precursor, in the hypothalamus was increased in rats given corn oil for five consecutive days. Interestingly, the increase of POMC mRNA was observed just before the ingestion of corn oil, while after corn oil ingestion, it was decreased (Figure 10.5). If the rats were kept away from corn oil on the test day, a high level of POMC mRNA was maintained for more than 30 min (Mizushige et al., 2006). These results suggest that the beta-endorphin system is related to the anticipation of corn oil intake. In accordance with these results, daily corn oil ingestion paired with a daily treatment of naloxone

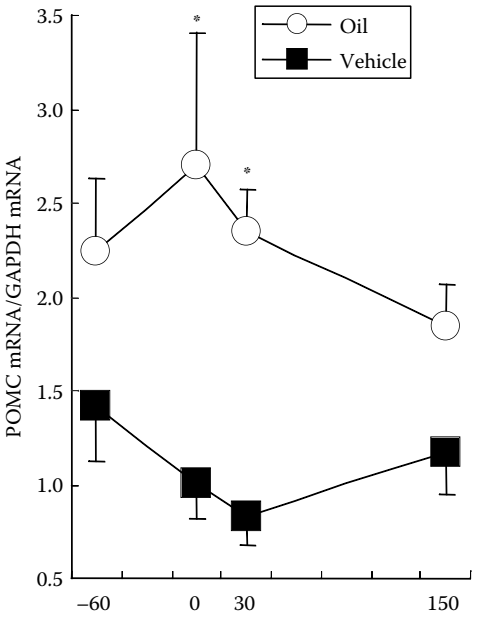

FIGURE 10.5 Rats were provided with either a vehicle (0.3% xanthan gum solution) or 5% corn oil at the same time each day for five consecutive days. On test day (day 6), the hypothalamus was removed 60 min before presentation, 0 (just before presentation), 30, or 150 min after presentation of 5% corn oil and RNA was extracted. POMC mRNA in the rats conditioned to be offered 5% corn oil at the same time each day was increased before the presentation of corn oil solution on the test day. After the ingestion of the corn oil solution, the POMC mRNA level was decreased. (Data from Mizushige, T. et al., *Biomed. Res.*, 27, 227, 2006.)

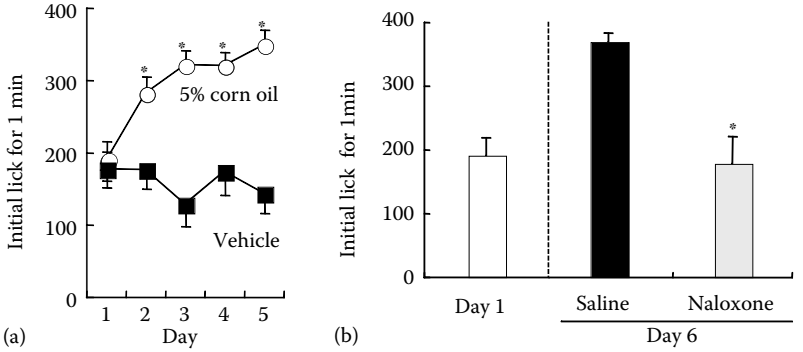

FIGURE 10.6 Effect of an opioid receptor antagonist on the preference for corn oil. (a) Animals were provided with 5% corn oil or a vehicle (mineral oil) for 5 days. Licking rates for 5% corn oil were gradually increased over 5 days, but not those for the vehicle. (b) Rats were injected intraperitoneally with the opioid receptor blocker naloxone or with saline as a control for 5 days before the licking test for 5% corn oil. On day 6, licking rates for corn oil without administration of the drug were measured. Licking rates for 5% corn oil in the naloxone-injected group did not increase, but licking rates did increase in the saline-injected group (Mizushige et al. unpublished data).

for 5 consecutive days did not lead to an increased preference for corn oil in the licking test (Figure 10.6), suggesting that the strong palatability of corn oil was supported by the opioidergic system. Those results also support our conclusion that brain mechanisms taking place after the ingestion of corn oil are important for inducing an appetite for corn oil.

It remains unclear whether the dopaminergic system also plays a role in the appetite for corn oil. It is possible that both the opioid and dopaminergic systems cooperate to elicit a strong appetite for oil.

10.5 CONCLUSION

Rodents prefer fatty foods. Taste, smell, and texture are all important orosensory factors behind the high palatability of dietary fats. Of particular interest are FAs released from triglycerides by the lingual lipase on the tongue and possibly recognized by a receptor in the circumvallate papillae (Gpr120, CD36). Via these receptors, signals are transmitted to the brain through the taste nerves innervating the taste buds. Subsequently, ingested oil is not only digested and absorbed in the gastrointestinal tract, but also sends signals to the brain through an unknown mechanism. The information from orosensory receptors and peripheral tissue is integrated in the brain, resulting in a strong appetite for fatty foods (Figure 10.7). Understanding the mechanism of fat recognition will help us develop a strategy for coping with the high palatability of attractive foods, which will be conducive towards the prevention of overeating.

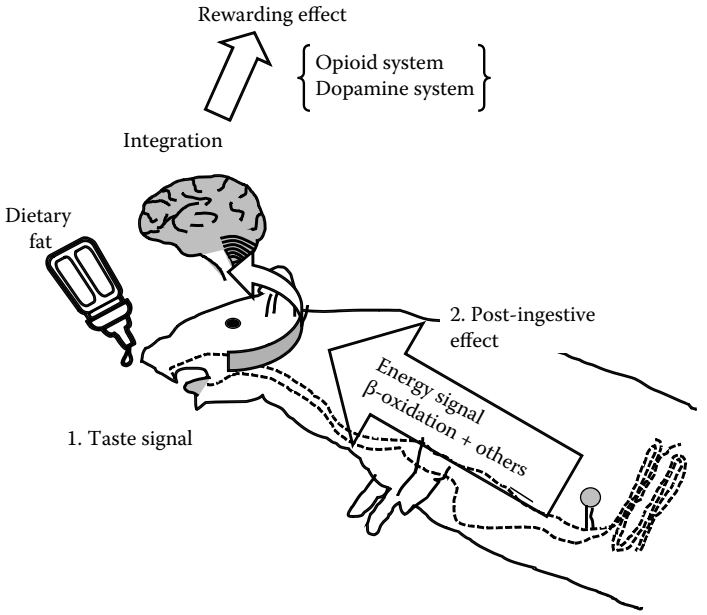

FIGURE 10.7 Schematic overview of the mechanisms underlying the high palatability of dietary fats. When animals eat dietary fats, sensory signals such as taste, smell, and texture are transmitted to the brain. The taste signal from the tongue is especially important for palatability. Triacylglycerols in the dietary fat are digested by the lingual lipase on the tongue and release FAs, which, by binding to FA receptors such as CD36 or GPR120, send a signal to the brain through the taste nerve. After ingestion of fat, information about their caloric content is collected by peripheral organs and transmitted to the brain through still unknown mechanisms. This information is integrated in the brain and elicits the rewarding or reinforcing effect for fat.

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11 Human Perceptions and Preferences for Fat-Rich Foods

Adam Drewnowski and Eva Almiron-Roig

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11.1 INTRODUCTION

Fat plays a unique role in the human diet. In addition to being the most concentrated source of dietary energy, fat contributes to the texture, flavor, and aroma of a wide variety of foods. In general, the most palatable foods are those that are both energy-dense and high in fat content (Drewnowski, 1997a,b). The taste, smell, mouthfeel, and hedonic properties of fat all contribute to the popular concept of fat "taste" (Drewnowski, 1997a).

Fat is one reason why palatability and energy density of foods are closely intertwined (Drewnowski, 1997a,b). Energy density of foods is largely determined by their water and fat content (Drewnowski, 1998). By providing weight without energy, water has more impact on energy density of foods than does any macronutrient,

even fat (Drewnowski, 1997a). Together, water and fat account for over 95% of the variance in the energy density of foods in the food supply.

Energy-dense foods and diets have been associated with higher energy intakes (Andrieu et al., 2006) and with higher prevalence of obesity and the metabolic syndrome (Mendoza et al., 2007). By all reports, the energy density of the Western diet is on the rise, as is the consumption of dietary fats (Drewnowski and Popkin, 1997; Popkin et al., 2001; Drewnowski, 2005). In the United Kingdom, dietary fat accounted for an average of 36% of dietary energy for men and 35% for women (Henderson et al., 2003). In France, dietary fat accounted for 37%–42% of dietary energy, with saturated fat providing 16% (Drewnowski et al., 1996; Perrin et al., 2002; Astorg et al., 2004). In the United States, dietary fat accounted for 33%–36% of dietary energy (Allred, 1995; CDC, 2004). Despite public health efforts to lower fat consumption, global consumption of both animal and vegetable fats continues to rise (CDC, 2004; Amuna and Zotor, 2008). Innate preferences for dietary fats seem to be tempered only by incomes.

As incomes rise, developing nations typically replace plant-based diets with more animal fats, vegetable oils, and caloric sweeteners, a phenomenon known as the "nutrition transition" (Drewnowski and Popkin, 1997). Paradoxically, developed nations recommend replacing fat-rich foods with water-laden grains, vegetables, and fruit in order to increase bulk and so reduce the energy density of the diet. Recommendations to reduce dietary energy density are one way to reduce energy intakes in an effort to address the global obesity epidemic (WHO Report, 2003).

The problem is that the palatability and enjoyment of foods are often tied to their energy density and therefore fat content. Energy-dense foods that are rich in fat are more palatable than are many low-energy-density vegetables and fruit (Drewnowski, 1998). High-fat foods, many containing sugar or salt, have an undeniable sensory appeal and are difficult to resist (Folkenberg and Martens, 2003). Energy-dense high-fat diets are consumed in preference to plant-based diets of grains, pulses, and legumes.

There are many explanations for why humans like fat (Drewnowski, 1997a,b). Several physiological mechanisms have been proposed, many of which are based on the strong links found between fat content, palatability, satiety, and energy density. The orosensory properties of fat or fat "taste" seem to be perceived through a combination of taste, texture, and olfaction (Drewnowski, 1997a; Schiffman et al., 1998). Fat is a concentrated source of energy with rewarding postingestive effects (Drewnowski, 1995). The learning of food preferences may be based on associating sensory attributes with the physiologic consequences of ingestion, such as satiety and well-being (Birch, 1999).

Parallels have been drawn between sugar and fat preferences. Sensory preference for sweet taste is present at birth, and the "sweet tooth" of early childhood helps to introduce new foods into the children's diet (Birch, 1999). Children learn quickly to prefer flavors associated with high-energy content and begin to select high-fat foods early in life (Johnson et al., 1991; Birch, 1992). Genetic predisposition, metabolic needs, and behavioral or emotional factors can influence the liking for fats. Human preferences for fat-rich foods may also be influenced by economic factors and sociocultural values (Drewnowski, 1995; Drewnowski, 1997a; Tuorila and Pangborn, 1988).

This chapter will cover recent advances in the understanding of the genetic, physiological and behavioral factors related to the perception of and preference for fat in humans. As will be discussed further, human liking for fats may be a consequence of evolutionary pressures to select energy-dense foods to assure nutrition and survival (Drewnowski and Rock, 1995).

11.2 PERCEPTION OF FAT-RICH FOODS: TASTE, OLFACTION, AND TEXTURE

Sensory perception of foods involves the initial stage of chemosensation, which includes the detection of tastants, odorants, and textural attributes of foods, followed by the integration of sensory signals by the brain. Sensory processes begin with the placement of food in the mouth, the fracturing of the food by the teeth and its dilution with saliva, oral perception of temperature and texture, and the binding of taste and flavor molecules to receptors in the oral and nasal cavities. Activated receptors act on secondary messengers or other systems, sending a signal through the sensory nerves to the brain. Upon integration of sensory input, we become aware of the taste, aroma, and texture of foods (Chandrashekar et al., 2006; Engelen and Van der Bilt, 2008). The hedonic component of sensation, known as palatability or the "pleasantness" of food is an integral part of a complete sensory profile for a given food (Yeomans, 1998).

Fats are perceived by a variety of sensory mechanisms (Drewnowski 1989a; Mattes, 2005). The concept of food "taste" as understood by consumers includes the chemical sense of taste (gustation); smell/aroma (olfaction), and the perception of food texture in the mouth. The first sensory response tends to be the olfactory perception through the nose or mouth of fat-soluble volatile flavor molecules (Drewnowski et al., 1989; Drewnowski, 1990a). Texture, defined as the overall measure of oral sensations associated with placing food in the mouth (Drewnowski, 1997a), adds to the sensation of fatty taste (Drewnowski et al., 1989). The physical form that fat takes at different temperatures can also influence mouthfeel and contribute to the enjoyment of high-fat foods.

Traditionally, studies in sensory psychophysics have examined human taste acuity (detection and recognition thresholds) or taste sensitivity (intensity scaling), using aqueous solutions of sweet, salty, sour, or bitter compounds. However, such techniques have not been applied to the study of fats in foods. One reason is that the chemical structure of triglycerides makes them insoluble in saliva such that the delivery of fat molecules to receptors in aqueous solutions is not possible. Therefore, many early studies have focused on the texture of fat-containing foods in relation to food quality and food preference.

11.2.1 Perception and Preferences for Fat Taste

The first sensation of fat flavor is olfactory perception of volatile, fat-soluble molecules through the nose, also known as orthonasal olfaction. Olfactory perception of already-ingested foods that occurs in the oral cavity is referred to as retronasal olfaction and may employ distinct brain pathways (Heilmann and Hummel,

2004; Mattes, 2005). Food texture and mouthfeel, which may change as a result of chewing and mastication are also detected in the oral cavity (Drewnowski, 1987, 1991a, 1995).

Recent evidence indicates that the sensation of fat can be mediated through chemoreception and not just through mechanical perception of texture. Evidence that free fatty acids are perceived through chemoreception comes largely from animal studies. Rats may be able to detect free fatty acids in the oral cavity through fatty acid-sensitive receptors (Gilbertson et al., 1997, 2005) in a sex-dependent manner (Pittman et al., 2008). A specific fatty acid transporter (CD36) for linoleic acid has been described in mice (Laugerette et al., 2005) and a potential pathway for the detection of a "fatty taste" by the mouse has been described as well (Gaillard et al., 2008).

A variety of fat-digesting lipases may assist in the chemosensory process. Human lipases digest dietary fat (mostly triacylglycerides) into glycerol and fatty acids. Lipases are present in the digestive juices (lingual lipase, gastric lipase, and pancreatic lipase); inside cells (hormone-sensitive lipase and lysosomal acid lipase); and in endothelial cells (lipoprotein lipase and hepatic lipase) (Hamosh, 1990). In newborns, lingual lipase breaks down the fat globules in milk, releasing free fatty acids to begin the digestive process. In adults, the role of lingual lipase is less clear (Schiffman et al., 1998), since enzyme levels in adult humans are apparently too low to digest dietary triglycerides (Spielman et al., 1993). In any case, sensory studies have long demonstrated that free fatty acids are perceived as acrid, pungent, nauseous, and generally repulsive (Schiffman and Dackis., 1975). Free fatty acids are usually removed from processed food for health, safety, and stability reasons. Free fatty acids such as linoleic acid and other unpleasant fatty acids are involved in product deterioration (Hansen and Rose, 1996; Refsgaard et al., 2000) and are generally bitter.

Indeed, the ability to perceive free fatty acids ought to lead to product rejection rather than acceptance. For example, there is 0.6–1.5 mg of free fatty acid in 1 g of milk. Fatty acid levels above 3.5 mg/g or 5.6 mg/g in fresh milk were in fact rejected by a sensory panel (Nasser et al., 2001). A recent study (Chalé-Rush et al., 2007) evaluated the sensitivity to linoleic, oleic, and stearic acids of 22 healthy adults, using taste, orthonasal, and retronasal olfaction. Oral and nasal irritancy were also measured. Orthonasal perception had markedly lower thresholds, suggesting that being able to detect free fatty acids prior to ingestion (Heilmann and Hummel, 2004) may have had evolutionary importance in the detection of spoilt food by primates.

Human ability to detect bitter compounds may act as a warning signal, leading to the rejection of toxic plant compounds (Drewnowski, 2000), or spoiled food containing oxidized fat (Mattes, 2005). The detection systems for free fatty acids may reflect evolutionary adaptation against the ingestion of undesirable or toxic compounds. Even if humans do have one or more sensory mechanisms for detecting free fatty acids, such mechanisms appear more tied to avoidance and survival than to sensory pleasure and enjoyment.

The olfactory perception of fat-soluble flavor molecules may be more closely linked to hedonic value. Food odors may increase appetite and induce salivation and promote the release of gastric acid and insulin (Yeomans, 2006). Warwick et al. (1993) showed

that combining smell and taste sensations (using vanilla and aspartame) enhanced subjects' satiety after a high-fat meal, compared with a bland version of the same meal. The neuroanatomical overlap of brain structures processing taste, satiety, and emotion could explain why some fat-soluble volatile compounds can produce feelings of pleasure or disgust, without affecting our ability to detect fat content (Schiffman et al., 1998). Recent neuroimaging studies identified brain loci for sensory information on the mouthfeel, viscosity, odor, and even the pleasantness of fat. These regions are also accessed by satiety and physiological signals (De Araujo and Rolls, 2004; Rolls, 2004).

Both humans and rats are capable of detecting low concentrations of triglycerides in emulsions. Rats were able to discriminate a 0.78% corn oil emulsion from water (Mindell et al., 1990). Among humans, detection thresholds ranged from 5.3% (vol/vol) in young adults to 15.8% (vol/vol) in elderly subjects (Schiffman et al., 1998), depending on the type of emulsifier. Emulsifiers, naturally present in food, may modulate the interaction of the fat globules with the taste receptors (Schiffman et al., 1998). Young subjects (mean age 23 years) were able to discriminate the fat content of whole milk versus skim milk with difference thresholds ranging from 1.4% to 2.1%. These thresholds were not lowered by odor cues (Mela, 1988; Schiffman et al., 1998). Humans were better at detecting the fat content of liquids compared to solid foods (Mela and Christensen, 1987).

Recent studies suggest that oral exposure to fat *per se*, before ingestion, may influence metabolic processes related to digestion and energy storage, and may have implications for long-term health status (Mattes, 2002, 2005). Olfactory detection of pleasant, fatty flavors may also be linked with induction of preparatory digestive mechanisms, or satisfaction and satiety after consumption of a meal. Sensory perception of fats, whether mediated by taste, texture, olfaction, or by some combination of all three, seems to have important consequences for fat metabolism (Mattes, 2005).

11.2.2 Perception and Preference for Fat Texture

Fats endow foods with some key textural properties, including viscosity (thickness) and lubricity (slipperiness or oiliness) (Drewnowski and Greenwood, 1983; Mela, 1988; Schiffman et al., 1998). Food texture studies focused on the viscosity, elasticity, and orientation and elongation of food particles, often in relation to food acceptance. Texture studies showed, for example, that consumer preferences for yogurts and other dairy products were driven by perception of smooth or creamy textures that depended on fat content (Folkenberg and Martens, 2003).

The classic definition of food texture (Szczesniak, 1963) held that the perception of product texture arises from the dynamic interaction between the food and the consumer, occurring along the dimension of time. Multiple senses were involved. Whereas oral sensation or mouthfeel refer to the sense of touch, the perception of texture could also involve both sight and hearing, and the manner in which the food responds to the applied forces (Engelen and Van der Bilt, 2008). Another definition held texture to be a dynamic interaction with time between the food's physical properties and the senses of touch, sight, and hearing (Engelen and Van der Bilt, 2008).

Mastication and swallowing played an essential role in the dynamic perception of texture in semisolid foods (Engelen and Van der Bilt, 2008). Semisolid is a texture represented by such foods as sauces, mayonnaise, cream, spread cheeses, ice creams, and margarines. The presence of particles, the size of particles, and their orientation are important, but only when combined with physiological processes taking place in the oral cavity, such as salivation (saliva flow, rate, and composition), tongue movements, and temperature exchanges between food and oral cavity (Engelen and Van der Bilt, 2008).

Because fats endow foods with a wide range of taste and texture properties, it is difficult to distinguish which particular oral sensations contribute to perception of fat content. Sensory evaluation of fats in solid foods is further complicated by the fact that the fat may be an integral part of the food (such as marbling in meat) or fats may be added during preparation. Fat globules in dairy products cannot be individually perceived in the oral cavity, but the substance may feel creamy (Cooper, 1987). Fat in frozen dairy products contributes to creaminess and smoothness by preventing the formation of large ice crystals. The principal sensory cue for fat content in milk or cream is stimulus thickness, smoothness, or viscosity (Drewnowski, 1987). The fat content of dairy products (Mela, 1988), peanut butter, margarine, and mayonnaise were also associated with thickness, smoothness, and viscosity (Kokini et al., 1977; Cussler et al., 1979). Temperature of the food, or temperature of the oral cavity does not seem to influence perception of fat content of oil-and-water emulsions (Mela et al., 1994a); but particle size (degree of emulsification) does, with smaller particle size slightly enhancing perception of fat content (Mela et al., 1994b).

Some of the terms used to characterize the perception of texture in the oral cavity, and particularly fat texture, and their definitions are summarized in Table 11.1. Table 11.2 shows how descriptive terms, classified into different categories may apply to characterizing the mouthfeel of beverages.

The general foods (GF) Texture Profile was created to standardize evaluation criteria for product texture (Brandt et al., 1963; Szczesniak et al., 1963). This model evaluated sensory properties of foods over time, from placement in the mouth to complete mastication. It identified primary mechanical characteristics of food such as hardness, cohesiveness, adhesiveness, and viscosity; and secondary characteristics such as brittleness, chewiness, and gumminess. Further, geometrical characteristics were defined based on the orientation, shape and size of the food particles (gritty, grainy, and coarse), and mouthfeel characteristics were defined based on perception of moisture or fat (wet, oily, and greasy). The use of the texture profile entailed the introduction of a number of anchored rating scales (Szczesniak et al., 1963), such as for hardness, fracturability, chewiness, gumminess, adhesiveness, and viscosity. Each scale represented a wide range of textures, so that many different foods could be evaluated (reviewed in Drewnowski, 1991a). It is important to note that some of these descriptors are not hedonically neutral, i.e., while "creaminess" may be a desirable property, "greasiness" generally is not. Table 11.3 summarizes the GF Texture Profile scales for two attributes, viscosity, and adhesiveness, together with product anchors.

TABLE 11.1
Terms Used to Characterize the Oral Sensation of Dietary Fats

Term	Descriptor
Body	The mouthfeel sensation of a substance
Creamy	Sensation of miscible, thick, smooth liquid in oral cavity
Gelatinous	Absence of structural elements in a springy solid
Greasy	Sensation of thick immiscible liquid or plastic solid
Melting	Sensation of a change in texture during testing
Mouthfeel	Tactile sensation on the surfaces of the oral cavity
Oily	Sensation of thin immiscible liquid in oral cavity
Slimy	Sensation of wet slipperiness on surfaces of oral cavity
Smooth	Absence of detectable solid particles
Sticky	Tendency to adhere to palate, teeth and tongue during mastication
Thick	Reluctance to flow (also viscous)
Thin	Readiness to flow
Waxy	Sensation of immiscible solid in oral cavity
Wet	Sensation of a rise in free fluids during mastication

Source: Modified from Drewnowski, A., *Food Texture*, Marcel Dekker Inc., New York, 1987.

TABLE 11.2
Terms Used to Characterize the Mouthfeel of Beverages

Category	Descriptor	Example
Body-related terms	Heavy, watery, light	Milk, eggnog
Afterfeel-mouth	Clean, lingering	Water, hot chocolate
Carbonation	Foamy	Ice-cream soda
Coating of oral cavity	Fatty, oily, clinging	Ice-cream soda
Feel on soft tissue	Smooth, creamy	Milk, half-and-half, light cream
Resistance	Slimy, syrupy, sticky	Heavy cream, eggnog
Temperature	Cool, warm	Milk, liqueur
Viscosity	Thin, viscous, thick	Milkshake, eggnog
Wetness	Wet, dry	Water, coffee

Source: Modified from Drewnowski, A., *Food Texture*, Marcel Dekker Inc., New York, 1987.

Different attribute scales reflect different aspects of fat texture perception. In one study, 25 normal weight men and women rated seven liquid dairy products along multiple 9-point attribute scales. Attribute scales included the standard texture and mouthfeel terms above as well as more abstract evaluations of the energy density of

TABLE 11.3
Descriptors for Oral Sensations Associated with Fat and the Viscosity Scale from the General Foods Texture Profile

Panel Rating	Product	Fat Content g/100 g	Carbohydrate Content g/100 g	Energy Density kcal/100 g
Viscosity				
1	Water	0	0	0
2	Light cream	19	3	195
3	Heavy cream	37	3	345
4	Evaporated milk	8	10	134
5	Maple syrup	0	64	262
6	Chocolate syrup	1	49	279
7	Mayonnaise (1/2 cup) and heavy cream (2 tbs)	34	24	330
8	Condensed milk	9	54	321
Adhesiveness				
1	Vegetable oil	100	0	884
3	Cream cheese	35	3	349
4	Marshmallow topping	0	79	322
6	Peanut butter	51	19	593

Sources: Brandt, M.S. et al., *J. Food. Sci*., 28, 404, 1963; Szczesniak, A.S., *J. Food. Sci*., 28, 385, 1963; Szczesniak, A.S., *J. Texture Stud*., 2, 196, 1971; Yoshikawa, S. et al., *J. Texture Stud*., 1, 452, 1970.

the sample (rich, fattening). There was a log-linear relation between attribute ratings and stimulus fat content, best seen when the graphs were plotted on a logarithmic scale. These data are summarized in Figure 11.1.

Fat can also affect the texture of cooked or processed food. Frying in fat brings the food to temperatures above the boiling point of water and contributes to crispiness or crunchiness in cakes, pastries, and cookies. Fat contributes to freshness and moisture by binding water molecules (Cooper, 1987). Appropriate terms for describing those texture attributes were also a part of the GF Texture Profile.

The reliance on texture cues means that an illusion of fat content can be created by making the stimulus viscous, either by gelling or by the addition of hydrocolloid thickeners. This becomes far more complex when the stimulus is solid as opposed to liquid. Drewnowski and Schwartz (1990) asked 50 young women to rate the sweetness and fat content of 15 stimuli resembling cake frostings. The samples were composed of sucrose (20%–77%, wt/wt), polydextrose (a bland, partly metabolizable starch), unsalted butter (15%–35%, wt/wt), and water.

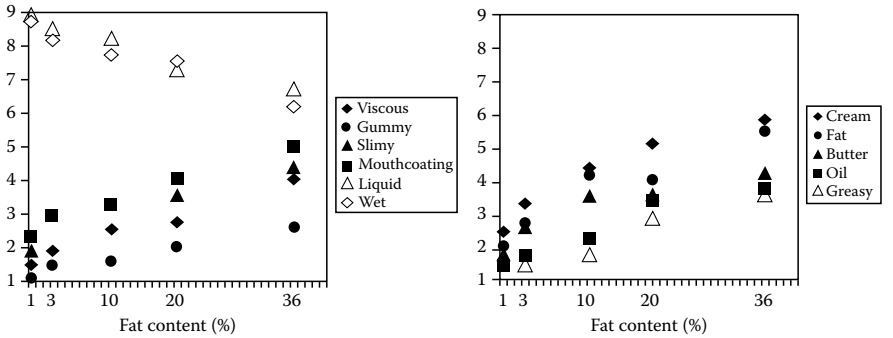

FIGURE 11.1 Relation between texture attributes and fat content of fluid dairy products. Sensory evaluation was carried out using 9-point category scales for each attribute, where 1, "not at all –" and 9, "extremely –". Subjects (13 women and 12 men) were tested in the morning, approximately 3 h following their habitual breakfast meal. (Data from Drewnowski, A. et al., *Physiol. Behav.*, 45, 177, 1989.)

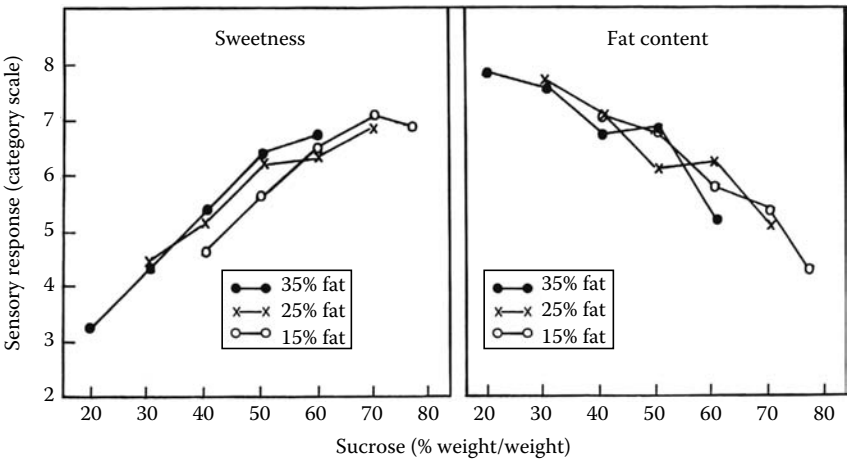

FIGURE 11.2 Ratings of perceived sweetness intensity (left panel) and fat content (right panel) as a function of stimulus sucrose and fat levels. (From Drewnowski, A. and Schwartz, M., *Appetite* 14, 203, 1990. With permission.)

Figure 11.2 (left panel) shows that the perception of sweetness was largely unaffected by fat content. Sweetness ratings accurately tracked increasing sweetness concentration. By contrast, the perception of fat content plunged as the sugar content increased (right panel). Perceived fatness was a combined function of fat and sugar contents that was heavily dependent on product texture. The addition of water caused a sharp decrease in fatness ratings, with the sweetest stimuli, being rated as lowest

in fat content. The presence of sugar clearly distorted the ability to perceive the fat content of solid foods (reviewed in Drewnowski, 1991a).

However, preferences for fat may be independent of the conscious ability to detect or assess the fat content of solid foods. Another study compared the ability of 25 young men and women to rate the sweetness, creaminess, and fat content of solid and liquid foods of differing sugar and fat content (Drewnowski et al., 1989). Liquid dairy products included sweetened skim milk (<0.5% fat), whole milk (around 3.5% milk fat), half-and-half (12.5% milk fat), and heavy cream (36% milk fat). Solid foods consisted of cottage cheese, cream cheese, or the two blended together to produce mixtures of comparable fat content (0.1%–52%). The sweetened blends were spread over white bread and cut into small pieces to produce the solid food samples (Drewnowski et al., 1989). Subjects reliably estimated sweetness intensity for both solid and liquid samples, but they were unable to track the increasing fat content of the solid foods. Preferences were estimated using the 9-point hedonic preference scale (Peryam and Pilgim, 1957). Hedonic preferences for high-fat samples remained high despite difficulties in estimating stimulus fat content.

Despite impaired ability to track the fat content of solid foods, as opposed to liquids, subjects generally preferred stimuli with the higher fat content (Drewnowski, 1989a). Sensory assessment of fat content in foods can be a challenge, since it relies so much on the integration of multiple interrelated sensorial signals. Further, such assessment is system-specific, and may be affected by other product attributes, such as sugar content. Finally, given that the perception of food texture is driven by the interaction between the food and the consumer, it is not surprising that humans sometimes lack an accurate, conscious judgment of how much fat is contained in the food (Blundell and McDiarmid, 1997; Schiffman et al., 1998).

11.3 INTERACTIONS BETWEEN FAT, SUGAR, AND SALT

Fat content contributes to the food's acceptability, palatability, and enjoyment. The creation of fat replacement products with a desirable and well-rounded sensory profile is a particular challenge for food chemists (Drewnowski, 1990b). Depending on the product, desired sensations may range from creaminess and smoothness (in dairy products), to tenderness and moisture (in cakes), to crispiness and crunchiness (in baked or fried foods). A particular hedonic synergy is obtained by pairing sugar and fat (Drewnowski and Greenwood, 1983; Drewnowski, 1991a, 1995; Schiffman et al., 1998).

When it comes to sweet liquids or solids, the hedonic response typically follows an inverted U-curve as preferences increase with added sweetness and then decline when the product is perceived as too sweet. The sensory optimum, usually reached at 8%–10% of sugar in aqueous solutions, is generally referred to as the "hedonic breakpoint." Some individual variation is observed as hedonic responses to sugar may rise and then decline (type 1 response) or rise and plateau (type 2 response). In general, children prefer much sweeter solutions than do adults and show little evidence of a hedonic breakpoint. By contrast, hedonic response curves for older adults may actually decrease as a function of sugar concentration (Drewnowski, 1991a). The hedonic breakpoint for solids is found at a much higher sugar concentration than for liquids.

Sensory evaluations of mixtures of sweetened dairy products of variable sugar and fat concentration showed no evidence for a hedonic breakpoint for fat. In these studies, normal weight men and women were asked to taste and rate 20 mixtures of milk cream and sugar. Sugar content varied from 0% to 20%, whereas fat content varied from 0.1% to 36% (Drewnowski and Greenwood, 1983). Subjects were asked to rate sweetness, creaminess, perceived fat content, and preference (hedonic response) on a 9-point category scale. Later studies, based on identical design were conducted with massively obese patients, patients who had recently lost weight and normal weight controls (Drewnowski et al., 1985).

The sensory system was highly interactive when it came to hedonic preferences. Whereas hedonic ratings for sweetness peaked at 8%–10% sugar, depending on fat concentration, no sensory breakpoint for dairy fat was observed. There was also a synergy between sugar and fat: highest preference ratings were obtained for light cream with sugar (Drewnowski and Greenwood, 1983). Hedonic preference ratings for obese patients, as compared to normal weight controls, are shown in Figure 11.3. The graphs illustrate that obese patients disliked high concentrations of sucrose in skim milk, but showed increased acceptance when the sucrose was presented in heavy cream. For both normal weight and for obese subjects, hedonic preferences for taste stimuli were a joint function of sugar and fat content.

Modeling hedonic responses to a two-dimensional sensory system required the use of a mathematical modeling technique, the response surface method (RSM) (Drewnowski and Greenwood, 1983). This analytical procedure can be used to predict and plot the response to multiple stimuli for any combination of ingredient levels. Used in food optimization studies, RSM can determine the combination of ingredients that yield the most preferred product and can graph hedonic optima

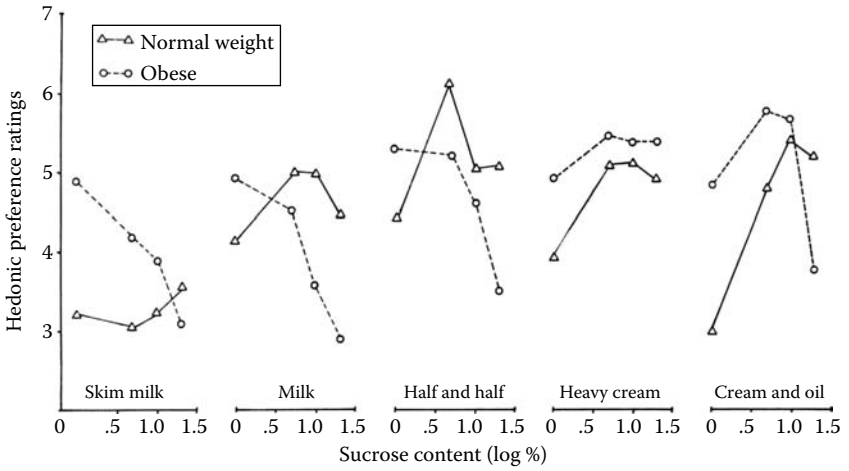

FIGURE 11.3 Hedonic preference ratings for dairy products sweetened with different amounts of sucrose. Ratings are for obese patients and normal weight controls. (Data from Drewnowski, A. et al., *Am. J. Clin. Nutr.* 61, 1206, 1995.)

in three dimensions (Drewnowski and Greenwood, 1983; Drewnowski et al., 1985, 1987b). A contour map, sometimes called "isobologram," joins points representing the same level of response to variable stimulus levels of sugar and fat (Gessner, 1995). Drewnowski and Greenwood (1983) showed that the peak hedonic response was obtained at high, but not highest, fat and sugar content (8% sugar and 20% fat). There was a strong interaction between the sweet and the fat component. Whereas sweetened skim milk and unsweetened heavy cream were not highly preferred, highest hedonic ratings were obtained for sweetened light cream (reviewed in Drewnowski, 1995).

Figure 11.4 (top panels) shows the three-dimensional representation of the hedonic response surface for obese patients, reduced obese patients, and normal weight controls. Isopreference contours are shown directly below (bottom panels). The data show that reduced obese patients had the highest hedonic ratings, selecting high energy density stimuli that were highest in sugar and fat content (Drewnowski et al., 1985).

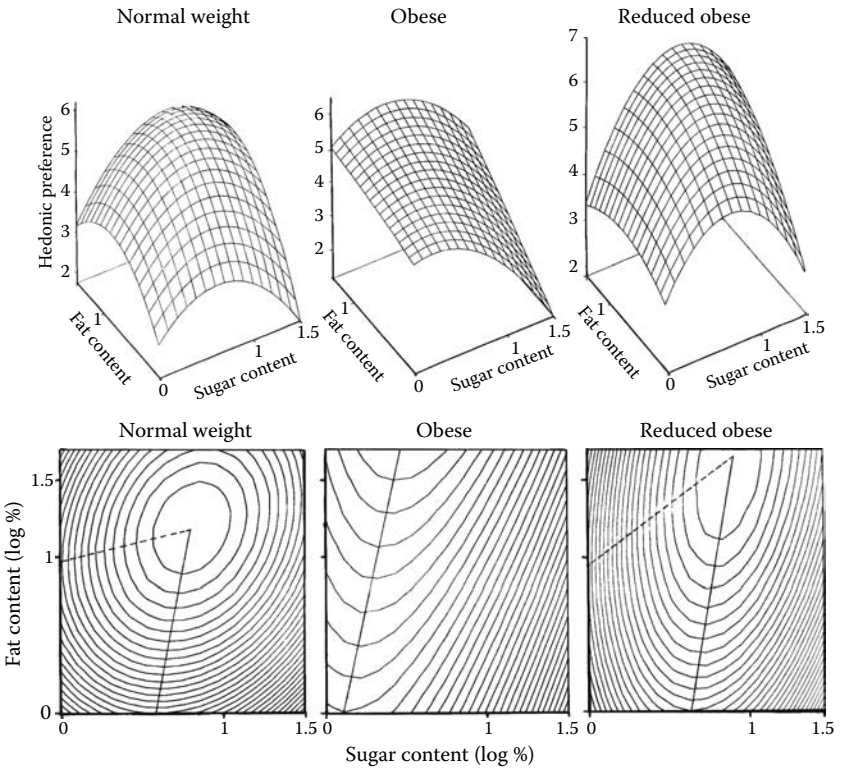

FIGURE 11.4 Hedonic preference ratings for sweetened dairy products of differing sugar and fat content. The data are for normal weight subjects, obese patients, and reduced obese patients. Data are presented as three-dimensional hedonic response surfaces (top panels) and as isopreference contours (bottom panels). (From Drewnowski, A. et al., *Physiol. Behav.* 35, 617, 1985. With permission.)

Sensory responses to sugar/fat mixtures were further explored as a function of extremely low body weight (Drewnowski et al. 1987a,b). Those clinical studies investigated female patients with anorexia nervosa and bulimia nervosa, as compared to normal weight age-matched female controls. A negative relation ($r = -0.43$) between sugar/fat ratios and degree of overweight confirmed clinical reports of differential response to dietary fat by anorectic and obese women. Whereas anorectic patients tended to reject fat-rich foods, obese patients, on the contrary showed enhanced fat preferences. An association between high preference for fats and overweight had also been shown in other studies (Mela and Sacchetti, 1991; Drewnowski and Holden-Wiltse, 1992). Formerly obese patients maintaining weight loss showed enhanced sensory preferences for both sugar and fat (reviewed in Drewnowski, 1991a).

A majority of laboratory studies have supported the link between obesity and liking for higher fat foods (Drewnowski and Greenwood, 1983; Drewnowski et al., 1985; Mela and Rogers, 1998; Rissanen et al., 2002; De Graaf, 2005). However, the findings were not universal (Salbe et al., 2004). Obese persons tend to consume more energy-dense diets and formerly obese subjects show enhanced responsiveness to fat (Drewnowski et al., 1985; Cox et al., 1999; Rissanen et al., 2002). However, some cross-sectional and longitudinal studies failed to show a convincing link between BMI values and self-reported liking for high-fat foods.

Sensory studies on fat and salt mixtures are extremely limited. Warwick and Schiffman (1990) studied sensory responses for fat/sugar and fat/salt mixtures in young and elderly subjects of different body weights. The stimuli contained 0.5%–36% fat by weight, and 0%–20% sucrose, or 0%–0.6% NaCl. Younger subjects preferred sweeter stimuli, consistent with previous studies (Drewnowski, 1991a; Cox et al., 1999). Hedonic ratings of elderly subjects were not influenced by fat content. Younger subjects showed a link between preferences for fat/salt mixtures but the elderly did not.

11.4 MECHANISMS UNDERLYING PREFERENCES FOR FAT: PALATABILITY OR SATIETY?

There is no question that dietary sugars and fats are associated with overconsumption of energy. Whether this is due to excess palatability or deficient satiety is an unresolved issue. On one hand, fat and sugar mixtures are among the most preferred of foods (Drewnowski and Greenwood, 1983; Drewnowski, 1991a). The foods most preferred by young children are energy-dense, familiar, and sweet. Typically, such foods include chocolate, cookies, jellies, and candy (Drewnowski, 1989a). High-fat foods were not only the most preferred, but induced the least neophobia (Rankin and Mattes, 1996). Children quickly learned to select flavors associated with high-energy content, that is, foods rich in fat (Drewnowski, 1989a; Johnson et al., 1991; Birch, 1992).

Highly preferred foods can stimulate consumption independent of energy deficit or perceived hunger (Sørensen et al., 2003). Palatable foods can stimulate appetite and eating rate, leading to excessive food consumption (Yeomans et al., 1997, 2001, 2004). This overriding of hedonic responses over metabolic responses has been

termed passive overconsumption (Blundell and Mcdiarmid, 1997), or more recently, nonhomeostatic eating (Mela, 2006).

Research has begun to integrate the homeostatic (energy) and the hedonic (pleasure) components of appetite control, assigning a role to weight status. Mela (2006) has noted that obesity seems to be linked to an increased "wanting" of highly palatable foods (motivation for eating) that is not necessarily linked to higher "liking" of those foods. Studies by Blundell et al. support the notion that wanting (how hard you are prepared to work for a food) and liking (the actual pleasure effects of eating the food) are two independent components of appetite, which can be measured separately (Blundell and Finlayson, 2004; Finlayson et al., 2007).

Studies on endogenous opioid peptides seem to support the hedonic or "liking" viewpoint (Reid, 1985; Cooper et al., 1988; Drewnowski et al., 1992a, 1995; Will et al., 2004; Ward and Dykstra, 2005). Endogenous opioid peptides are pleasure-enhancing molecules produced in the human brain. Early studies in rodents identified the link between endogenous opioid peptides and sugar and fat intake (Blass, 1987). Later clinical studies (Drewnowski et al., 1992a, 1995) examined sensory preferences for fat and sugar following administration of opioid peptide agonists (butorphanol) and antagonists (naloxone).

Those studies were conducted in normal weight and obese bulimic patients and nonbinging controls. Bulimic women consumed more sweet, high-fat foods under laboratory conditions than did nonbinging controls. Following naloxone administration, taste preferences for sugar/fat mixtures were suppressed by the opiate blockade. Food consumption of sweet high-fat foods (mostly chocolate) was also selectively suppressed, but only in bulimic females. The conclusion was that the opoid antagonist naloxone selectively affected the hedonic component of overeating: Stronger suppression was obtained for sweet- and fat-containing foods than for bland breadsticks and popcorn. Since sensory preferences were affected as well, the conclusion was that naloxone exerted its effects by reducing the hedonic appeal of palatable foods. By contrast, no differences in sensory or consumption responses were obtained between obese women and women of normal body weight. In retrospect, body weight was unrelated to the wanting of palatable foods, at least, under those particular laboratory conditions.

Butorphanol, an opiate agonist, administered in very small doses, had no impact on taste preferences or food selection. These clinical studies showed that sensory preferences for sweet and high-fat foods did involve opioid peptides that are implicated in food reward. Naloxone had less effect on foods that were high in either sugar or fat than on foods that contained both. The sweet high-fat foods, mostly chocolate, were the most preferred, most palatable combinations. In other studies also, naltrexone, another opioid antagonist, only had an effect on the consumption of most preferred foods (Yeomans and Gray, 1996).

Considering the growing body of research linking food reward with the endogenous opioid system in humans (Reid, 1985; Drewnowski et al., 1985, 1992a; De Araujo and Rolls, 2004; Davies et al., 2007) and animals (Will et al., 2004; Mizushige et al., 2006, 2007, Sclafani, 2007), it seems safe to conclude that both oral (including hedonics) and postingestive (including neuropeptides) stimuli are likely to play an interactive role and that hedonic factors are likely to override homeostatic controls

on food intake at least under particular psychological conditions. In particular, dopamine, a neurotransmitter involved in the reward response, has been implicated in fat overconsumption and positive energy balance in humans (Davis et al., 2007). Low levels of brain dopamine have been shown to predict overeating and obesity in women (Goldfield et al., 2007). Dopamine has also been implicated in the concept of food addictions, generally involving sweet and high-fat foods (Drewnowski and Bellisle, 2007).

Additional information has come from recent neuroimaging studies. The anorectic peptide YY (PYY), associated with satiety, may downregulate preferences for high-fat foods in laboratory animals (Chandarama and Batterham, 2008). Functional brain imaging studies indicate that an exogenous analog to PYY is able to activate the orbitofrontal cortex; reduce motivation for seeking high-fat foods, and induce weight loss. These findings highlight the tightly interconnected roles for the hedonic and homeostatic pathways. In fact, recent studies in animals have shown that neurons in the homeostatic ("metabolic") and reward ("cognitive") brain areas are in close anatomic contact (Zheng et al., 2007).

Satiety is another mechanism advanced to explain human overconsumption of fats. Fats are also said to be less satiating than either carbohydrate or protein (Blundell et al., 1993; Rolls, 1995). The last point is something of a puzzle, since fats contain more energy per gram than does protein; slow down gastric emptying, and very effectively delay the onset of the next meal. Furthermore, palatability and satiety are generally measured in terms of food consumption. Higher intakes mean that a food is palatable; lower intakes mean that it is satiating. By definition then, palatability is the inverse of satiety, such that having a satiating yet palatable food is a contradiction in terms.

Distinguishing between satiety and satiation resolved the paradox. Whereas satiation refers to internal sensations responsible for terminating the current meal, satiety refers to processes that influence the time delay until the following meal and subsequent meal size. Fats were decreed to have an impact on satiety but not on satiation, neatly sidestepping the paradox. Of course, this makes it impossible to distinguish between high palatability and low satiation, especially since they are both measured in terms of food consumption and meal size.

The concept of sensory-specific satiety (SSS), first described by Rolls et al. (1981), integrates current pleasure and past experience. The current response may be influenced by postingestive consequences of recently consumed foods. SSS is defined as a decrease in pleasantness for foods just eaten, without a decrease in pleasantness for new foods (Rolls et al., 1981). The salient sensory mechanism may be taste, but flavor/aroma, texture, and even color may contribute as well (Johnson and Vickers, 1992; Guinard and Brun, 1998). Sensory satiety can be prolonged, lasting from up to 24 h (single exposure) to 2 weeks for repeated exposures (Kamphuis et al., 2001; Weenen et al., 2005). It is not clear if ingestion of calories is even necessary to induce sensory satiety. Drewnowski et al. (1982) had called a similar phenomenon flavor fatigue, as it applied to reduced preferences following repeated tasting without ingestion of a variety of soft drinks.

The evolutionary significance of sensory satiety may lie in variety seeking and the drive to secure a diet containing all essential nutrients (Rolls, 2007). Exposure

to a wide variety of palatable foods, representing an array of tastes, flavors, and textures, has led to higher food intakes in rats (Rolls et al., 1983) and may reduce SSS in humans (Romer et al., 2006). However, studies have found that taste can have a stronger effect on SSS than fat content (Snoek et al., 2004) and texture (Guinard and Brun, 1998). A study of pleasantness ratings comparing ham sandwiches made with soft versus crusty bread and apples versus apple sauce showed that subjects could experience texture fatigue, independent of the taste fatigue. Another study of potato chips (Maier et al., 2007) showed that flavor was a strong component of SSS in salted high-fat foods; however different textures were not compared.

The distinction between palatability and satiety makes the argument that fats are highly palatable but have little impact on satiation (as distinct from satiety). However, in purely practical terms, palatability and satiation are inextricably linked since both are measured in terms of meal size and food consumption. Studies have failed to show differences in SSS profiles between obese subjects and lean controls (Snoek et al., 2004; Brondel et al., 2007).

The newer distinction between wanting and liking attempts to account for the fact that the obese are equally sensitive to taste sensations and like high-fat foods not more that lean controls, based on hedonic ratings and self-reports. Yet the obese appear more susceptible (or less resistant) to the drive to eat, that is, to the wanting component of appetite for energy-dense high-fat foods. Judging by published reports, this wanting seems to be unconscious and distinct from cognitive, conscious wanting, and may be related to the postingestive effects of eating pleasurable food itself, rather than oronsensory stimulation (Berridge, 2004; Mela, 2006). In addition to liking and wanting, it is evident that learned behaviors (Herman et al., 2005; Brunstrom, 2007), as well as external associations and cues (cultural background, time, and social environment for example) also modulate eating behavior.

11.5 FAT PREFERENCES AND AVERSIONS IN EATING DISORDERS, STRESS, AND OVERWEIGHT

11.5.1 Eating Disorders and Stress

Stress and eating disorders have been associated with food cravings and food aversions. Stress (Torres and Nowson, 2008), dietary restraint (Drewnowski et al., 1987b; Drewnowski, 1991b) and concerns with health, and body image or body weight (Bowen et al., 2003) all seem to have an impact on the selection of sweet or high-fat foods.

The most dramatic effects are observed in eating disorders. Studies in anorectic and bulimic patients showed how sugar and fat preference can be used as a biological marker to discriminate between clinical patient populations at extremes of body weight. Anorectic patients exhibit both carbohydrate and fat phobia. While giving high sensory preference ratings to intensely sweet solutions of sugar in water, anorectic patients viewed sweet foods as forbidden in their regular diets. By contrast, sensory preferences for fat were extremely low. Anorectic patients disliked mixtures of milk, cream, and sugar and avoided both meat and milk products in their diets. The avoidance of high-calorie foods was most likely linked to the avoidance of fat (Drewnowski et al., 1987b, 1988). Whether this fat phobia was physiologically or psychologically based was unclear.

Female patients suffering from bulimia nervosa showed a similar sensory and preference profile (Drewnowski, 1989b). When asked to rate the sweetness, creaminess, and preferences for mixtures of milk cream, and sugar, they showed the same ability to detect increasing sucrose concentration as controls and enhanced sensory response to fat. They too showed an aversion to sensory stimuli that were sweet and rich in fat (Drewnowski et al., 1987a; Sunday and Halmi, 1990). The application of the RSM technique (discussed earlier), showed that anorectic-restrictor and anorectic-bulimic patients liked sweet stimuli but disliked those with a high-fat content (Drewnowski et al., 1987a,b).

Dietary restraint that is concerned with weight or dieting, can also influence reported food preferences. Tuorila et al. (2001) showed that women with higher restraint scores chose fat-free versions of a high sugar/high-fat food (fudge). The choices were further modulated by the hedonic quality of the fudge and by the subjects' perceived hunger. By contrast, obese patients with high restraint scores showed elevated preferences for the taste of fat (Elfhag and Erlanson-Albertsson, 2006). Drewnowski and Holden-Wiltse (1992) recruited 37 obese female weight-cyclers, and classified them as low-flux cyclers (weight changes between 0 and 5.5 kg over the last year) or high-flux cyclers (weight changes between 5.6 and 30 kg). The high-flux group showed higher preference ratings for nine ice creams of varying sugar and fat content than did the low-flux group and showed higher preference ratings for sweet desserts on a food preference questionnaire. By contrast, the two groups did not differ in their preference ratings for five sucrose solutions. The authors concluded that fat, not sugar, preferences were more closely tied to the weight-cycling syndrome.

Exploring the relationship between stress and eating behavior, Torres and Nowson (2008) identified sweet, high-fat foods as those that were most likely to be consumed under stressful conditions. Although the number of human studies was very limited, all five showed that both chronic and acute stressors induced the consumption of M&M candy, peanuts, chocolate, and ice cream by obese subjects. However, the effects of obesity and stress may have been confounded; stress was measured only with subjective rather than physiologic measures; and the studies were conducted in the laboratory or under free-living conditions.

Despite these limitations, there seems to be some agreement that acute and chronic stressors may have differential effects on food consumption. Only chronic stressors are associated with higher preference for energy-dense foods, which may be mediated by an increase in circulating cortisol (Torres and Nowson, 2008). Current studies in animals confirm that stress selectively elevates the intake of a preferred high-fat diet in mice (Teegarden and Bale, 2008). These findings prompt the question of whether a higher preference for fat would ensure better preparedness for the fight or flight response under stress conditions in mammals, evolutionary enhancing their survival. Taken together, these data point out the strong influence of the reward system in food intake (dys)regulation.

11.5.2 Fat Preference in Smoking Cessation, Diabetes, and Pregnancy

Smoking cessation has been associated with increased appetite for sweet and high-fat foods and with weight gain. Perkins et al. (1990) showed that long-term exposure to nicotine suppressed hedonic ratings for sugar and fat. In that study, 10 smokers

and 10 nonsmokers (all male) rated sweetened mixtures of milk, cream, and sugar containing between 0% and 20% sucrose and 0.1%–37.6% fat (Drewnowski and Greenwood, 1983). Acute nicotine exposure decreased perceived intensity of fat taste, but had no impact on sweet taste perception or on hedonic ratings for the stimuli. However, current smokers liked sweet/fat mixtures less regardless of nicotine exposure. The authors concluded that long-term exposure to nicotine seems to suppress sensory preferences for sugar and fat and may play a role in weight control.

Pregnancy has been associated with changes in taste perception, food cravings, and food aversions (Pope et al., 1992; Drewnowski, 1997a; Duffy et al., 1998; Tepper and Seldner, 1999; Bartoshuk et al., 2006;). Foods typically craved by pregnant women include sweet and salty foods, notably chocolate, sweets, desserts, fruits, fruit juices, ice cream; pizza; breads, noodles, and grains; pickles and salty snacks (Pope et al., 1992). On the other hand, aversions include coffee, meats, eggs, and pizza. Whereas cravings turn to the basic tastes, sugar, and salt, aversions are formed toward foods with a strong aromatic component. The aroma of coffee, meats, and other fat-soluble volatile molecules may be a warning system denoting potential dietary danger from foods that are bitter, spoiled, and potentially toxic. In other words, the aversions are to odors, many of which are emitted by fat-containing animal foods. The mechanisms by which these taste alterations happen during pregnancy are not fully understood, but they seem to be related to hormonal fluctuations and to the support of healthy pregnancy outcomes (Frye and Demolar, 1994; Duffy et al., 1998). Given that fat is more slowly emptied from the stomach compared with carbohydrate, high-fat foods may induce more feelings of fullness or nausea than carbohydrate-rich foods.

Alterations in sweet taste perception may also occur in type 1 and type 2 diabetes mellitus (DM) (Settle, 1991; Perros et al., 1996). Very limited studies have examined changes in fat perception or fat preference under these conditions. Laitinen et al. (1991) reported no changes in preference for fatty foods (milk and cheese) during the first 3 months of dietary therapy for patients newly diagnosed with type 2 DM. After this time, preferences for fat and sugar decreased accompanied by decreased intake of such foods. These patients were on a clinical diet, which may have accelerated taste changes due to imposed dietary restrictions. Diabetes may not affect preferences for dietary fats. Campbell et al. (1994) alternated 10 patients with diabetes mellitus between a high-monounsaturated fatty acid (MUFA) diet and a high-carbohydrate diet as advised for DM, for a period of 2 weeks each. Both diets were accepted equally well.

11.5.3 Fat Taste Response and Overweight

There is agreement that combinations of sugar and fat have unique hedonic properties and are able to activate the system of reward (Drewnowski, 1991a, 1997b). The question is whether hedonic qualities of such products are a direct cause of overeating and weight gain. Earlier studies on sensory properties of sugar and fat mixtures and the hedonic response showed that the sugar-to-fat preference ratios were tied to extremes of body weight. Massively obese women preferred fat to sugar, whereas anorectic women preferred sugar to fat, while showing reduced hedonic responses overall. However, preferences for dietary sugars and fats have not been directly

linked to weight gain in any longitudinal cohort study. Rather, links between taste responsiveness, hedonic preferences, and body weight are still based on a series of small, clinical, cross-sectional studies.

Patients with BMI in the range of morbid obesity preferred stimuli richer in fat than in sugar, while anorectic patients preferred intensively sweet stimuli and reported aversion to dietary fat (Drewnowski et al., 1985, 1987a,b). Mela and Sacchetti (1991) further reported a direct association between percent fat in most preferred foods (mashed potato and scrambled eggs) and the participants' own percent body fat. Furthermore, a retrospective history of weight cycling was associated with higher preferences for fatty, sweet foods such as ice cream and cakes (Drewnowski and Holden-Wiltse, 1992).

Subsequent studies attempted to identify fat-seeking phenotypes using clusters of characteristics as opposed to single traits (Cooling and Blundell, 1998; Blundell et al., 2005). Such clusters included a strong palatability and weak satiety response to fats; no suppression in liking for energy-dense foods after a meal, and high scores for hunger and disinhibition on the Three-Factor Eating Questionnaire (Blundell et al., 2005). The hypothesis was that the two phenotypes (high fat and low fat) would differ in their ability to distinguish between high- and low-fat foods and in their fat consumption (Cooling and Blundell, 1998). Data analyses showed that the high-fat or thrifty phenotypes had a long-term positive energy balance of approx. 0.5 MJ or 120 kcal/day.

However, the two phenotypes did not differ in their pleasantness ratings for fat or sugar, nor in their ability to estimate fat content based on ratings of creaminess (Cooling and Blundell, 2001). That study compared responses of the two groups of young males (n = 8 per group) to 18 solutions varying in sugar and fat content (Drewnowski et al., 1987b). Both groups correctly identified increasing levels of creaminess and sweetness with increasing fat and sugar concentrations, prompting the question of what is it that causes individuals with low-fat phenotypes to choose different foods from individuals with high-fat phenotypes, when their taste preferences do not seem to differ (Cooling and Blundell, 2001).

Obese men and women appear to differ in their food preferences (Drewnowski, 1997a). A clinical sample of 386 obese women and 93 obese men identified beef steaks and roasts, doughnuts, cookies, and cake as the foods they liked the most and had most trouble with when it came to weight control (Drewnowski et al., 1992b).

Whereas obese women typically preferred fat and sugar mixtures such as chocolate, cookies, doughnuts, and cakes, obese men tended to prefer fat and protein mixtures such as meats, fish, and eggs (Drewnowski et al., 1992b). These patterns of food preferences corresponded to the patterns of food consumption in the broader population (CDC, 2004; Serra-Majem et al., 2007). Data for United Kingdom in 2003 showed that men derived a higher proportion (25%) of their average daily total fat intake from meat and meat products than did women (20%); but similar amounts from spreads, cereals and cereal products, milk and milk products, and potatoes and savory snacks (Henderson et al., 2003). Within food groups, men consumed slightly more bacon, ham, sausages, meat pies, and pastries than women but the differences were small; by contrast, women tended to consume more chocolate, but again the differences were small (1% of total daily fat intake).

In short, differences in fat preference by men and women exist and are more prominent in the obese population. Overall, it seems that men of all sizes tend to derive more fat from savory sources of food; while women (more so if obese) tend to derive more fat from sugar/fat mixtures such as chocolates and desserts. As all preferred foods in the study with the obese population contained fat, a "fat tooth" rather than a "sweet tooth" may the characteristic feature of human obesity (Drewnowski, 1995).

11.6 CONCLUDING REMARKS

The aroma, texture, and mouthfeel of foods are often influenced by their fat content. Human preferences for fat in foods shape dietary choices and may affect energy balance and the long-term regulation of body weight. Understanding the mechanisms by which we detect, perceive, and enjoy fat-rich foods may help us predict food choices and their multiple influences on diets and health.

This chapter explored the perceptions and preferences for dietary fats, using findings from genetic, physiological, and behavioral research on food preference. Traditionally, studies on the sensory perception of fat have focused on diverse aspects of food texture; however, the response to fats also depends on taste and olfaction and on synergistic interactions between fats and other food components. Given that fat in foods can give rise to a variety of textures and flavors, it can be difficult to decide which particular percept is most closely associated with fat content. Fat perception is also influenced by physical form (liquid or solid) and by the presence of other gustatory cues, such as sweetness.

Genetic, developmental, attitudinal, and physiological factors also influence fat preferences. Fat preferences are shaped by postchildhood experiences, remain stable in adult life and do not decline with age. Hedonic preferences for fat-containing foods among adults are influenced by multiple sensory stimuli. In particular, studies have identified a hedonic synergy in sugar/fat mixtures. Mixtures of cream and sugar were universally better liked than sweetened skim milk or unsweetened heavy cream (Drewnowski and Greenwood, 1983). Although studies have generally failed to link sugar consumption to overweight, several cross-sectional studies have pointed to an association between overweight and excess consumption of dietary fats.

Human preferences for energy-dense sweet and high-fat foods may have evolved for reasons of survival. The relative contributions of hedonic (preference or "liking") versus homeostatic (physiological or "wanting") processes in determining preference and choice for high-fat foods have not been defined (Mela, 2006). The hedonic response to fat seems to be strongly linked to the endogenous opioid reward system. This reward system may be independent from motivation to eat at least in the obese population, contributing to increased energy intakes.

Our understanding of fat chemosensation has helped to model the development of a preference for dietary fats in humans. Physiological and gender variations in fat consumption need to be examined further in the context of food culture. The amount and type of fat consumed is strongly influenced by geography, culture, and cost (Perrin et al., 2002; Serra-Majem et al., 2007). Fat consumption is a classic example of how human innate preferences have been aligned with current trends in the food supply and the economics of food choice.

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Part V

Control of Food Intake as a Function of Fat

12 Oral and Postoral Determinants of Dietary Fat Appetite

Karen Ackroff and Anthony Sclafani

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12.1 INTRODUCTION

12.1.1 General Issues in the Study of Fat Appetite

Dietary fat is an important determinant of palatability and energy density in a wide variety of foods. The appetite for fat can be traced to both unlearned attraction to orosensory factors and learned appreciation of high-fat foods based on postoral factors. While these two factors normally operate together, they can be separated experimentally by various techniques. This chapter describes the appetite for dietary fat as a function of oral and postoral effects, and discusses the emerging information about the genetics of fat appetite. Much of the data comes from animal models (rats and mice), but studies of human fat appetite are also noted.

Understanding the basis for fat appetite is important, as it is thought to contribute to the increasing levels of obesity in affluent nations. This stems in part from the higher metabolizable energy content of fat, which is more than twice that of carbohydrate and protein. Foods that are high in fat are thus more energy-dense than lower-fat foods (an exception might be high-fat foods that are also high in water or fiber content). Inexpensive, palatable high-fat foods are widely available. In addition, high-fat food is often found to be less satiating than an equal volume of high-carbohydrate food, which can exacerbate the overconsumption of high-fat foods.

The measurement of appetite for fat can be done by comparing intake of fat to that of other foods presented at the same time (preference) or by absolute amounts consumed, as compared to other foods offered at other times (acceptance). A potential drawback of foods with a mixture of nutrients is that it is difficult to ascribe preference or acceptance to particular components of the food. Accordingly, much of the work that evaluates fat appetite uses isolated nutrients to simplify the analysis. However, foods can be constructed to vary in fat and carbohydrate content, while holding the other nutrients constant, which makes them reasonable alternatives to single-nutrient analysis.

12.1.2 Rodent Models to Study Orosensory and Visceral Determinants of Fat Appetite

Rodent models of feeding behavior have provided extensive information on the origin of fat appetite and the metabolic consequences of eating high-fat diets. Early studies focused on the weight gains and adiposity produced by feeding rats and mice on high-fat composite diets, i.e., a single diet varying in fat content (e.g., Corbit and Stellar 1964; Mickelsen et al. 1955). Later experiments investigated the feeding responses of rodents given access to a pure fat source such as vegetable shortening (e.g., Corwin et al. 1998; Lucas et al. 1989), corn oil, or oil emulsion (Lucas et al. 1989; Takeda et al. 2001) in addition to their low-fat maintenance diet. High-fat and fat option diets typically, but not always, promote overeating, which led to investigations of the determinants of fat-induced overeating.

High-fat diets may increase energy consumption in part because the conversion of dietary fat to body fat reduces its availability for oxidation (Ramirez et al. 1989). That is, foods high in fat may be less satiating than low-fat foods. In addition, high-fat diets

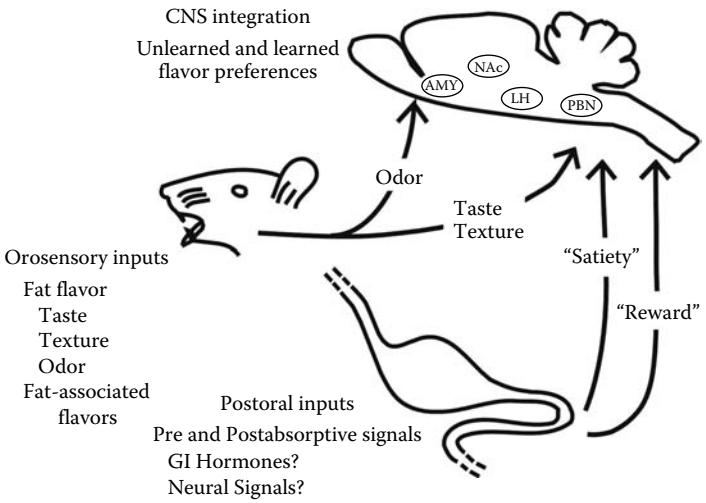

FIGURE 12.1 Oral and postoral determinants of fat appetite. Both unlearned and learned sources of flavor reward contribute to the appetite for fat. Orosensory detection of fat flavor has olfactory, somatosensory (trigeminal), and taste components. Candidate taste signaling elements include CD36, GPR120, Trpm5, and P2X2/P2X3. These and related flavors in fat-containing foods may acquire greater attractiveness by association with the postoral reward signals of fat. The latter may be derived from intestinal "taste" detection. Intestinal signaling elements are not yet known but may include GPR120 and Trpm5 and may be relayed to the brain by neural and/or hormonal pathways. In the brain, the lateral hypothalamus (LH) and amygdala (AMY) are involved in fat-conditioned flavor preferences. Other brain areas implicated in flavor-nutrient conditioning include the parabrachial nucleus (PBN) and nucleus accumbens (NAc). Gut satiety signals generated by the postoral actions of fat suppress fat intake and do not appear to contribute to fat reward.

may have a palatable flavor that promotes overeating and postoral rewarding effects that, via a conditioning process, further increase the attractiveness of the diet's flavor. The availability of inbred mouse strains that differ in their avidity for fat as well as the development of knockout (KO) mice missing components of taste transduction systems have provided new data on the orosensory controls of fat intake. The following sections review the evidence that both orosensory (flavor) and postoral (conditioning) factors are important determinants of fat appetite in rodents (see Figure 12.1).

12.2 OROSENSORY FACTORS

12.2.1 Fat Preference and Texture

12.2.1.1 Nutritive vs. Nonnutritive Oil

In choice tests, rats typically prefer high-fat to low-fat foods (Hamilton 1964; Rockwood and Bhathena 1990). This preference is based, at least initially, on the flavor (taste, texture, and odor) of the high-fat food because it is apparent in the very first test session. Furthermore, rats prefer foods made oily or greasy with the addition of

noncaloric mineral oil or petrolatum over dry, powdered food in choice tests (Carlisle and Stellar 1969; Hamilton 1964). These early findings indicated that the oily/greasy texture of fat was palatable to rats even when separated from the postoral nutritive actions of fat. Hamilton (1964) observed that rats given the choice between a 30% fat (lard) diet and a 30% petrolatum diet initially consumed similar amounts but developed a strong preference for the fat diet over the course of several days. Similar results were observed in rats given the choice of a 25% corn oil diet vs. a 25% mineral oil diet: rats equally preferred the diets for 2 days but then developed an 80% preference for the corn oil diet by day 8 of testing (Carlisle and Stellar 1969). The late developing preference for the fat-rich food over the food containing the nonnutritive fat substitute provided early evidence that the postoral effects of dietary fat influence food preferences in animals via a learning process.

More detailed studies of the orosensory contribution to fat appetite have been conducted using nutritive and nonnutritive oils presented as emulsions or suspensions. Rats as young as 12 days of age displayed comparable ingestive responses to intraoral infusions of 30% corn oil and 30% mineral oil emulsions, which exceeded their response to water infusions (Ackroff et al. 1990). Ingestion by 21-day-old weanling rats was stimulated by corn oil emulsions as low as 6.25% and peak intakes were obtained with a 50% emulsion (Ackerman et al. 1992). Adult rats with no prior experience with oils consumed equivalent amounts of 30% corn oil and 30% mineral oil emulsions during 3 min one-bottle intake tests that minimized postoral nutritive effects (Ackroff et al. 1990). The rats displayed a slight preference for corn oil over mineral oil emulsions in 3 min two-bottle tests, which developed into a small (62%) but significant preference when the test session was extended to 30 min. Food restriction, i.e., maintenance on limited daily chow rations, increased the corn oil emulsion preference to 80%. This strong preference persisted when the animals were no longer food restricted. Other oil-naive adult rats displayed similar deprivation-induced increases in corn oil and mineral oil emulsion intakes during daily 30 min one-bottle tests. These findings indicate that nutritive and nonnutritive oil emulsions are equally acceptable to neonatal and adult rats, that nutritive oil is only slightly preferred by freely fed animals in short-term choice tests, and that deprivation increases the acceptance of both oils. However, when food deprived, animals develop a strong preference for the nutritive oil.

The potent feeding stimulatory effects of nutritive and nonnutritive oil have also been revealed in sham-feeding experiments. With this preparation, the ingested fluid drains out an open gastric fistula which greatly reduces but does not completely eliminate postoral nutritive effects (Mindell et al. 1990). Food-restricted rats with no prior oil experience displayed identical increases in their one-bottle sham-feeding intakes of pure corn oil and mineral oil over successive test sessions. In two-bottle tests, however, the rats preferred the corn oil to the mineral oil, which indicates that in pure form, the nutritive oil has a more attractive flavor than the nonnutritive oil. The sham-feeding response to different corn oil emulsions revealed that a 25% corn oil emulsion stimulates greater intakes than do higher (50% or 100%) and lower (0.78%–12.5%) concentrations. Long-term (24 h/day) real-intake tests have also revealed higher intakes of midrange concentrations of oil emulsions than higher or lower concentrations (Castonguay et al. 1984; Lucas et al. 1989).

The findings that neonatal and adult rats with no prior experience with corn oil or mineral oil display ingestive responses to these oils when first exposed suggest that there is an unlearned attraction to the orosensory properties of oil (Ackerman et al. 1992; Ackroff et al. 1990; Mindell et al. 1990). However, it is possible that the rat's response to oil flavor is conditioned by the animal's very early experience with mother's milk (Ackerman et al. 1992; Ackroff et al. 1990). Newborn rat pups without natural suckling experience ingest more milk (commercial "half and half") and sweet solutions (saccharin or sucrose) than water from a surrogate nipple (Petrov et al. 2004). A comparison of the ingestive responses of milk-naive and milk-experienced rat pups to oil emulsions would be most informative on the issue of the innate basis of fat appetite.

The ingestive responses of mice to different oils and oil emulsions have been investigated in some detail. In 10 min two-bottle tests, mice (ddY strain) displayed strong preferences for 1% emulsions of corn oil, canola oil, or vegetable oil over the xanthan gum vehicle (Takeda et al. 2000). In another study, ddY mice displayed comparable preferences for 2% nutritive corn oil and 2% nonnutritive sorbitol fatty acid ester oil over xanthan gum vehicle during 10 min tests. They also displayed equal preferences for the nutritive and nonnutritive oils over a range of concentrations (2%–100%) in 10 min tests. In a 24 h choice test with pure oils, however, the mice preferred the nutritive oil, which presumably reflects the differential postoral effects of the two oils (Suzuki et al. 2003). In contrast to these results, BALB/c mice consistently preferred corn oil to mineral oil in 10 min two-bottle tests at corn oil concentrations of 1%–100% (Yoneda et al. 2007). An unusual aspect of this test was that the animals had the choice between different concentrations of corn oil mixed into mineral oil vs. pure mineral oil. This suggests that mice, like rats, prefer nutritive to nonnutritive oils when the oils are in pure form but not necessarily when they are presented as emulsions or suspensions.

Some work has attempted to determine the particular textural quality that rodents use to discriminate oils. Ramirez (1994) showed that rats trained to avoid an oil suspension (triglyceride, silicone, or mineral oil) generalized their avoidance to the other oils but not to a nonoily gum with similar viscosity. These results suggested that a textural property other than viscosity, such as lubricity, was critical for oil detection and preference. Consistent with this interpretation, whereas nondeprived and deprived rats preferred oil emulsions (0.5%–30%) to water in two-bottle tests, they did not prefer the gum vehicle to water (Kimura et al. 2003). Studies of the central neural response to orosensation in the primate orbitofrontal cortex revealed that individual nerve cells respond to the texture of nutritive and nonnutritive oils but not to nonoily stimuli of similar viscosity (Rolls et al. 1999; Verhagen et al. 2003).

12.2.1.2 Strain Differences: Oil vs. Vehicle Tests

It is well established that inbred mouse strains differ in their preference (or aversion) for sapid solutions including salt, bitter, sweet, and umami, so it is not surprising that they also differ in their preference for oil emulsions (Bodnar et al. 2008). These studies have generally used 24 h two-bottle tests comparing emulsion intake vs. water or the emulsion/suspension vehicle. In an early study, SWR/J mice displayed stronger preferences and higher intakes than did AKR/J mice at several corn oil emulsion

concentrations during 24 h two-bottle tests (0.005%–50% oil emulsion vs. emulsifier vehicle) (Smith et al. 2001). In another study, C57BL/6ByJ mice had higher intakes and preferences for soybean oil than did 129P3/J mice at low (1%–10%) but not high (30%–100%) concentrations vs. xanthan gum vehicle (Bachmanov et al. 2001). Similar results were obtained with C57BL/6J and 129 (129P3/J, 129X1/SvJ) mice tested with oil emulsions prepared using Intralipid, a stable, commercially available soybean oil emulsion (Sclafani 2007b, see below). These findings were confirmed and extended in a study of 11 inbred mouse strains tested with a wide range of dilute Intralipid emulsions (0.00001%–5%) vs. water (Lewis et al. 2007). Interestingly, the mouse strains displaying stronger oil preference and/or acceptance in these experiments were those that also showed stronger preferences for sucrose and saccharin (Bachmanov et al. 2001; Bodnar et al. 2008; Smith et al. 2001). However, an earlier study comparing macronutrient intake patterns in 13 inbred mouse strains found that variations in fat selection appeared unrelated to sweetener preferences (Smith et al. 2000).

Strain differences in sweetener preference are due in part to allelic variations in the Tas1r3 gene that codes for the T1R3 sweet taste receptor. Glendinning et al. (2008) explicitly examined the relationship between the avidity for sugar and oil by comparing the initial (5 s) licking response to sucrose and Intralipid in inbred strains with the sensitive form of the T1R3 receptor (C57BL/6J, FVB/NJ, SWR/J, SM/J) and the subsensitive form (129P3/J, BALB/cJ, DBA/2J, AKR/J). Consistent with prior work, the sweet sensitive strains licked more vigorously than did the sweet subsensitive strains for low sucrose concentrations (≤ 0.3 M, ~10%). There was, however, no clear relationship between sweet taste sensitivity and the licking response to the oil emulsions (1%–20%). Nevertheless, there were significant strain differences in the initial oil licking as well as in 24 h oil preferences (Intralipid vs. water). Licking rates and 24 h oil preferences were highly correlated for 1% oil but not at the higher concentrations.

Taken together, these data indicate that responsiveness to orosensory cues primarily determines the intake of dilute oil emulsions but that postoral mechanisms influence the intake of more concentrated oil emulsions. This is not unique to fat consumption because sweet taste sensitivity does not fully account for the intake of concentrated sugar solutions (Glendinning et al. 2005; Sclafani 2006). Further evidence that sweet taste sensitivity does not account for oil consumption is indicated by the finding that mice lacking the T1R3 sweet receptor, which are indifferent to dilute sugar solutions, do not differ from wild-type (WT) mice in their intake and preference for 1% Intralipid (S. Zukerman and A. Sclafani, unpublished findings).

The source of the mouse strain differences in the orosensory response to oils is not known, but it may be related in part to differences in oral somatosensation. In the Intralipid study mentioned above (Sclafani 2007b), C57BL/6J (B6) and 129 (129P3/J, 129X1/SvJ) mice were first tested with the nonnutritive oil Sefa Soyate, a sucrose polyester oil used in the preparation of olestra. In 24 h oil vs. vehicle tests, nonnutritive oil intake and preference were greater in B6 mice than 129 mice at 0.313%–2.5% concentrations. Similar strain differences were obtained with low (0.313%–5%) but not high (10%–20%) Intralipid concentrations. However, when retested for their Intralipid preferences, the B6 and 129 mice showed robust (>90%) preferences for all oil concentrations, although B6 mice still consumed more oil at low

concentrations. A second test with the nonnutritive oil revealed increased preference and acceptance in the B6 and 129X1/SvJ mice. The nonnutritive oil preference and intake were much less than that for the nutritive oil. We attributed this experiential effect to a conditioned increase in the animals' evaluation of oil flavor due to the postoral nutritive effects of Intralipid. As discussed below, odor or taste stimuli may mediate the increased preference for nutritive over nonnutritive oils.

12.2.2 Fat Detection and Odor

Although pure, processed oils have virtually no odor, oil and other fats develop odors as they interact with oxygen, enzymes, and chemicals in food (Mela and Marshall 1992). This led to the conjecture that animals could distinguish among nutritive oils, and discriminate them from nonnutritive oils, on the basis of odor. Larue (1978) investigated the role of olfaction in fat detection using a conditioned aversion paradigm. Rats were poisoned after they consumed a 20% fat chow (butter, margarine, lard) or chow containing a nonnutritive fat substitute (20% vaseline). In rats with intact olfaction, aversions generalized from one nutritive fat to others but not to vaseline. Rats made anosmic with zinc sulfate treatment showed smaller aversions when trained with vaseline and little difference from intact rats when trained with the nutritive fats. These results were taken as evidence against the idea that a fatty odor is importantly involved in fat appetite.

Other studies investigated the role of olfaction in the preference for oil emulsions. Rats made anosmic by removal of the olfactory bulb lost their preference for 0.5% corn oil or triolein oil emulsions but not for 1% oil emulsions (Ramirez 1993). In contrast, mice with zinc-induced anosmia, unlike control mice, did not prefer 1% and 3% corn oil emulsions, although they did prefer 5% and 10% oil emulsions (Takeda et al. 2001). Anosmic mice, unlike control mice, also failed to prefer triolein oil (0.1%–2%) or oleate (0.1%–0.2%) to xanthan gum vehicle (Fukuwatari et al. 2003). In evaluating these results, however, it should be noted that anosmia in rats also reduced the preference for dilute sucrose, starch, and Polycose (maltodextrin) solutions (Ramirez 1993) while anosmia in mice blocked the preference for a concentrated sucrose solution (0.5 M) (Uebayashi et al. 2001). Thus, experimentally induced anosmia produces a generalized reduction in responsiveness to nutrient flavors.

Distinguishing nutritive from nonnutritive fat may be aided by olfaction when the fat is embedded in a mixed food. After mice given corn oil and mineral oil adulterated chows acquired a preference for the corn oil version, the olfactory nerve was sectioned in one group. These mice did not distinguish between the nutritive and nonnutritive chows until nerve regrowth occurred, suggesting that mice may rely on odor cues to detect nutritive oil in a mixed food (Kinney and Antill 1996). Postoral conditioning studies discussed below also demonstrate that animals can use odor cues to learn to prefer nutritive oils.

12.2.3 Fatty Acid Taste

Fat had long been considered to be "tasteless" (Mela 1992), but there is accumulating evidence for the existence of a fat taste mediated by a gustatory response to fatty

acids (Abumrad 2005; Fukuwatari et al. 1997; Gilbertson et al. 1997; Laugerette et al. 2005). In rodents, the taste of fat depends on the presence of long-chain free fatty acids. In 5 min two-bottle tests, rats preferred 1% linolenic acid, linoleic acid, and oleic acid, in that order, to xanthan gum vehicle but did not prefer caprylic acid, a medium-chain fatty acid (Tsuruta et al. 1999). The attraction to triglycerides appears to be mediated, in part, by fatty acids released in the mouth by the action of salivary lipase (Kawai and Fushiki 2003). Rats preferred a 2% emulsion of purified triolein to a triolein emulsion containing the lipase inhibitor orlistat during a 5 min two-bottle choice. In contrast to this finding, rats equally preferred a 2% corn oil emulsion and a corn oil + orlistat emulsion. This indicates that attractiveness of natural food oils does not depend upon lipolysis in the mouth. Also, long-chain free fatty acids are attractive to rodents at dilute concentrations but they lose their appeal at higher concentrations. In one study, BALB/c mice showed a monotonic increase in their 60 s licking response to corn oil as concentration increased from 1% to 100%, whereas their peak response to linoleic acid occurred at the 1% concentration (Yoneda et al. 2007). In separate 24 h intake tests, C57BL6/J mice consumed substantially more of 2% soybean oil than 2% linoleic acid when both were suspended in a xanthan gum vehicle (19.3 vs. 8.0 g/day) (Sclafani et al. 2007a). Free fatty acids may have irritant effects in the mouth that reduce their palatability at higher concentrations (see Chale-Rush et al. 2007).

Evidence that free fatty acids are detected by the gustatory system is reviewed in the Chapters 3, 4 and 5 by Gilbertson, Pittman, and Contreras. The following section reviews work from our laboratory investigating fat preferences in genetically modified mice missing different taste signaling elements.

12.2.3.1 Fatty Acid Receptors in Taste Buds

In 1997, Fukuwatari et al. reported the localization of the fatty acid binding protein CD36 in taste buds and they speculated that it participates in the oral detection of fat. Laugerette et al. (2005) subsequently confirmed the localization of CD36 in the taste buds of the rodent circumvallate papillae where it was colocalized in taste cells with alpha-gustducin. Further work demonstrated that linoleic acid increases intracellular calcium and neurotransmitter release in CD36-positive taste cells and activates gustatory neurons in the nucleus of the solitary tract (El-Yassimi et al. 2008; Gaillard et al. 2008). Evidence for CD36 involvement in fat preference was indicated by the failure of CD36 KO mice, unlike WT mice, to prefer 2% linoleic acid to xanthan gum vehicle in 0.5 or 24 h two-bottle tests (Laugerette et al. 2005). Linoleic acid preference was also blocked by gustatory nerve transection in WT mice (Gaillard et al. 2008). CD36 is also implicated in the cephalic phase digestive response to fat (Hiraoka et al. 2003). Lingual stimulation with linoleic acid promoted pancreatobiliary secretion in WT mice but not in CD36 KO mice (Laugerette et al. 2005).

We further investigated the role of CD36 in fat appetite by comparing the 24 h preferences of CD36 KO and C57BL/6J WT mice for nutritive soybean oil and non-nutritive Sefa Soyate oil as well as linoleic acid (Sclafani et al. 2007a). In an initial oil vs. vehicle test, CD36 KO mice showed no preference for 0.313%–2.5% Sefa Soyate oil, whereas WT mice preferred Sefa Soyate at the 2.5% concentration. In a

second test, CD36 KO mice showed only weak preferences for 0.313%–1.25% soybean oil but displayed a significant preference for 2.5% oil. In contrast, WT mice strongly preferred even the lowest concentration. In a choice between 0.625% soybean and Soyate oils, the B6 mice strongly preferred the nutritive oil whereas the KO mice were indifferent to the oils. However, after experience with the 2.5% soybean oil, the CD36 KO displayed a significant, albeit attenuated, preference for 0.625% soybean oil over Soyate oil. The oil-experienced CD36 KO mice also strongly preferred 0.313%–20% Intralipid although they consumed less oil than the WT mice at 2.5%–20% concentrations.

In confirmation of prior findings (Laugerette et al. 2005), naive CD36 KO mice were indifferent to 1%–2% linoleic acid emulsions preferred by WT mice (Sclafani et al. 2007a). However, after they were tested with, and developed a preference for soybean oil emulsions, the experienced CD36 KO mice preferred a range of linoleic acid emulsions (0.025%–2%) over the xanthan gum vehicle and did not differ from WT mice in their fatty acid preference or intake. Thus, despite their initial deficit in fat and fatty acid preference, the KO mice developed significant preferences for soybean oil and linoleic acid even at low concentrations. This late-developing preference was probably due to a postoral conditioning effect. As discussed below, CD36 KO as well as WT mice learn to prefer flavors paired with intragastric (IG) infusions of soybean oil. The failure of CD36 KO mice to prefer the nonnutritive Soyate oil was surprising, as nonnutritive oil preference is assumed to be mediated by oily texture rather than taste. Conceivably, Soyate oil may have a fat-like taste to WT mice because it consists of fatty acids attached to a sucrose core as well as containing a small amount of unbound free fatty acids. However, in a follow-up experiment, naive CD36 KO mice displayed weaker preferences for dilute (0.313%–2.5%) mineral oil emulsions compared to WT mice and the reason for this deficit is not clear.

The deficits displayed by CD36 KO mice in initial preference tests with soybean oil and linoleic acid support the view that this fatty acid binding protein is involved in fat taste. Nevertheless, our findings that with experience CD36 KO mice were indistinguishable from WT mice in their preference for dilute soybean oil or linoleic acid emulsions demonstrates that this protein is not essential for the detection or preference for fat. It may be that the mice learn to prefer oils by associating their odor or texture (e.g., lubricity) with their postoral nutritive effects. However, it is also possible that rodents have other fatty acid sensors that function independently of CD36. The role of delayed rectifying potassium channels in taste cells in fatty acid detection is discussed in the Chapter 3. The G protein–coupled receptor GPR120, which is thought to be a free fatty acid receptor in the gut (e.g., Hirasawa et al. 2005), was recently identified in the circumvallate papillae where it may serve as a fatty acid taste receptor (Matsumura et al. 2007).

12.2.3.2 Gustducin Signaling

Gustducin is a G protein found in taste cells that mediates the neural and behavioral response to sweet, umami, and bitter tastants (Nelson et al. 2002; Ruiz-Avila et al. 2001). In particular, gustducin KO mice display reduced preferences for sucrose, glutamate, and quinine solutions. Gustducin is colocalized with CD36 in circumvallate taste cells, which suggests a role for the G protein in fatty acid taste (Laugerette et al.

2005). We therefore compared the preference for nutritive and nonnutritive oils of gustducin KO mice with that of C57BL/6J WT mice (Sclafani et al. 2007b). In 24 h two-bottle tests, gustducin KO mice were similar to WT mice in their preference for Sefa Soyate oil (0.313%–2.5%) and the two genotypes showed identical preferences and intakes for soybean oil over a wide range of concentrations (0.039%–20%). Thus, despite its presence in CD36-containing taste cells, these data indicate that gustducin does not have a critical role in fat preference.

12.2.3.3 Trpm5 Channel Signaling

Trpm5 (transient receptor potential family) is a Ca^{2+} activated cation channel mediating the chemosensory transduction cascade in gustatory sensory cells. Like gustducin, it is important in mediating sweet, umami, and bitter tastes. To determine if Trpm5 is involved in fat appetite, we compared the oil preferences of Trpm5 KO and WT mice (Sclafani et al. 2007b). In initial tests with 0.313%–2.5% oil emulsions, Trpm5 KO mice failed to prefer either soybean oil or Sefa Soyate oil to vehicle. In a subsequent test with soybean oil in the form of Intralipid, Trpm5 KO showed weak preferences for 0.313%–1.25% oil but developed a significant preference for 2.5% and higher concentrations. Nevertheless, they underconsumed 2.5%–20% Intralipid compared to WT mice. Given that the Trpm5 KO mice developed a strong (95%) preference for concentrated Intralipid emulsions, they were retested with dilute emulsions. In this final test series, the Trpm5 KO mice displayed preferences even at the lowest concentration (0.039%); the only difference from WT behavior was lower intake of Intralipid at the highest concentrations in the series (0.625%–2.5%). The similarities to CD36 KO mice (initial indifference to soybean and Sefa Soyate oil, and persistent lower acceptance even after preferences had been acquired) (Sclafani et al. 2007a) suggest that Trpm5 is part of the CD36 fatty acid signaling pathway.

In a subsequent experiment (A. Sclafani, unpublished) that directly compared the oil preferences of CD36 KO, Trpm5 KO, and WT mice, the Trpm5 KO mice displayed the most profound deficit. In an initial test with 0.313%–2.5% mineral oil, Trpm5 KO mice were completely indifferent to the nonnutritive oil whereas CD36 KO mice displayed an attenuated preference compared to WT mice. Unlike CD36 KO and WT mice, the Trpm5 KO mice were indifferent to 0.313%–2.5% soybean oil. Although Trpm5 KO intakes were lower, all strains displayed preferences for 5%–20% oil. Finally, whereas CD36 KO and WT mice preferred 0.625% soybean oil to mineral oil and Sefa Soyate oil, the Trpm5 KO mice did not discriminate between the nutritive and nonnutritive oils. These data indicate that Trpm5 is more essential for the detection of and preference for dilute oil emulsions than is CD36, which is consistent with the possible existence of other fatty acid taste receptors.

12.2.3.4 Purinergic Receptor Signaling

Taste receptor cell communication with other taste cells and gustatory nerves appears quite complex (Roper 2007). Recent evidence indicates that taste receptor cells use ATP as a neurotransmitter to communicate with gustatory nerves via P2X2 and P2X3 heteromeric receptors. P2X2/P2X3 double KO (P2X Dbl KO) mice show no

gustatory nerve response to bitter, sour, salty, or umami stimuli. They also showed greatly reduced two-bottle preference/aversion responses to sweeteners, glutamate, and bitter substances (Finger et al. 2005). Given that P2X Dbl KO mice appear to have a near-total ageusia, we investigated their preference response to dietary fat (Sclafani 2007a). In 24 h two-bottle tests with 0.313%–20% soybean oil emulsions vs. vehicle, P2X Dbl KO mice, unlike WT controls, failed to prefer the intermediate 0.625%–5% concentrations. They displayed a marginal preference for 10% oil but a strong preference (93%) for 20% soybean oil comparable to that of the WT mice (94%). When retested with the same oil concentrations, the P2X Dbl KO mice, like WT controls, displayed robust preferences (>90%) for all concentrations, and the two genotypes consumed comparable amounts of oil.

Thus, the soybean oil preference deficit of the P2X Dbl KO mice was slightly greater than that observed with Trpm5 KO mice and more profound than that of CD36 KO mice in our other studies. However, like the Trpm5 KO and CD36 KO mice, the P2X Dbl KO mice displayed near total preferences for even dilute soybean oil emulsions after experience with concentrated oil. The KO mice presumably associated the nontaste flavor components (odor, texture) of the oil emulsions with their postoral nutrient reinforcing actions.

Together with previously reported data (Laugerette et al. 2005), the findings of our KO mouse studies implicate CD36 in the preference for free fatty acids and triglycerides but revealed an even more important role for Trpm5 and P2X2/P2X3 taste signaling elements. In all cases, however, the initial oil preference deficit displayed by these KO mice disappeared after they had an experience with the concentrated nutritive oil emulsions. This indicates that the postoral nutritive effects of fat can overcome orosensory deficits in fat detection. As discussed next, the potent preference conditioning actions of postoral nutrients are documented by the studies involving IG nutrient infusions.

12.3 POSTORAL FACTORS

As discussed in Section 12.2.1, a learned component to fat appetite was first suggested by early reports of rats developing a preference with repeated testing for a high-fat food over a food containing a nonnutritive fat substitute (Carlisle and Stellar 1969; Hamilton 1964). Similar preference shifts were observed in rats and mice tested with nutritive and nonnutritive oils (Ackroff et al. 1990; Suzuki et al. 2003). In addition, mice show significant increases in their preferences for dilute oil emulsions after consuming concentrated nutritive oil emulsions (Sclafani 2007a,b; Sclafani et al. 2007a,b). Such findings provide indirect evidence that postoral effects of fat produced learned changes in the attractiveness of high-fat foods. There are other possible explanations, however. For example, intake of a high-fat food may have digestive and metabolic effects that allow animals to increase their consumption of fat-rich foods and thereby express a preexisting fat flavor preference (e.g., Reed et al. 1991). The postoral conditioning actions of fat are now well established in numerous learning experiments using flavor or place preference paradigms. This section reviews the literature of fat-conditioned flavor preference; a discussion of place preference conditioning by fat is provided in Chapter 10.

12.3.1 Oral Conditioning Studies

Preference conditioning by dietary fat has been formally investigated using a Pavlovian conditioning procedure in which an arbitrary flavor (the conditioned stimulus or CS+, e.g., grape) is associated with a fat source and a different flavor (the CS−, e.g., cherry) is paired with a nonnutritive alternative (e.g., water or a saccharin solution). In an early application of this paradigm, Mehiel and Bolles (1988) trained food-restricted rats to drink, on alternate days, a CS+ flavored corn oil emulsion (3%) and CS− flavored saccharin (0.25%) solution. In a two-bottle choice test with both flavors presented in water, the rats consumed more of the CS+ flavor solution than the CS− flavor solution. Mehiel concluded that the CS+ preference was reinforced by the postoral caloric effects of the fat rather than its flavor, because a separate test indicated that the corn oil emulsion was less preferred than the saccharin solution. In a subsequent study, we obtained a fat-conditioned preference in food-restricted rats trained with a CS+ flavor added to a 7.1% corn oil emulsion (Elizalde and Sclafani 1990). However, we also observed a preference in rats trained with a flavor added to a nonnutritive 7.1% mineral oil emulsion, although the preference was less pronounced than that displayed by the corn oil trained animals (72% vs. 89%). Our results indicated that the mineral oil had a palatable flavor that, even in the absence of postoral nutritive feedback, was sufficient to condition a flavor preference. The process by which a palatable flavor can produce a preference for a neutral flavor is known as "flavor–flavor" conditioning.

In a second experiment, new groups of rats had a CS+ flavor paired with the consumption of corn oil or mineral oil emulsions, but in this case, a delayed conditioning procedure was used. That is, the rats consumed a CS+ flavored saccharin solution for 10 min followed, after a 10 min delay, by the consumption of an oil emulsion without added flavor. (Consumption of a CS− saccharin solution was not followed by another solution.) With this procedure, only rats trained with the corn oil emulsion acquired a preference for the oil-associated flavor (80%, compared to 44% for the mineral oil group) (Elizalde and Sclafani 1990). The effectiveness of nutritive, but not nonnutritive oil to condition a flavor preference over a delay is consistent with earlier results obtained with nutritive (glucose) and nonnutritive (saccharin) sweeteners (Holman 1975). In that study, only rats trained with a CS+ flavor paired with delayed intake of a glucose solution acquired a preference for the CS+ flavor. Other rats trained with a CS+ flavor paired with the delayed intake of a nonnutritive saccharin solution failed to acquire a preference for the CS+ flavor. Flavor-nutrient learning over a delay presumably evolved to tolerate the natural delay that occurs between orosensory stimulation and postoral effects of food.

Delay conditioning is a useful method to separate the oral vs. postoral effects of nutrients and is not unique to fat. We have conditioned similar flavor preferences based on fat, carbohydrate, and protein sources using this procedure (Pérez et al. 1995). An alternate way to separate oral from postoral effects of nutrients is pharmacological inhibition of digestive enzymes. We trained rats to eat two different flavored fat diets (vegetable shortening supplemented with vitamins, minerals, and fiber) during alternate 30 min/day meals (Ackroff and Sclafani 1996). One flavored diet (e.g., grape) contained the lipase inhibitor orlistat and the other flavored diet

(e.g., cherry) was drug free (plain diet). In subsequent two-choice tests, the animals consumed substantially more (84%) of flavored plain diet than flavored orlistat diet whether or not the latter diet contained orlistat during the choice test. We attributed the learned flavor preference to the positive postoral effects of the digested fat in the plain diet. Alternately, the animals may have learned an aversion to the flavored orlistat diet due to direct actions of drug or of the undigested fat in the gut. This seemed unlikely, however, because the animals consumed comparable amounts of the two diets during initial training. Another possibility is that the orlistat diet was less tasty than the plain diet because orlistat prevented lingual lipase from producing free fatty acids in the mouth. However, other animals given a two-choice test between unflavored plain and orlistat fat diets consumed comparable amounts during the first two 30 min tests. Thus, as in the case with corn oil (Kawai and Fushiki 2003), inhibiting lingual lipase does not appear to have an immediate effect on the palatability of vegetable shortening.

12.3.2 Gastric Conditioning Studies

A more direct approach to studying postoral conditioning by dietary fat is to pair the intake of a CS+ flavor solution with IG infusion of nutritive oils. This eliminates the possibility that the palatable flavor of the oil contributed to the learning of the CS+ preference. In the first study to report flavor preferences conditioned by IG fat infusions (Lucas and Sclafani 1989), we trained food-restricted rats with a CS+ flavored saccharin solution paired with IG infusions of 7.1% corn oil emulsion, and a CS− flavored saccharin solution paired with IG water during daily 10 min one-bottle sessions; IG infusion volumes were fixed at 7 mL. In a subsequent 10 min choice test conducted without IG infusions, the rats showed a significant, but modest (61%) CS+ preference. Other animals were given 24 h/day access to food and, on alternate days, CS+ and CS− flavored saccharin solutions paired with IG corn oil and water infusions, respectively. In this case, the amount of infused IG was automatically matched to the oral intake of the CS solution, i.e., whenever the animal licked the CS+ or CS− solution, a computer turned on the oil or water infusion pump, respectively. In a subsequent two-bottle choice test during which both CS solutions remained paired with their appropriate IG infusions (reinforced test), the animals displayed a 76% CS+ preference. The rats continued to prefer the CS+ flavor during four additional days of testing during which intake of both solutions was paired with IG water infusions (extinction test). The latter finding demonstrates that, once learned, fat-conditioned flavor preferences are quite persistent even in the absence of continued reinforcement.

We have replicated the effectiveness of IG fat infusions to condition flavor preferences in many experiments that investigated various aspects of the conditioning process. Although significant, the 61% CS+ preference obtained in our original corn oil short-term conditioning experiment was weaker than the preferences (>85%) typically obtained in studies involving IG carbohydrate infusions (Drucker and Sclafani 1997; Lucas and Sclafani 1996a; Lucas et al. 1998b; Sclafani and Lucas 1996). We attempted to improve fat conditioning by using IG infusions of "preingested" fat, that is, a corn oil emulsion that was ingested by a donor rat and then collected from

its stomach. The preingested oil contained salivary and gastric secretions thought to facilitate nutrient conditioning (Molina et al. 1977; Puerto et al. 1976). The preingested fat emulsion was no more effective than a fresh emulsion in conditioning a flavor preference (Lucas and Sclafani 1989). We also determined if adding a small amount of oil to the CS solution would enhance IG conditioning by activating fatty taste-induced cephalic reflexes that facilitate fat digestion (see Laugerette et al. 2005). Training animals with 2% corn oil added to the CS solutions did not enhance the conditioning response to IG oil infusions (Lucas and Sclafani 1989). Perhaps, the addition of a free fatty acid (e.g., linoleic) rather than a triglyceride would be more effective in light of the recent findings of Laugerette et al. (2005).

One manipulation that did improve flavor conditioning was feeding animals a high-fat chow diet rather than the standard low-fat chow used in our laboratory. It is well documented that diets high in fat enhance lipase secretion, fat digestion, and absorption as well as voluntary fat consumption (see Reed and Friedman 1990; Reed et al. 1991). We therefore compared flavor conditioning in rats maintained on a low-fat (LFC, 12% fat) standard chow diet with rats fed a high-fat (HFC, 48% fat) chow-oil diet (Lucas and Sclafani, 1996b). The food-restricted rats were trained in 30 min daily sessions with CS+ and CS− solutions paired with matched IG infusions of 7.1% corn oil and water, respectively. By the end of training, the HFC and LFC groups displayed CS+ preferences of 90% and 62%. Interestingly, the rats in the HFC group retained their strong CS+ preference after being switched to the LFC diet. This indicates that a high-fat diet enhances the acquisition of a fat-based preference and is not necessary for its continued expression.

We also determined if fats with different fatty acid compositions (chain length and saturation) differ in their ability to condition flavor preferences (Ackroff et al. 2005). The food-restricted rats were trained (30 min/day) with three flavored solutions: two CS+ flavors were paired with IG infusions of two different fat sources (7.1% emulsion) and a third flavor (CS−) was paired with IG water infusion. The results revealed that corn oil conditioned a stronger preference than did a medium-chain triglyceride mixture (84% vs. 65%). In contrast, emulsions of corn oil, beef tallow, vegetable shortening, or safflower oil produced similar CS+ preference relative to the CS− flavor. In direct comparisons, however, the rats preferred the corn oil-paired CS+ flavor to the CS+ flavors paired with beef tallow and vegetable oil. The flavors paired with corn oil and safflower oil were equally preferred. Taken together, the results demonstrate that a variety of fat sources can condition flavor preferences, but fats with high polyunsaturated content and/or lower saturated fat content are the most effective. Another notable aspect of this study is that all the oils, except for the medium-chain triglyceride oil, produced relatively strong flavor preferences (~85%) even though the animals were fed a low-fat chow diet. This would appear inconsistent with the maintenance diet study cited above. It may be that training animals with two different oils within an experiment facilitates learning in animals on a low-fat diet. Another potential factor is that our ability to prepare stable emulsions has improved over the years as new techniques have been adopted which may enhance the digestion of the infused oil emulsions (Ledeboer et al. 1999).

Mice, like rats, are sensitive to the postoral reinforcing actions of dietary fat. It is possible, therefore, that the different fat preferences observed in inbred mouse

strains may be due, in part, to differences in the postoral nutritive effects of fat. We investigated this possibility in C57B6/6J (B6) and 129 mice, which displayed strong and weak preferences, respectively, for dilute nutritive oil emulsions (Bachmanov et al. 2001; Glendinning et al. 2008; Lewis et al. 2007; Sclafani 2007b). The mice were fitted with a chronic gastric catheter and trained to drink flavored CS+ and CS− saccharin solutions paired with IG infusions of 5% Intralipid and water, respectively. The animals had *ad libitum* access to low-fat chow during the 23 h/day training sessions. In our initial experiment, the CS+ solutions were sweetened with saccharin and the B6 mice acquired a stronger preference (96%) for the CS+ flavor than did the 129 mice (80%). However, the 129 mice drank only half as much of the CS solutions as did the B6 mice during training, which we attributed to their less avid response to the saccharin-sweetened solutions.

In a second experiment, therefore, we matched the attractiveness of the CS solutions for the two strains by adding more sweetener in the solutions offered to the 129 mice. This equated the CS solution training intakes of the two strains and resulted in equally strong CS+ preferences in the 129 mice (98%) and B6 mice (93%). Furthermore, the 129 mice overconsumed the oil-paired CS+ solution relative to the water-paired CS− solution to the same degree as did the B6 mice. This contrasts with the finding that 129 mice consume about half as much of a comparably dilute Intralipid solution (Sclafani 2007b). Taken together, these findings indicate that the B6 and 129 mice are equally responsive to the postoral reinforcing actions of Intralipid and 129 mice underconsume the oil emulsion because of a reduced orosensory response to the emulsion. Whether other inbred strains differ in their oral or postoral response to fat remains to be determined, and the IG conditioning procedure provides a powerful tool to dissect out the origin of the strain differences.

12.3.2.1 Fat vs. Carbohydrate Conditioning

The overconsumption of high-fat foods is often attributed to their palatable flavor, but given the IG conditioning results reviewed above, it is also possible that postoral factors contribute to the palatability of such foods. Yet, in early experiments the flavor conditioning effects produced by IG fat infusions were weaker than those obtained in comparable studies using IG carbohydrate infusions (Elizalde and Sclafani 1990; Lucas and Sclafani 1989, 1996b; Ramirez 1997). To directly compare the postoral reinforcing effects of fat and carbohydrate, we used a conditioning paradigm in which rats were trained with one flavor (the CS+F) paired with IG fat, a second flavor (the CS+C) paired with IG carbohydrate and a third flavor (the CS−) paired with IG water infusions. This allows two kinds of comparisons: the relative strength of preferences for each CS+ over the CS−, and the preference between the CS+F vs. CS+C flavors.

In one experiment (Lucas and Sclafani 1999a), we trained food-restricted rats 30 min/day, with intake of the CS+F solution paired with IG 7.1% corn oil and intake of the CS+C solution paired with IG 16% Polycose (a maltodextrin isocaloric to corn oil at this concentration). Half of the rats were fed a low-fat chow ration (LFC; 12% fat) and the other half was fed a high-fat ration (HFC; 48% fat energy) throughout the experiment. Overall, in the posttraining choice tests, the rats consumed more CS+C and CS+F solution than CS− solution. The preferences, relative

to the CS−, were slightly greater for CS+C than CS+F, and in the HFC rats than the LFC rats but the differences were not significant (HFC: CS+C vs. CS+F, 83% and 76%; LFC: 73% vs. 68%). In the critical CS+C vs. CS+F choice test, however, CS+C was significantly preferred by both the HFC (68%) and LFC (70%) groups. We used a similar training procedure in another experiment in which rats were fed restricted rations of low-fat chow or a varying menu of palatable foods including high-fat items (e.g., cookies, cheese, meat). Both diet groups significantly preferred the CS+C to the CS+F (76%–78%). Other investigators reported a similar preference for CS+C over CS+F in chow-fed rats (Tracy et al. 2004).

To determine if food restriction and/or short-training sessions influenced the preference for a carbohydrate-paired flavor over a fat-paired flavor, we studied flavor conditioning in nondeprived rats trained 22 h/day. The animals, which had *ad libitum* access to either a LFC or HFC diet, were trained with CS solutions paired with IG infusions of fat (7.1% corn oil), carbohydrate (16% sucrose), or water (Ackroff and Sclafani 2007). The chow diet did not affect preferences for the CS+F over CS− (86% LFC vs. 82% HFC), but the CS+C vs. CS− preference was stronger in the LFC group (86% vs. 77%). In the choice test with the two nutrient-paired CS+ solutions, the rats in both diet groups preferred the CS+C to the CS+F (60% or 72%). Thus, the results of this series of experiments were quite consistent: irrespective of maintenance diet, food deprivation state, or session length, rats develop stronger preferences for the CS+C than the CS+F. This indicates that the postoral reinforcing actions of fat are less pronounced than those of carbohydrate at the concentrations tested.

12.3.2.2 High-Fat vs. High-Carbohydrate Diet Conditioning

The results obtained with IG fat and carbohydrate infusions would seem to imply that the preference rats display for high-fat over high-carbohydrate diets can not be attributed to the postoral actions of the diets. However, further research revealed that the conditioning actions of fat and carbohydrate depend upon nutrient context and energy density. This research evolved from a seminal study by Warwick and Weingarten (1995) that dissected the oral and postoral determinants of high-fat diet overeating. These investigators developed a pair of isocaloric liquid diets based on evaporated milk supplemented with corn oil and sucrose that were high in fat (HF: 60% of energy) or carbohydrate (HC: 78% of energy), but equal in protein. In an oral feeding experiment, rats fed the HF diet consumed more energy and gained more weight than rats fed the HC diet. In a sham-feeding choice test, which eliminated the postoral effects of the two diets, rats drank significantly more of the HF than HC diet; this demonstrates the greater palatability of the HF diet. In a final IG feeding experiment, rats were given a saccharin solution to drink that was paired with matched IG infusions of either the HF or HC diet as their only nutrient source. The HF rats self-infused more diet and gained more weight than did the HC rats demonstrating that, in the absence of a flavor difference, the postoral actions of the HF diet were sufficient to promote overeating. The investigators hypothesized that the rats overconsumed the saccharin solution paired with the HF diet infusions because the diet was less satiating than the HC diet.

Another possible explanation for the HF-induced overeating, not incompatible with the reduced satiety hypothesis, is that the HF diet has a more reinforcing postoral effect

than does the HC diet. This alternative hypothesis would seem inconsistent with the findings summarized above that IG fat infusions condition weaker, not stronger flavor preferences than do IG carbohydrate infusions. However, these data were obtained with pure nutrient infusions and it seemed possible that different results would be obtained with high-fat and high-carbohydrate mixed diets. Therefore, we compared the flavor conditioning produced by IG infusions of the HF and HC diets (Lucas et al. 1998a). Rats were fed chow *ad libitum* and trained, on different days, with three flavored saccharin solutions (CS+HF, CS+HC, and CS−) paired with IG infusions of HF diet, HC diet, and water, respectively. After 12 training days, flavor preferences were evaluated in a series of two-bottle tests. During training, the rats consumed more of the CS+HF solution than CS+HC solution, and consequently were infused with more HF diet than HC diet. In choice tests with the CS−, the rats strongly preferred both the CS+HF (91%) and CS+HC (84%) flavors. However, in the critical test with the two CS+ solutions, the animals preferred the CS+HF to the CS+HC by 72%.

Because the animals consumed more CS+HF than CS+HC during training, we considered the possibility that their increased familiarity with this flavor was responsible for the CS+HF preference. This was not the case; rats trained with fixed amounts of the CS+HF and CS+HC solutions (30 mL/day) still preferred the CS+HF in a subsequent choice test with the two CS+ solutions. A final experiment determined if the reduced satiating effect of the CS+HF diet was responsible for flavor conditioning effects. Towards this end, rats were trained with an HC diet that was less calorically dense than the HF diet (1.4 vs. 2.1 kcal/mL). This treatment successfully equated the satiating actions of the HC diet and HF diets, as assessed by the similar intakes and meal patterns of CS+HC and CS+HF solutions during training. In the critical CS+HF vs. CS+HC choice test, the rats did not significantly differ in their intake of the two solutions and their CS+HF preference was only 58%.

Subsequent research further explored the training and test parameters that influence the flavor preferences obtained with HF and HC diets. In one study, we examined the effect of deprivation state and session length (Lucas and Sclafani 1999b). Food-restricted rats trained 30 min/day with flavor solutions paired with isocaloric (2.1 kcal/mL) HF and HC diet infusions tended to prefer the HC to the HF paired flavor. Yet, the same rats given 22 h/day training with the same flavors and infusions developed a significant CS+HF preference (72%). However, this CS+HF preference was only expressed in 22 h/day tests; the animals consumed comparable amounts of the CS+HF and CS+HC solutions during the initial 30 min of testing.

A second study further explored the influence of caloric density on the preferences conditioned by HF and HC diets (Ackroff and Sclafani 2006). *Ad libitum* fed rats were trained 22 h/day with flavored solutions paired with IG infusions of dilute (0.5 kcal/mL) versions of the HF and HC diets. During one-bottle training, the rats consumed similar amounts of the CS+HF and CS+HC flavored solutions paired with these diets. In posttraining choice tests, they strongly preferred the CS+HF (93%) and CS+HC (97%) solutions to the CS− solution, but preferred the CS+HC to CS+HF by 81%. The same animals were next trained with new flavors paired with the calorically dense versions (2.1 kcal/mL) of the diets and they displayed a significant preference for the CS+HF (67%) confirming our original results (Lucas et al. 1998a).

The results of these studies, although complex, were quite consistent. The energy-dense, high-fat liquid diet promoted greater intake than the isocaloric high-carbohydrate diet whether it was consumed by mouth or by IG self-infusion (Ackroff and Sclafani 2006; Lucas and Sclafani 1999b; Lucas et al. 1998a; Warwick and Weingarten 1995). CS flavors paired with infusions of either diet were significantly preferred to a water-paired CS− flavor, with the preference being stronger for the CS+HF solution, and the rats preferred the CS+HF to the CS+HC in 22 h/day choice tests. This preference was not displayed in short (30 min) tests, and food-restricted animals actually preferred the CS+HC to CS+HF. When trained with the energy-dilute versions of the diet, the rats self-infused similar amounts of HF and HC diets, yet, developed a strong preference for the CS+HC over the CS+HF. Finally, when trained with an energy-dense HF diet (2.1 kcal/mL) but diluted HC diet (1.4 kcal/mL), the animals self-infused similar volumes of the two diets (but less HC in calories) and displayed similar preferences for the CS+HF and CS+HC. Thus, the postoral effects of a high-fat mixed diet are not invariably more or less reinforcing than a high-carbohydrate diet. The HF diet was more reinforcing than the HC diet when the diets were energy-dense and available 22 h/day. We attribute this to the reduced satiating action of the energy-dense HF diet relative to the HC diet. As discussed in detail elsewhere (Sclafani and Ackroff 2004), the postoral reinforcing effects of food are separate from their satiating actions which may, in some cases, actually reduce food reinforcement.

12.3.3 Postoral Conditioning Mechanisms

The physiological mechanisms that mediate flavor preference conditioning by dietary fat have been investigated at several levels but remain incompletely understood. One commonly held view is that nutrient reinforcement involves satiety or energy repletion signals. In fact, some investigators hypothesize that the postoral actions of nutrients are reinforcing only in food-restricted animals (Davidson 1998; Harris et al. 2000). Flavor-nutrient learning is often studied in food-restricted animals, but this is not necessary; such learning occurs in animals given unlimited access to food (Ackroff and Sclafani 2006, 2007; Lucas et al. 1998a; Lucas and Sclafani 1989). In one study, we specifically investigated the influence of energy deprivation state on flavor conditioning by fat (Yiin et al. 2005). Rats were fed either unlimited or limited amounts of a high-fat chow diet and trained 30 min/day to drink CS flavored saccharin solutions paired with IG infusions of 7.1% corn oil or water. Both the hungry (food-restricted) and satiated (unrestricted) groups learned preferences for the fat-paired CS+ flavor that did not significantly differ (85% vs. 78%).

It is adaptive for an animal to recognize the postoral nutritive value of a new food even if it is not hungry at the time. However, it is also important for animals with particular nutritional needs to develop preferences for foods that satisfy those needs. Evidence for such nutritional wisdom is provided by the report that diabetic rats with impaired ability to utilize carbohydrates learned to prefer a flavored food associated with the oral intake of a corn oil emulsion over a different flavored food associated with the intake of a sugar solution (Tordoff et al. 1987). Even in the absence of metabolic disorder, preferential intake of high-fat food

would still be valuable under many circumstances, allowing the animal to meet its energy requirements more efficiently. The human predilection for energy-dense foods has been interpreted as an evolved response to seasonal food availability (Ulijaszek 2002).

12.3.3.1 Peripheral Sites of Action

The postoral site at which fat acts to condition flavor preferences is not certain. In most conditioning studies, oil emulsions were infused into the stomach, but intraduodenal (ID) infusions of corn oil emulsion are also effective in producing CS+ flavor preferences (Lucas and Sclafani 1996a). This suggests that the stomach is not a critical site of action. Whether ID fat infusions act in the intestines or a postabsorptive site to condition flavor preferences is not known; there are no studies comparing the conditioning effects of intestinal vs. intravenous fat infusions. With respect to satiety, available evidence indicates that fat-induced satiation is preabsorptive: ID, but not intravenous, infusions of Intralipid suppressed sham feeding in rats (Greenberg et al. 1993; Greenberg and Weatherford 1990). As we have noted in Sections 12.3.2.2 and 12.3.3.3, fat satiety effects are distinct from, and may counteract, the reinforcing actions of fat. The satiating effects of fat are further discussed in Chapter 15.

The nutrient specificity of preference learning is apparent in the differential strength of preferences for isocaloric infusions of fats vs. carbohydrates, as reviewed in Section 12.3.2.1. Other indications that rats can discriminate the postoral effects of nutrients were provided by a conditioned taste aversion study (Tracy et al. 2004). Rats were poisoned after IG or ID infusions of carbohydrate (Polycose) or fat (corn oil emulsion) and subsequently showed reduced acceptance of the poisoned nutrient when given the opportunity to consume it orally. These findings were taken as evidence that the intestinal tract can "taste" nutrients in a manner analogous to the lingual gustatory system, but the identity of the intestinal taste receptors was not revealed.

As discussed in Section 12.2.3.1, the fatty acid binding protein CD36 is a putative lingual fat taste receptor. Some investigators also proposed that CD36 functions as a fatty acid detector in the intestinal tract (Chen et al. 2001; Drover et al. 2005; Fukuwatari et al. 1997). We investigated the role of intestinal CD36 in fat-conditioned flavor preferences using CD36 KO and WT mice (Sclafani et al. 2007a). The animals were fitted with chronic IG catheters and trained to associate CS+ and CS− flavored solutions with IG infusions of 5% Intralipid and water, respectively. Although training and testing intakes were lower in KO mice than in WT mice, the animals did not differ in their conditioned preference for the fat-paired CS+ flavor (99% vs. 94%). The calcium channel Trpm5, which appears to be more essential than CD36 in the mediation of oral fat preferences (Section 12.2.3.3) is also found in intestinal cells (Bezençon et al. 2007). In initial oil vs. water two-bottle tests, Trpm5 KO mice were indifferent to nutritive oil emulsions but after extensive experience they developed strong oil preferences (Section 12.2.3.3). These data suggest that the KO mice learned to prefer the oil emulsions based on the postoral reinforcing actions fat. However, the conditioning response of Trpm5 KO mice to IG fat infusions remains to be documented. The role of the GPR120 receptor in the gut in flavor conditioning by IG fat infusions also requires investigation.

12.3.3.2 Peripheral Lesion Studies

The postoral reinforcing signal generated by fats that condition flavor preferences could be transmitted to the brain via neural or humoral pathways. To date, evidence for a neural route is lacking. In one study, we treated rats with capsaicin, a neurotoxin that produces partial visceral deafferentation, and trained them to associate a CS+ flavor with ID 3.5% corn oil infusions (Lucas and Sclafani 1996a). The capsaicin-treated rats acquired a CS+ preference that was lower, but not significantly so, than that of control animals (72% vs. 87%). Intake of the CS+ solution was suppressed by the ID fat infusions in the controls but not in the capsaicin-treated animals, confirming earlier findings (Yox and Ritter 1988) that capsaicin blocks the satiating action of infused lipids.

We also investigated the potential role of vagal transmission in fat conditioning using a selective sensory vagotomy procedure (Sclafani et al. 2003). The rats were given restricted rations of high-fat chow, and ID infusions of 3.5% corn oil and water were paired with the consumption of CS+ and CS− solutions. Deafferented rats and sham controls displayed the same 69% preference for the fat-paired CS+ solution in the posttraining choice tests. In a satiation test, however, only the sham rats suppressed their intake of a palatable solution in response to ID fat infusions. These findings indicated that the selective vagotomy disrupted fat satiation, but not reinforcement. Other groups of rats were given celiac-superior mesenteric ganglionectomy alone (to remove nonvagal sensory afferents) or ganglionectomy plus vagal deafferentation. The combined treatment appeared to block fat conditioning (57% CS+ preference) but the results were inconclusive because the sham controls displayed a rather weak CS+ preference (66%) which did not improve with further training. Thus, vagal afferents do not appear to mediate fat-conditioned flavor preferences, but the role of nonvagal afferents is uncertain.

The only potential hormonal signal examined thus far in flavor preference learning is cholecystokinin (CCK). An early study by Mehiel and Bolles (1988) proposed that CCK released by nutrients in the gut may signal nutrient reinforcement to the brain. Some support for this idea was provided by our finding that rats learned a preference for a flavored solution paired with a low dose of exogenous CCK (Pérez and Sclafani 1991). However, in a subsequent study, we found that the CCK-A receptor antagonist devazepide did not prevent flavor preference conditioned by ID carbohydrate infusions (Pérez et al. 1998). Preliminary results also indicated that flavor conditioning by ID fat infusions is not blocked by devazepide treatment (C. Pérez, F. Lucas, and A. Sclafani, unpublished results).

In addition to CCK, there are several other intestinal hormones released by fat, including apolipoprotein A-IV, GLP-1, PYY, and enterostatin (Woods 2004). These hormones are all implicated in the satiating response to dietary fat. Their role, if any, in fat reinforcement is not known. The capsaicin and vagotomy findings reviewed above suggest that different physiological processes mediate fat satiation and reinforcement, but this requires further study.

12.3.3.3 Central Studies

The central neural structures involved in flavor-nutrient learning have been investigated in recent studies. Lesions of the lateral hypothalamus (LH) (Touzani and Sclafani 2001) reduced the magnitude of conditioned preferences for CS+ flavors

paired with IG carbohydrate (67%, compared to 84% in controls) and fat infusions (63%, compared to 95% in controls) relative to the water-paired CS− flavor. Choice tests between the two CS+ flavors revealed CS+ carbohydrate preferences in the lateral hypothalamic lesion (67%) and control (84%) groups. Thus, LH lesions attenuated but did not eliminate nutrient-based learning. Another study (Touzani and Sclafani 2002) showed that lesions of the *area postrema*, which is involved in flavor aversion learning, did not alter conditioned preferences for a CS+ flavor paired with 7.1% corn oil infusions (80% AP lesioned, 74% controls).

The amygdala is also implicated in flavor aversion learning; its combination of orosensory and viscerosensory inputs makes it a potentially important site for flavor learning as well. Lesions of the basolateral nucleus reduced but did not eliminate preferences for carbohydrate-paired flavors (78%, vs. 91% in controls) or fat-paired flavors (73%, vs. 89% in controls) (Touzani and Sclafani 2005). Other rats given large lesions that destroyed the central, corticomedial, and basolateral nuclei, failed to learn a carbohydrate-based preference (52%, compared to 95% in controls) or a fat-based preference (54%, compared to 93% in controls). Further work with carbohydrate infusions in animals with large amygdala lesions showed that they could acquire taste-nutrient preferences (bitter-sweet and salty-sweet CS solutions, 74%, vs. 86% in controls) but not flavor-nutrient (sweet-sour odors, saccharin Kool-Aid, nonsignificant 63%, vs. 87% in controls). This indicated that the preference learning deficit was specific to orosensory cues: the large lesions prevented learning involving olfactory cues, but had only minor effects on association of taste cues with postoral nutrient effects.

Some studies have tested the involvement of central circuits only for carbohydrate-conditioned flavor preferences. Lesions of insular cortex, which receives both gustatory and visceral input, did not prevent flavor conditioning with IG carbohydrate infusions (Touzani and Sclafani 2007). Parabrachial nucleus lesions blocked taste but not flavor preference conditioning with IG carbohydrate infusions, suggesting that its importance is primarily in the integration of gustatory cues with postoral information (Sclafani et al. 2001). Pharmacological blockade of dopamine D1 receptors in nucleus accumbens shell and core prevented the acquisition of preference for flavors paired with IG carbohydrate infusions (Touzani and Sclafani 2008). More work is needed to determine whether these and other areas play a role in fat reinforcement of flavor preferences.

12.4 HUMAN STUDIES

The orosensory response of humans to dietary fat has been studied extensively and is reviewed in Chapters 7 and 11. On the other hand, very few studies have been designed to investigate conditioned changes in food preferences based on the postoral actions of dietary fat in humans. Two early studies from Leann Birch's laboratory have provided evidence that children acquire preferences for the flavors of high-fat yogurt drinks (Johnson et al. 1991; Kern et al. 1993). The investigators trained children as young as 2–3 years of age to indicate their ranking for flavors, using simple cartoon faces (smiling, neutral, frowning) to establish preferred, neutral, and unpreferred flavors. Following an initial assessment, the children repeatedly consumed two flavors of yogurt drink on separate occasions, in a design parallel to our rodent studies. One flavor was high-fat, and a serving provided about twice the energy of the

alternate low-fat flavor; the textural characteristics of the two drinks were matched so that the target flavors were the salient cues. Posttraining evaluation of the high-fat flavor was enhanced but the ranking of the low-fat flavor did not change significantly. Children also ate less in test meals following the high-fat yogurt (Johnson et al. 1991); this effect was only apparent after training, suggesting that it was a learned response. When 4–5-year-old children were trained while hungry (overnight fasted), there was enhanced ranking of the high-fat flavor when the children were also hungry at test, but not when they had just eaten a meal (Kern et al. 1993). Retested 2 months later in the hungry state, the children still tended to show enhanced preference for the high-fat flavor. A recent study of college students investigated flavor conditioning using high-fat and low-fat cream cheese spreads (Capaldi and Privitera 2007). Both spreads contained a cue flavor (orange or banana extract) as well as a bitter taste (quinine). The bitter taste was added to reduce the possibility that a conditioned preference for the cue flavor was due to its association with the palatable flavor of the cream cheese spread. Subjects trained with the high-fat spread rated the pleasantness of the cue flavor higher than those trained with the low-fat spread, which the authors took as evidence for flavor-nutrient learning.

12.5 CONCLUSIONS

Studies of fat appetite in animals have demonstrated clearly that there are both oral and postoral sources of fat reward. Recent advances in knowledge of fat detection have begun to reveal the mechanisms for orosensory appetite for fats. Research findings obtained with KO mice missing taste signaling elements (present chapter) and with gustatory nerve transected animals (Chapters 4 and 5) demonstrate the involvement of the gustatory system in fat preference. The olfactory system is implicated in fat appetite by studies of animals with experimentally induced anosmia. The trigeminal system is also importantly involved in the detection of and preference for dietary fat, and new findings are noted in Chapter 3. The postoral influences on fat preference and acceptance are well documented by experiments involving IG fat infusions in rats and mice. However, the transduction of appetitive information about fat in the gut has been more elusive, and thus far little is known about how this information reaches the brain. Further work in animals should fill the gaps in knowledge and contribute to a better understanding of fat appetite.

Both animal and human studies suggest that the postoral consequences of dietary fat, by enhancing preferences for their associated flavors, could contribute to the appetite for high-fat foods. However, it should be noted that the human studies do not establish a clear role for fat *per se*, since energy density covaried with fat content. Studies comparing the effects of isocaloric high-fat and high-carbohydrate foods on flavor preferences are needed, to determine whether humans find these nutrients differentially rewarding in the absence of energy differences.

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13 Control of Fat Intake by Striatal Opioids*

Brian A. Baldo, Wayne E. Pratt, and Ann E. Kelley

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13.1 INTRODUCTION

It is well established that opioid systems in the brain exert an important modulatory influence on food intake. Even before the discovery of the endogenous opioid peptides or the cloning of their receptors, it was known that opiate drugs such as morphine produce marked hyperphagia (Martin et al., 1963). As this phenomenon was

* We would like to dedicate this chapter to the memory of Professor Ann E. Kelley, who died of metastatic colon cancer in August 2007. She was widely regarded as one of the foremost behavioral neuroscientists in the world. Among her many contributions was the discovery that striatal opioids strongly regulate the intake of palatable foods, particularly foods high in sugar and fat content. Professor Kelley was an inspirational scientist and mentor with a deep concern about the devastating societal impact of drug abuse and dysregulated eating, which she referred to as "disorders of appetitive motivation," and which she believed to share important common neural substrates. This chapter represents a review of several of the important themes underlying her work, with a particular emphasis on her contributions to understanding the control of food intake by endogenous opioid systems in the brain.

studied further, it became apparent that pharmacological manipulations of opioid systems exhibit some degree of specificity for altering the intake of highly palatable foods. Accordingly, blockade of opiate receptors with drugs such as naloxone or naltrexone diminish the intake and perceived "pleasantness" of preferred foods in humans (Yeomans and Gray, 2002). Animal studies have confirmed these results; for example, doses of opiate receptor antagonists given either systemically or directly into the brain markedly reduce intake of palatable foods and tastants such as sweetened lard, sucrose, and palatable saccharin and salt solutions, without affecting intake of standard laboratory chow (Kelley et al., 2002).

Based on these and other findings to be discussed below, an influential theory has been advanced that opioid systems within the central nervous system play an important role in modulating the orosensory reward and taste hedonics associated with the ingestion of preferred foods (Calcagnetti and Reid, 1983; Cooper and Kirkham, 1993; Gosnell and Levine, 1996; Pecina and Berridge, 2000; Kelley et al., 2002). The emphasis of the present chapter will be on three interrelated issues: (1) the anatomical localization of opioid-mediated effects on gustatory reward within subregions of the striatum, (2) the specific behavioral processes affected by intrastriatal opioid manipulations, particularly with regard to dissociations between weak effects on the appetitive/preparatory aspects of feeding vs. stronger, consistent effects on the reinforcing and hedonic aspects of consummatory behavior, and (3) emerging theories of striatal function that could account for the selective effects of striatal opioids on gustatory reward. Related to these themes, and germane to the subject of this book, we will discuss evidence that the fat content of a particular food is an important, albeit not obligatory, feature that predicts sensitivity to opioid-mediated changes in intake.

13.2 MODULATION OF FOOD INTAKE BY STRIATAL OPIOID PEPTIDES: SPECIFICITY FOR HIGHLY PREFERRED MACRONUTRIENTS AND TASTANTS

The striatal complex contains dense concentrations of opioid peptides and opiate receptors (Pickel et al., 1980; Jongen-Relo et al., 1993; Mansour et al., 1994; Akil et al., 1998; Van Ree et al., 2000). Striatal opioid peptides are synthesized in medium-spiny neurons, the major neuron type intrinsic to the striatum, and are presumably released from local intrastriatal axon collaterals (Pickel et al., 1980). Medium-spiny neurons synthesize both enkephalins and dynorphins, although these peptides are found within segregated cell populations with distinguishable efferent connectivity. Opioid peptides released locally in the striatum access mu-and delta-type receptors localized on the soma of medium-spiny neurons (Wang et al., 1996; Wang et al., 1999) or kappa receptors on axonal terminals that modulate glutamatergic and dopaminergic transmission (You et al., 1999; Meshul and McGinty, 2000).

The global organization of striatal circuitry involves parallel loops originating with glutamatergic projections from the cortex that innervate topographically distinct striatal sectors (Alexander and Crutcher, 1990; Groenewegen et al., 1990; Haber, 2003). These striatal areas then project to corresponding subregions of

the pallidal complex, which in turn innervate thalamic nuclei that send projections to frontal cortical regions involved in motor planning and executive control of behavioral and affective function. These somewhat segregated, yet interacting pathways form functional cortico-striato-pallido-thalamic loops (Koob and Swerdlow, 1988; Alexander et al., 1990; Haber, 2003). The loop through the ventral striatum, in particular the nucleus accumbens (Acb), is thought to play a particularly important role in translating central motivational states into goal-seeking behavior. A particularly influential model of Acb function was proposed by G. Mogenson, who postulated that this structure serves as "limbic–motor interface" between allocortical areas comprising the classic "limbic loop," such as the amygdala and hippocampus, and downstream basal ganglia motor effectors (Mogenson et al., 1980). In general agreement with this theory, it has been shown that the Acb plays a critical role in mediating the effects of "natural" rewards such as eating, drinking, sexual behavior, etc., as well as the effects of drugs of abuse (Wise and Hoffman, 1992; Koob, 1996; Robbins and Everitt, 1996; Kelley and Berridge, 2002). Hence, the Acb is thought to constitute a critical node in the forebrain system that enables behavior directed toward goal objects critical for the survival of the organism or the species.

Considering the high concentration of opioid peptides and receptors in the Acb and the established role of this structure in governing goal-directed behaviors, it is perhaps unsurprising that opioid transmission in the Acb exerts an important influence on feeding behavior. Among the first demonstrations of the critical role of striatal opioids in modulating food intake were studies by Mucha and Iversen and Majeed et al. showing that infusions of morphine into the Acb markedly increased chow intake (Majeed et al., 1986; Mucha and Iversen, 1986). Expanding on these results, Bakshi and Kelley carried out a detailed microinfusion mapping study that revealed an anatomical gradient in which more ventrally placed morphine infusions (including infusions in the Acb) produced larger increases in chow intake than injections placed dorsally (Bakshi and Kelley, 1993b). Similarly, a mapping study conducted with the mu-receptor-selective opioid peptide, D-Ala, nMe-Phe, Glyol-enkephalin (DAMGO) showed that large increases in fat intake were obtained with DAMGO infusions into the core and shell subregions of the Acb, and in a ventrolateral sector of the striatum, in contrast to infusions into the dorsal striatum which produced no effect (Zhang and Kelley, 2000). The orexigenic effects of intra-Acb opioid agonist infusions appear to be due primarily to mu receptor activation; thus, the effects of morphine are mimicked fully by DAMGO, only partially by the delta receptor-selective peptide, D-Pen-enkephalin (DPEN), and not at all by the kappa-selective agonist, U50-488 (Bakshi and Kelley, 1993a,b). Conversely, the mu receptor-selective antagonist B-FNA decreases feeding provoked by food deprivation or 2-deoxyglucose administration (Bodnar et al., 1995). Taken together, these findings indicate that opioid receptors in the Acb play an important role in modulating food intake, and that the mu receptor subtype may be particularly important for this process.

Further studies, conducted predominantly in male rats, revealed that intra-Acb opioid manipulations produce somewhat selective effects on the intake of palatable foods and tastants. For example, intra-Acb administration of the opioid

antagonists naloxone or naltrexone diminished sucrose drinking at doses that did not affect chow intake in food-deprived rats (Kelley et al., 1996); conversely, intra-Acb morphine markedly increased sucrose intake at doses that produced negligible effects on standard chow or sweetened chow (Evans and Vaccarino, 1990). These results are consistent with earlier findings that systemic treatment with opiate agonists increase (Calcagnetti and Reid, 1983; Czirr and Reid, 1986) and antagonists decrease (Apfelbaum and Mandenoff, 1981; Levine et al., 1982; Cooper et al., 1985; Giraudo et al., 1993) intake of palatable foodstuffs or tastants. Hence, it would appear that the Acb is one critical neural locus at which systemic opiate antagonists act to selectively modulate palatable feeding.

These results raise the question as to which specific component of food confers sensitivity to opioid-induced alterations of food intake. Is it the critical variable taste hedonics, macronutrient content, postingestive effects, energy density, or a combination of these interrelated variables? The results of several studies would suggest that the critical factor is an enhancement of the rewarding properties of preferred flavors or foods, even if the food/tastant lacks caloric content or postingestive effects. For example, systemic administration of naloxone reduces sucrose and saccharine intake, as well as sucrose sham feeding (Apfelbaum and Mandenoff, 1981; Cooper, 1983; Lynch and Libby, 1983; Kirkham and Cooper, 1988). Similar effects have been obtained with intra-Acb naloxone infusions. In an influential study, Zhang, Gosnell, and Kelley showed that when rats were given a concurrent choice between a carbohydrate-enriched test diet and a fat-enriched diet, intra-Acb DAMGO produced a selective increase in fat intake even in rats showing a baseline preference for the high-carbohydrate diet (Zhang et al., 1998). Nevertheless, DAMGO enhanced intake of both diets when they were presented separately. Notably, even when rats were tested under food-deprived conditions, intra-Acb DAMGO treatment preferentially increased fat intake relative to the high-carbohydrate diet (see Figure 13.1a). Similar Acb naltrexone infusions resulted in a preferential reduction of the intake of high-fat diet under deprivation conditions (Figure 13.1b). Intra-Acb DAMGO administration also enhanced the intake of palatable sucrose, saccharin, or salt solutions (Zhang and Kelley, 1997, 2002). These results have been interpreted as indicating a general enhancement of the rewarding properties of those foodstuffs that the animal finds intrinsically palatable, regardless of macronutrient content.

This idea was directly tested in a recent study in which palatability was varied but macronutrient content held constant. Woolly et al. tested the effects of intra-Acb DAMGO infusions on the intake of two different sweetened chow pellets made from a common meal substrate, but prepared with either banana or chocolate flavor (Woolley et al., 2006). Rats showed baseline preferences for the chocolate-flavored pellets. Intra-Acb infusions of DAMGO enhanced intake of either pellet when the pellets were presented independently. But when the pellets were presented together, intra-Acb DAMGO enhanced and intra-Acb naltrexone attenuated intake of the chocolate pellets selectively. This outcome supports the hypothesis that mu-opioid receptor stimulation in the Acb codes for a process related to flavor preference, regardless of macronutrient content.

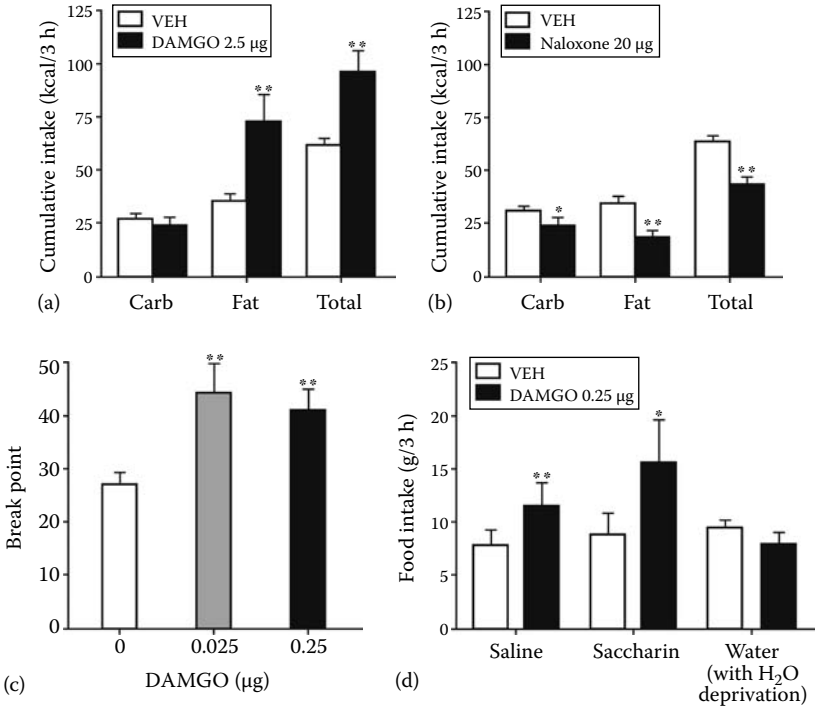

FIGURE 13.1 Stimulation of mu-opioid receptors with DAMGO favors the intake of highly palatable high-fat diets. Even animals that have been food-deprived for 24 h preferentially increase consumption of a high-fat diet under the influence of Acb mu receptor stimulation (a). Similarly, naloxone antagonism of opioid receptors of the Acb reduces the intake of fat diet under deprivation conditions (b). Mu receptor stimulation of the Acb increases breakpoint for earning sucrose pellets within a progressive ratio paradigm (c). DAMGO treatment also increases intake of palatable, but noncaloric, saccharin, and saline solutions, without affecting water intake, even in mildly water-deprived animals (d). Together, these data suggest that mu receptor activation of the Acb causes a general enhancement of the rewarding properties of foods that rats find intrinsically palatable. (Adapted from Kelley, A.E. et al., *Physiol Behav.*, 86, 773, 2005.)

13.3 EVIDENCE THAT INTRA-ACB OPIOID TRANSMISSION MODULATES THE REWARDING PROPERTIES OF FEEDING

The evidence reviewed above supports the hypothesis that opioid transmission in the Acb plays an important role in the "rewarding" properties (in a general sense) of foods that the animal finds intrinsically palatable. The question then arises as to the specific process or processes that are regulated by opioid transmission. Recent theories of motivation have proposed that the incentive properties of appetitive goals and goal-associated conditioned stimuli are governed by neural processes that are

distinguishable from those that control the positive affective states arising from commerce with the goal (Kelley et al., 2005b; Salamone et al., 2005; Baldo and Kelley, 2007; Berridge, 2007). The colloquial terms "wanting" and "liking" have been applied to the former and latter process, respectively (Berridge, 1996). As applied to feeding behavior, "wanting" would refer to food-seeking behaviors (lever pressing, maze running, or, under more naturalistic conditions, foraging and hoarding, etc.) and "liking" to the hedonic experience arising from feeding-related sensory inputs. Not unrelated to this theory are ethological frameworks emphasizing distinctions between the more flexible, adaptable behaviors during goal-seeking (variously termed "approach," "preparatory," or "appetitive" behaviors) and the more inflexible, stereotyped patterns associated with interactions with the goal (consummatory behaviors, in the sense of "consummation of the goal-seeking phase") (Craig, 1918). That appetitive and consummatory behaviors are phenomenologically distinguishable could imply distinct neural mechanisms of control.

There are several converging lines of evidence to suggest that the mechanism underlying the regulation of palatable feeding by Acb opioid systems involves an enhancement of the hedonic experience of consummatory behavior, which then influences a closely cooperative yet distinct system for acutely energizing goal-seeking behaviors directed at the foodstuff tasted. Evidence that these two systems are separable comes largely from comparing the effects of local intra-Acb pharmacological manipulations of the opioid and dopamine systems on behavioral tests assaying food intake, instrumental food-seeking behaviors, and taste reactivity—a procedure designed to evaluate stereotyped orofacial motor responses to palatable or aversive tastants.

Overall, the evidence has shown that enhancing dopaminergic transmission in the Acb markedly energizes instrumental food-seeking behaviors, while producing small and often inconsistent effects on actual intake (for reviews, see Kelley et al., 2005a,b; Salamone et al., 2005; Berridge, 2007). For example, intra-Acb administration of amphetamine, which enhances dopamine release, augments responding for sucrose pellets in the progressive ratio paradigm in which animals are required to emit increasingly larger numbers of responses to receive a reinforcer (Zhang et al., 2003). Intra-Acb amphetamine, in a similar dose range, also markedly increases lever pressing for a compound stimulus (activation of a light and the click of the food hopper) that was previously paired with food delivery in a Pavlovian conditioning procedure (Taylor and Robbins, 1984; Cador et al., 1991; Kelley and Delfs, 1991). Nevertheless, similar amphetamine treatments produce inconsistent effects on food intake, actually decreasing intake of standard chow in hungry rats in some studies (Evans and Vaccarino, 1986; Bakshi and Kelley, 1991). Conversely, blockade of Acb dopamine receptors has been shown to produce no effect or actually increase food intake, possibly by shifting behavioral repertoires toward less effortful activities and/or inhibiting the process of switching away from feeding (Bakshi and Kelley, 1991; Nowend et al., 2001; Baldo et al., 2002). Based on these considerations, it has been suggested that dopamine transmission in the Acb is closely connected to the process of assigning motivational significance to and energizing goal-seeking behaviors directed toward appetitive goals ("wanting") (Berridge and Robinson, 1998; Salamone and Correa, 2002; Baldo and Kelley, 2007).

The behavioral pattern associated with intra-Acb opioid receptor manipulations differs from dopamine-mediated effects in several important ways. First and foremost, unlike the inconsistent effects on food intake of intra-Acb amphetamine, infusion of opioid receptor agonists into the Acb reliably increases food intake (as discussed above), despite the fact that, like amphetamine, intra-Acb infusions of opioid agonists promote hyperactivity (Cunningham and Kelley, 1992b). With regard to operant responding for food reward, intra-Acb mu-opioid receptor stimulation augments progressive ratio responding for sugar pellets (Zhang et al., 2003) but does not influence lever pressing for a food-associated conditioned reinforcer (Cunningham and Kelley, 1992a,b; but see Phillips et al., 1994). These findings suggest that the influence of Acb opioid manipulations on food-seeking behavior depend upon the animal tasting food in the context of the behavioral testing session. In a similar vein, it has been found that intra-Acb opioid receptor stimulation augments palatable reactions to sucrose in the taste reactivity test (Pecina and Berridge, 2000, 2005), while intra-Acb amphetamine does not affect taste reactivity at doses that markedly enhance lever pressing for a Pavlovian conditioned stimulus for food delivery (Wyvell and Berridge, 2000). The augmentation of feeding mediated by Acb opioid transmission would therefore appear to result from an enhancement of the rewarding sensory experience of eating (liking), which then feeds into a closely cooperative, yet distinct, dopamine-dependent substrate for goal-directed action.

In further support of this hypothesis are the results of an important study comparing and contrasting the effects of systemic dopamine vs. opioid receptor antagonism on the intake of palatable food, goal-seeking behavior directed toward food in a runway paradigm, and conditioned hyperactivity associated with food anticipation. Barbano and Cador showed that blockade of dopamine receptors with *cis*-flupenthixol reduced anticipatory hyperactivity at doses that did not affect runway performance or food intake. In contrast, systemic treatment with naloxone reduced food intake, but only in *ad libitum*-fed animals given palatable food; food-deprived rats given standard chow were unaffected. Naloxone also slowed run time in rats traversing the runway to receive palatable food, and did not alter anticipatory hyperactivity. Hence, naloxone's effects were apparent in testing situations in which the animals' behavior was guided by interaction with palatable food, in agreement with the idea that opioid transmission selectively modulates the rewarding experience of tasting and eating preferred flavors and foodstuffs (Barbano and Cador, 2006).

13.4 FUNCTIONAL CHANGES IN STRIATAL EXPRESSION OF OPIOID PEPTIDE GENES: RELATIONSHIP TO FOOD MOTIVATION

As reviewed above, the activation of mu-opioid receptors throughout neural feeding networks promotes intake of palatable (fat–sweet) diets, most likely by enhancing the positive motivational properties of gustatory reward. The experiments described thus far have predominantly utilized systemic and intracranial infusions of opioid agents in order to assess their effects on feeding and food-directed behaviors. However, the activation of these receptors in humans and untreated animals is linked to the normal synthesis and release of opioid peptides, which is regulated by mRNA expression

within the opioid-releasing neurons. Of particular interest is the expression of preproenkephalin (PPE) mRNA, which represents the peptide precursor to the met- and leu-enkephalins, the endogenous ligands that preferentially bind the mu (and delta)-opioid receptor. Recently, we have begun to examine levels of striatal opioid gene expression as it relates to food-related central motivational states of the animal.

The first question that was asked was if a history of access to a palatable diet would affect striatal levels of PPE mRNA expression (Kelley et al., 2003). Rats were offered 3 h daily access to chocolate Ensure® over the course of 15 days, in addition to free access to rat chow in their home cage. A separate group of animals underwent the same procedure, but was only offered additional access to water. Ensure contains relatively high levels of fat and sugar (relative to rat chow), and is readily consumed by nonrestricted rats. Those animals that received access to the palatable Ensure diet steadily increased their intake over the days of exposure to the diet. On the 16th day, the rats were sacrificed, and *in situ* hybridization and northern blot analyses were performed on separate groups of animals to assess striatal PPE expression. Rats with a history of access to the Ensure diet had significantly reduced levels of striatal PPE than the animals that received an extra water bottle. Thus, chronic but restricted access to palatable diets induced plastic changes in striatal opioid mRNA expression. Intriguingly, the PPE reduction mirrored changes observed in animals exposed to chronic morphine or alcohol exposure (Uhl et al., 1988; Georges et al., 1999; Cowen and Lawrence, 2001), suggesting that palatable diets can impact neural circuits in a manner similar to drugs of abuse.

A second question of interest was whether or not striatal opioid mRNA expression would be affected by the current energy state of the animal (i.e., food restriction vs. *ad libitum* access to food) and/or the recent history of food intake. To address this issue, Will, Vanderheyden, and Kelley (Will et al., 2007), collected brain slices from *ad libitum*-fed or food-restricted rats 2 h following the onset of their dark cycle, during which rats normally take a meal. On the day of sacrifice, half of the animals from each group received food prior to lights-out, whereas the other half did not. Four sets of animals resulted: (1) animals with a history of free food access with a 2 h food restriction, (2) animals with a history of food restriction (with an additional 2 h food restriction prior to sacrifice), (3) animals with a history of *ad libitum* food that continued into the first two hours of their dark phase, and (4) animals with a history of food restriction that received 2 h of food access on the experimental day. *In situ* hybridization was used to evaluate the expression of PPE or preprodynorphin (PPD) within the striatum, as well as neuropeptide Y (NPY) within the arcuate nucleus of the hypothalamus. As would be expected based on the role of the arcuate nucleus in sensing peripheral energy stores (Williams et al., 2001), all animals that were chronically food deprived had increased hypothalamic NPY mRNA expression when compared to the rats that had not had a history of food deprivation. This pattern was not replicated with striatal PPE mRNA expression. Striatal PPE expression was reduced following access to food during the first 2 h of the dark cycle, independent from the initial food restriction condition (see Figure 13.2). PPD mRNA levels increased only in the Acb core, but varied as a result of deprivation condition, rather than recent history of food intake. Thus, while hypothalamic energy-sensing regions appear acutely attuned with the chronic state of deprivation of the rats, PPE

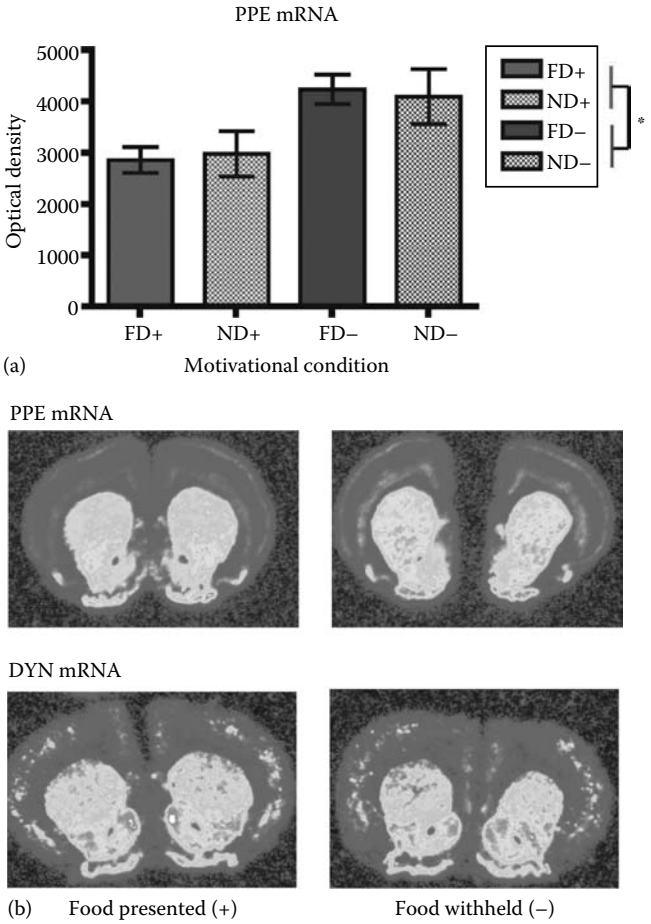

FIGURE 13.2 (See color insert following page 166.) Striatal enkephalin (PPE) gene expression fluctuates with specific aspects of food intake history. Different groups of rats were either food restricted (FD, solid bars) or maintained ad libitum (ND, stippled bars). On the evening of the test day, some rats were given food as usual (red shade), but for others the food was withheld (blue shade). An example is shown of the marked downregulation of PPE within the lateral accumbens shell that resulted from food access relative to deprivation (a). As can be observed, PPE mRNA levels appear to track acute satiety state rather than longer term energy deficit, as decreases in PPE expression were seen in both food-restricted and nonrestricted animals following food availability. Asterisk indicates significant difference in gene expression between groups. (b) PPE, but not PPD, downregulation was observed across the entire striatum following access to food. (Adapted from Kelley, A.E. et al., *J. Comp. Neurol*., 493, 72, 2005.)

expression within striatum appears to track short-term changes in satiety, regardless of deprivation condition.

In a separate paradigm, rats were fed *ad libitum*, but offered access to Ensure or water in two separate, distinct environments daily for 90 min each (Schiltz et al.,

2007). Following 15 days, all animals were food restricted for 12 h, and then placed into the environment initially paired with either Ensure or water exposure for 45 min (with no diet present), after which they were sacrificed. Levels of hnPENK were evaluated via *in situ* hybridization, to assess immediate expression of PPE resulting from the cue exposure. Rats that were exposed to cues predictive of the palatable diet showed increased striatal hnPENK expression relative to those exposed to the water-predictive cues, suggesting that PPE expression was elicited by the cues that predicted the palatable diet.

Together, the above two experiments suggest that PPE (but not necessarily PPD) mRNA levels rise in the striatum in response to cues that predict feeding, whether they be circadian cues (Will et al., 2007) or environmental cues predictive of palatable diet exposure (Schiltz et al., 2007). Furthermore, access to food during normal times of consumption (at the onset of the dark cycle for rats) results in decline in striatal PPE expression, which tracks more tightly with short-term satiety than it does with deprivation state. This pattern of data indicates that it may not be the deprivation state of the animal *per se* that promotes expression of PPE (the mRNA precursor to enkephalin), but rather a central motivational state elicited by the expectation of feeding (whether cued by circadian rhythms or the environment).

13.5 STRIATAL CHOLINERGIC INTERNEURONS: KEY REGULATORS OF FEEDING AND OPIOID EXPRESSION

It remains an open question as to how opioids within the striatum are modulated by its interactions within feeding circuitry. The enkephalin- and dynorphin-containing medium-spiny neurons of the striatum exist within a larger neural milieu, and integrate signals arising from the hypothalamus and ventral tegmentum, as well as from descending projections from limbic thalamic and cortical regions involved in memory and affect. It is known that the behavioral effects of locally applied mu-opioid agonists depend upon the integrity of other structures that communicate with the ventral striatum, such as the amygdala, ventral tegmentum, and regions of the hypothalamus (Glass et al., 2000; Will et al., 2003, 2004; Kim et al., 2004; Bodnar et al., 2005).

In addition to these established external influences on striatal opioid function, evidence is accumulating to support the idea that there may be an important role for striatal acetylcholine in the regulation of opioid expression within the striatum. In the Acb and caudate nucleus, acetylcholine is released predominantly from large local aspiny interneurons that extend their dendritic and axonal processes through up to a cubic millimeter of the neuropil (Zhou et al., 2002). Although these interneurons comprise only 1%–2% of the neurons within the striatum, they maintain a high level of cholinergic tone and serve to modulate striatal function via muscarinic and nicotinic receptors located on cell bodies and incoming axons from extrastriatal regions (Izzo and Bolam, 1988; Hersch et al., 1994). Opioid mRNA expression within the striatum has been shown to be modulated by action at the muscarinic receptor. Systemic administration of muscarinic agonists increases striatal PPE expression (Weisinger et al., 1992, 1998). In contrast, systemic antagonism of muscarinic receptors blocks the upregulation of PPE mRNA and increases the expression of PPD (Wang and

McGinty, 1996) that results from amphetamine administration or 6-OHDA lesions of striatal dopaminergic fibers (Nisenbaum et al., 1994; Wang and McGinty, 1996). Intrastriatal muscarinic receptor blockade (with scopolamine hydrobromide) similarly reduces the PPE mRNA expression following systemic amphetamine treatment (Wang and McGinty, 1997). Muscarinic acetylcholine receptors, therefore, appear to have an important modulatory role in the expression of striatal opioid peptides.

Might striatal acetylcholine then play an important role in feeding and appetitive behaviors? Substantial evidence suggests that this is the case. In fact, two decades of research from the laboratory of Hoebel and colleagues have implicated striatal acetylcholine in mechanisms underlying food satiation and in bingeing models of palatable food intake (Mark et al., 1992, 1995; Avena et al., 2006). Similarly, lesions of ventral striatum acetylcholine-containing interneurons have been shown to impact feeding behavior (Hajnal et al., 2000) and block learning in a number of paradigms that use food as the reinforcer (Kitabatake et al., 2003). Furthermore, activity of striatal tonically active neurons (putatively, the cholinergic striatal neurons) in monkeys have shown that the neurons respond to both food rewards and cues that reliably predict them (Apicella et al., 1997, 1998; Sardo et al., 2000; Matsumoto et al., 2001). We therefore became interested in applying a pharmacological approach to addressing the role of striatal acetylcholine in appetitive behavior.

Our initial experiments targeted the role of Acb acetylcholine receptors in an appetitive learning task. Hungry rats were placed in an instrumental chamber for daily 15 min sessions during which they had the opportunity to learn to press for sucrose pellet reward (Pratt and Kelley, 2004). Intra-Acb injections (into either the core or shell) of the muscarinic receptor antagonist scopolamine methyl bromide significantly impaired the learning of the lever press task during the first 5 days of the experiment. Notably, daily infusions of the nicotinic receptor antagonist mecamylamine (in separate groups of animals) did not impede rats' learning to lever press. The effects of muscarinic receptor blockade, however, were not limited to the learning phase of the experiment. Following the first 5 days of treatment, animals continued to be trained until they had reached maximal performance on the task. Following this training, an additional drug treatment was given. Rats that received the highest dose of scopolamine into the Acb core or shell (10 μg/side) significantly reduced their lever-pressing behavior. Utilizing a progressive ratio design in a separate group of animals, rats were able to perform the behaviors in the operant chamber at the same speed following scopolamine infusion, but demonstrated a reduction in the amount of effort that they would produce to earn a reinforcer (as shown by reduced breakpoint). This suggests that it was the motivation for the reinforcer, not motor impairment, which was responsible for the decreased lever pressing following maximal learning.

It was also discovered, serendipitously, that the rats that had undergone the scopolamine treatments within the Acb lost weight relative to vehicle-injected animals during the initial five treatments within the learning paradigm. The animals that had received the nicotinic receptor antagonist mecamylamine showed no such effect. Thus, in addition to the short-term learning and motivational deficits produced by muscarinic receptor antagonism of the ventral striatum, scopolamine treatment also appeared to induce a medium- to long-term effect on food intake.

This latter finding has been followed up in two ways. First, it was necessary to verify the reduction in feeding that resulted from striatal muscarinic blockade on animals that were not in a food restriction paradigm. Separate groups of rats were individually housed in order to assess the effect of scopolamine methyl bromide infusions into either the dorsal or ventral striatum on the overall amount of *ad libitum* food intake over a 24 h period (Pratt and Kelley, 2005). Following bilateral infusions of 5 or 10 µg scopolamine into the Acb or anterior dorsal striatum, rats reduced their subsequent daily intake of chow by as much as 50% from their intake in the days prior to the treatment (see Figure 13.3). Rats resumed normal intake of rat chow in the second 24 h period following treatment. Locomotor effects of the drug were also more specifically addressed; the motivational impact of muscarinic receptor blockade far outlasted the brief (approximately 30 min) increase in locomotion that occurred acutely following the drug infusion.

Second, as a means to assess which intrastriatal neurotransmitter systems may contribute to the modulation of mu-opioid-induced feeding on palatable food, we examined the effects of inhibitory pretreatment of several receptor antagonists upon the hyperphagic effects of intra-Acb treatment with the mu-opioid agonist DAMGO (Will et al., 2006). Toward this end, rats received various intra-Acb pretreatments prior to infusions of 0.25 µg/side of DAMGO. They were then allowed 2 h access to a high-fat diet. The individual pretreatments (tested on separate groups of animals) consisted of antagonists for opioid receptors (naltrexone), glutamate AMPA receptors (LY293558), dopamine D1, or D2 receptors (with SCH23390 or raclopride, respectively) or cholinergic, muscarinic, or nicotinic receptors (with scopolamine or mecamylamine, respectively). As had been shown previously (see above), DAMGO stimulation of Acb core significantly increased feeding on the fat diet. Blocking opioid receptors with naltrexone had the expected effect of eliminating DAMGO's enhancement of fat consumption. Inhibition of AMPA receptors (at doses that did not stimulate feeding), dopaminergic D1 or D2 receptors, or acetylcholine nicotinic receptors had no effect on either baseline intake or DAMGO-elicited feeding. However, blockade of acetylcholine muscarinic receptors with scopolamine reduced fat intake following treatments of DAMGO or when given alone. Stimulating Ach receptors directly with an Ach/physostigmine cocktail had no direct effect on fat diet intake. Thus, amongst the intrastriatal neurotransmitter systems tested thus far in concert with DAMGO treatment of the Acb, only muscarinic receptor antagonism appears to reduce the impact of opioid receptor stimulation on palatable food intake.

The above experiments suggest that muscarinic receptors within the ventral striatum modulate the intake of both chow and palatable diets in the laboratory rat. Additionally, even though muscarinic receptor blockade reduced both normal and DAMGO-induced fat intake, the discovery that antagonism of cholinergic signaling, but neither AMPA nor dopamine receptors, reduced palatable feeding suggests a functional link between acetylcholine and opioid systems within the striatum. As noted above, there is evidence suggesting a role for acetylcholine in modulating enkephalin gene expression within the striatum in response to systemic cholinergic activation or amphetamine-induced opioid mRNA expression. The functional relationship between striatal acetylcholine and opioids appears bidirectional, as mu-opioid

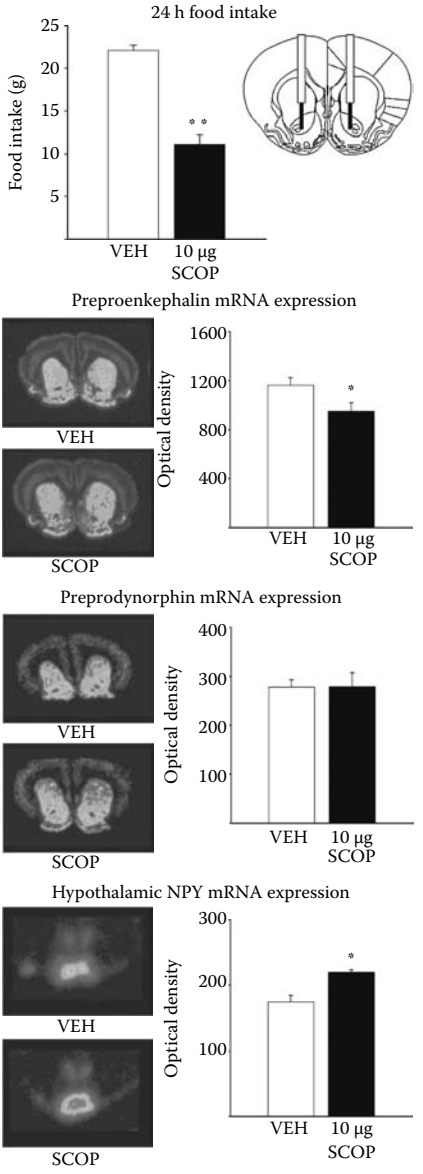

FIGURE 13.3 **(See color insert following page 166.)** Cholinergic muscarinic receptors play a major role in food intake and enkephalin gene expression. A single infusion of scopolamine into the Acb reduces food intake by almost half over the following 24 h period. This behavioral effect is associated with a significant downregulation of striatal PPE mRNA, but does not influence levels of PPD mRNA. Note the quantitatively similar reduction in PPE expression compared to that following food access in Figure 13.2. Hypothalamic NPY is increased by scopolamine treatment, reflective of negative energy balance. Asterisks indicate significant difference between groups or significant change in gene expression. (Adapted from Kelley, A.E. et al., *J. Comp. Neurol*., 493, 72, 2005.)

receptors have been shown to modulate the output of acetylcholine within striatum, and this relationship may be dependent upon circadian influences (Jabourian et al., 2004, 2005). Based on our findings that muscarinic receptor blockade reduced feeding on both chow and fat diets, we therefore wondered whether the 24 h decrease in food intake that we observed following muscarinic receptor antagonism of the Acb or anterior dorsal striatum might be mediated by a long-lasting decrease in striatal opioid function. To address this question, we examined the expression of PPE and PPD mRNA in the same animals that demonstrated reduced food intake 24 h following striatal muscarinic antagonism with scopolamine methyl bromide (Pratt and Kelley, 2005). The results were quite intriguing. Relative to vehicle-injected rats, animals with scopolamine infusions showed downregulation of striatal PPE mRNA that was apparent 24 h following the drug infusion (see Figure 13.3). Striatal PPD mRNA levels were either not altered (following Acb infusions) or increased near the injection site (in the case of anterior dorsal injections). This pattern of PPE expression is strikingly similar to the suppression seen following a meal in either deprived or nondeprived rats (Will et al., 2007). Opioid enkephalins may therefore mediate the perception of palatability as a function of recent feeding (Will et al., 2007) or following muscarinic receptor blockade of the striatum (as in Pratt and Kelley, 2005).

13.6 A POSSIBLE HYPOTHALAMIC–THALAMIC–STRIATAL LINK

Clearly, the regulation of food intake by striatal circuitry is complex. We have predominantly reviewed here the function of striatal opioids upon the promotion of palatable food intake. Activation of mu-opioid receptors within the striatum, as well as in other brain regions, promotes the intake of high-fat diets. Furthermore, expression of PPE is modulated by exposure to fat-rich diets, and PPE expression appears to vary as a result of recent access to food, rather than long-term energy restriction. This regulation may be modulated by the activation of muscarinic receptors (among other possible mechanisms), which serve to promote the expression of striatal PPE.

What then, could be the role of intrastriatal acetylcholine in the modulation of opioid expression and feeding behavior? Recently, we have proposed a central role for striatal cholinergic interneurons (as they relate to feeding behaviors) based upon our own research and a review of the functional connectivity of striatal acetylcholine-containing neurons. Central to the model is an examination of the anatomical position that these neurons have in relation to other feeding circuitry. Extensive anatomical examination from the laboratories of Paul Bolam and Gloria Meredith suggest that the predominant external inputs to the cholinergic interneurons of the striatum arise from the intralaminar thalamic nuclei (Dube et al., 1988; Meredith and Wouterlood, 1990; Lapper and Bolam, 1992; Zhou et al., 2002), although there is also evidence that cortical stimulation results in postsynaptic currents in these neurons (Reynolds and Wickens, 2004). These thalamic–striatal connections appear to be physiologically relevant. In the monkey, striatal tonically active (cholinergic) neurons respond with a characteristic decrease to juice rewards or stimuli which have come to predict them (Apicella et al., 1997; Apicella et al., 1998; Sardo et al., 2000). This decrease is significantly attenuated following the cooling of the parafasicular nucleus of the thalamus (Matsumoto et al., 2001). Additionally, lesions of this region in rats reduce

striatal acetylcholine output in response to dopamine receptor agonists (Consolo et al., 1996). The thalamic regions that project to cholinergic interneurons are heavily innervated by lateral hypothalamic regions, including neurons that release the feeding- and arousal-related peptide orexin/hypocretin (Peyron et al., 1998; Baldo et al., 2003). Thus, striatal cholinergic interneurons may receive hypothalamic energy state signals via a hypothalamic–thalamic–striatal axis.

We propose that one function of acetylcholine within striatum may be to modulate opioid expression based upon signals received via the midline thalamus, which is well positioned to provide the striatum with input based upon hunger and circadian signals arising from hypothalamic input (Kelley et al., 2005a,b). Tonic changes in acetylcholine availability may then modulate enkephalin production and availability, affecting food palatability and subsequent intake. Indirect support for this hypothesis can be found in the literature. Stimulating the lateral hypothalamus with orexin A agonists results in feeding in rats; this effect can be reversed with intra-Acb infusions of naltrexone (Sweet et al., 2004). It has been generally shown (see above) that muscarinic receptor stimulation increases striatal PPE mRNA expression while muscarinic antagonism decreases it. It is therefore possible that striatal blockade of muscarinic receptors may affect medium- to long-term food intake by directly or indirectly reducing enkephalin availability within the striatum. Such a downregulation may result in a general reduction of the hedonic aspects of all food, thus inhibiting food intake. The hedonic state change elicited by muscarinic receptor antagonism does not impact water intake in rats that are water restricted (Perry et al., 2008), although it can be associated with flavor cues and (to a lesser extent) spatial cues (Pratt et al., 2007). In the normal, untreated animal, energy state signaling from the hypothalamic–thalamic–striatal axis may result in an increase of enkephalin availability during times when feeding would normally occur (at the onset of darkness in rats, or in the presence of food-predictive cues, for example). Striatal PPE upregulation may serve to stimulate feeding (particularly on palatable substances) beyond that which would otherwise be required to maintain energy homeostasis. Within an evolutionary context, an opioid-driven motivation to overeat when presented with a highly palatable and caloric food source at a time when feeding is appropriate or expected would serve to increase fat stores and assist in promoting the survival of the individual in the event of future famine.

13.7 SUMMARY, CONCLUSIONS, AND FUTURE DIRECTIONS

We have reviewed evidence that the striatal opioid system represents a crucial component of the forebrain network that modulates feeding behavior. Although pharmacological stimulation of striatal mu-opioid receptors can affect the intake of all types of food when those foods are given in isolation, especially when higher doses of drugs are used, it would appear from the evidence discussed above that there is a particularly strong and selective impact on those foods that the organism perceives as inherently palatable, seemingly regardless of whether palatability arises from sugar content, the "mouthfeel" of fat, or the "tastiness" of sapid saline solutions.

As such, it would appear that the selectivity of intra-Acb opioid manipulations on palatable feeding does not derive from a certain type of gustatory input. Instead, it

could be hypothesized that the computation of whether a food or flavor is acceptable, regardless of its macronutrient composition or particular sensory characteristics, is antecedent to regulation by the striatal opioid system, but that striatal opioid release may come into play during commerce with the palatable food to establish a positive affective ("hedonic") state. This hypothesis is strongly supported by the human studies that have shown unaltered sensory perceptions of flavor but diminished "pleasantness" of those flavors after opiate receptor antagonism (for review, see Yeomans and Gray, 2002), and from animal studies that have shown augmented appetitive taste reactions to sucrose solutions after opiate receptor stimulation in the Acb (Pecina and Berridge, 2000, 2005). Thus, it is proposed that the hedonic state associated with Acb opioid transmission informs and directs subsequent behavioral output, whether it be eating, ambulation toward the food source, or invigorated responding in a progressive ratio operant paradigm. Nevertheless, doses of intra-Acb mu-opioid receptor agonists that promote palatable feeding or enhance appetitive taste reactions to sweet flavors do not augment lever pressing for conditioned stimuli predicting food delivery, nor do they enable the acquisition of a lever-press response for food delivery in *ad libitum*-fed rats (Hanlon et al., 2004). It would therefore seem that mu-opioid receptor stimulation in itself codes for the hedonic experience of palatable feeding, and that this feeds into a closely cooperative yet distinct system that energizes goal-seeking behaviors directed at food-predictive cues. It has been suggested that Acb regulation of goal-seeking motor behaviors is strongly dependent upon dopamine function, and that the coordinated activity of Acb opioid and dopamine systems is required for the full expression of behavioral sequences associated with palatable feeding. Uncovering the mechanisms underlying the interplay between these two neuropharmacological systems represents an important challenge for future studies.

Another crucial player in the striatal control of feeding is the cholinergic system. As we have reviewed, cholinergic tone exerts strong regulation over both opioid-mediated feeding behavior and the expression of opioid peptide genes. An important area of future research is the manner in which striatal cholinergic systems receive and process information relevant to food motivation and energy balance. One possible route of control, as outlined above, involves a pathway originating in the arcuate nucleus of the hypothalamus, which communicates with striatally projecting thalamic neurons via an orexin/hypocretin-coded relay in the lateral hypothalamus. As noted, the cholinergic interneurons of the striatum receive abundant synaptic connections of thalamic origin. Important areas of future work include testing the functional relevance of these anatomically described connections, and exploring whether the network of cholinergic processes throughout the striatal complex, by regulating overall opioid tone, could play a role in coordinating the activities of the opioid and dopamine systems. Such investigations could yield crucial insights about the neural control of nonhomeostatic intake of energy-dense, palatable foods.

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14 Fat-Rich Food Palatability and Appetite Regulation

Charlotte Erlanson-Albertsson

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14.1 INTRODUCTION

Two views are being debated around fat-rich food and appetite regulation. One is that fat intake has a weak satiety-signaling property, with the consequence being a passive overconsumption of fat-rich food, in turn leading to obesity (Westerterp, 2006). The other view is that fat intake is tightly regulated through specific signals, which when overstimulated leads to aversion (Jebb et al., 2006). Fat intake depends not only on the quantity but more importantly on the quality of fat ingested, whether it is saturated, monounsaturated, or polyunsaturated fat (Casas-Agustench et al., 2008). Another important feature relates to whether the fat is eaten with sucrose or with something that has a sweet taste (Erlanson-Albertsson, 2005a). In general this will lead to a blunted response. Endocannabinoids released after palatable food ingestion, such as food containing fat and sucrose, will promote hunger and energy storage. The following hormones have been found to regulate the appetite for fat. Galanin (Gaysinskaya et al., 2007), agouti-related peptide (AgRP) (Tracy et al., 2007), and ghrelin (Shimbara et al., 2004) stimulate fat intake, while enterostatin (Berger et al., 2004), apolipoprotein A-IV (Apo A-IV) (Tso and Liu, 2004a), peptide YY (PYY) (Boey et al., 2008), cholecystokinin (CCK) (Beglinger and Degen, 2004), and neuropeptide Y (NPY) (Primeaux et al., 2005) inhibit fat intake. Both galanin (Schneider et al., 2007) and ghrelin (Jerlhag et al., 2007) also stimulate the intake of ethanol, via pathways involving a link to the reward system. The inhibition of fat intake occurs through reduced gastric emptying and serotonin release (Ritter, 2004). A proper satiety for fat is possible only with complete fat digestion, fatty acids being important to release satiety hormones (Feinle-Bisset et al., 2005). For proper control of fat intake, fat digestion needs to be retarded without being inhibited (Albertsson et al., 2007).

Why we overeat fat?
- Energy dense
- Gastrointestinal processing too rapid
- Satiety signals too weak
- Hunger signals too strong

FIGURE 14.1 Reasons associated with overconsumption of fat: When satiety signals are weak the consumption of calorie-dense foods such as fat is promoted. Fat digestion is known to be rapid which leads to an empty intestine triggering hunger signals.

14.2 DIETARY FAT IS LINKED WITH HYPERPHAGIA

Several studies indicate that fat promotes overeating. The reasons for overconsumption are the high energy density coupled with the strong positive palatability of high-fat foods (Blundell and MacDiarmid, 1997) (Figure 14.1). However, there seems to be at least two types of responses to high-fat diets. Some do become obese, whereas others with a high-fat intake stay lean. These two types have been analyzed (Blundell et al., 2005). The overeaters are characterized by a strong liking or preference for fat and a strong attraction to palatable food, the palatability hence overriding any satiety signal being released from fat (Erlanson-Albertsson, 2005b). Such a scenario suggests a weak satiety for fat. It was also demonstrated that the high-fat eaters had higher levels of a diet-induced thermogenesis as well as higher leptin levels suggesting that some individuals stay lean with high-fat due to a specific genotype (Blundell and Cooling, 1999).

14.3 IMPORTANCE OF THE TYPE OF FAT

The most important issue regarding fat intake is the quality of the fat product consumed, whether it is saturated, monounsaturated, or polyunsaturated omega-3 fat or omega-6 fat. The saturated fats and trans fats are considered "bad" while the others are known as "good" fat. One reason is that saturated fat promotes fat accumulation in the body. Thus, a diet containing saturated fat in the form of tallow and corn oil was found to be more obesogenic than polyunsaturated fat diets (Jang et al., 2003). The unsaturated fatty acids promote satiety which will help the body to establish energy balance. In one study, Lawton et al. (2000) found that polyunsaturated fat exerted a relatively stronger satiety than monounsaturated fat and saturated fat. They studied the postingestive satiety for a fixed carbon length of the fat (C18). One meal consisted mainly of oleic acid (monounsaturated fat, C18:1 w9), another of linolenic acid (polyunsaturated fat, C18:2 w6), and the third of stearic acid (saturated fat, C18:0). Subjects gained significantly more energy after consumption of the lunch containing saturated fat than after the lunches containing either mono or polyunsaturated fat; additionally, there was a trend that these effects continued in the following days (Lawton et al., 2000).

14.4 DIFFERENTIAL EXPRESSION OF APPETITE REGULATING PEPTIDES BY DIETARY FAT TYPES

One explanation for the different satiety responses with saturated and unsaturated fat may be linked to a differential expression of appetite regulating peptides in the

brain. In one study (Dziedzic et al., 2007), it was found that rats fed with saturated fat became more obese compared with rats given either omega-3 fat or omega-6 fat, both of the polyunsaturated type. The rats receiving unsaturated fat had elevated levels of the satiety hormone pro-opiomelanocortin (POMC), whereas it was unchanged in the animals receiving saturated fat (Dziedzic et al., 2007). Such a difference in expression could explain the hyperphagia observed with the saturated fat. Furthermore the omega-3 fat caused a specific decrease in the expression of the hunger hormone melanin concentrating hormone (MCH), whereas there was no change in the other diet regimens. This suggests that there is a difference in appetite-regulating signals between the omega-3 fat and the omega-6 fat, omega-3 fat being more satiating (Dziedzic et al., 2007).

14.5 SECRETION OF INTESTINAL HORMONES

The secretion of satiety hormones in the intestine relating to the type of fat ingested may also be of importance. French et al. (2000) found that the satiety hormone CCK was released by infusion of fat into the intestine and that the degree of CCK released was related to the type of fat being infused. Intralipid, which is an emulsion of soybean oil (containing predominantly C18:2 fatty acids) with egg phospholipids, released the largest amount of CCK, whereas oleic acid, stearic acid, and linoleic acid gave a similar CCK response. All responses were significantly greater than the control. However, there was no direct relationship between CCK release and satiety, suggesting that other fat-specific peptides such as Apo A-IV or enterostatin might be involved. More importantly, Intralipid was the most satiating lipid, followed by linoleic acid, whereas stearic and oleic acid had no significant effect on satiety or food intake. It is thus clear that the satiety potency of fat is related to the small intestine area where receptors were exposed to fat. It is also clear that fatty acids, as opposed to triglycerides, are the critical stimuli for satiety response.

14.6 TRANS-FATTY ACIDS AFFECT FEEDING

Trans-fatty acids in the diet have been examined in pregnant rats as well as in their offspring (Albuquerque et al., 2006). It was found that during pregnancy, rats receiving trans fat had lower levels of eicosapentanoic acid as well as insulin receptor and insulin receptor substrate 1. Among the offspring, it was seen that control animals responded to insulin given to induce hypophagia but not the offspring of trans fat-fed mothers, even though they were now given a control diet. The data suggest that early exposure to hydrogenated fat rich in trans-fatty acids programs the hypothalamic feeding centre in such a way that insulin-induced satiety mechanisms are lost. Furthermore, it suggests that the type of fat eaten is relevant not only to the current satiety state but will also affect fetal satiety programming during pregnancy (Albuquerque et al., 2006).

The effect of different types of fat on single meal experiments could not be observed in a study involving overweight young men (Flint et al., 2003). After an overnight fast, these men were served a breakfast, with 60% of its energy coming from fat that varied only in the source of C18 fatty acids. Energy expenditure,

subjective satiety, and hunger were continuously measured for 5 h after feeding. There was no statistically significant effect using monounsaturated, trans fat or polyunsaturated fat on energy expenditure or on appetite ratings. It may be that overweight subjects have a blunted response to dietary fat, regarding both satiety and energy expenditure. Another explanation may be that the feeding protocol needs to be of a longer duration.

14.7 ENDOCANNABINOIDS PROMOTE OBESITY AFTER HIGH-FAT FEEDING

Another reason that high-fat diet promotes obesity in some individuals but not in others could be the role of endocannabinoids (Osei-Hyiaman et al., 2005). Endocannabinoids are released by high-fat feeding; their subsequent interaction with the hepatic endocannabinoid receptor CB1 increases the gene expression of the lipogenic transcription factor sterol regulatory element binding protein isoform 1c (SREBP-1c), which in turn activates lipogenic enzymes like acetyl-CoA-carboxylase-1 as well as fatty acid synthase (FAS). This could explain why high-fat feeding induces obesity. Such a hypothesis is supported by the failure of CB1-knockout mice to develop obesity following high-fat feeding as well as the reduced body weight gain observed after blockage of the CB1-receptor (Osei-Hyiaman et al., 2005). The endocannabinoids not only have a peripheral action in the liver, but also act centrally in the hypothalamus where FAS present in hypothalamic neurons was found to be activated by endocannabinoids. FAS is an important regulator of appetite as shown by studies using C75, an inhibitor of this enzyme which was found to curb the appetite (Loftus et al., 2000). Furthermore, C75 inhibited the expression of the hunger signal NPY in the hypothalamus, at the same time targeting malonyl-coenzyme A. Hence, fatty acid synthesis appears to be tightly linked to overeating and obesity, since inhibition of fatty acid synthesis in the hypothalamus inhibits feeding and promotes weight loss.

14.8 ENDOCANNABINOIDS ARE ELEVATED IN OBESE SUBJECTS

Since endocannabinoids play an important role in the promotion of obesity in animal models, is there reason to believe that they will have the same role in humans? In a study where normal weight subjects were compared to obese subjects, it was found that the obese subjects had elevated levels of the endocannabinoids *anandamide* and 2-*arachidonoylglycerol,* which were elevated by 35% and 52%, respectively compared to lean subjects (Engeli et al., 2005). Besides the brain, the CB1 receptor is highly expressed in the stomach as well as in the adipose tissue. In obese subjects, CB1 receptor gene expression is decreased, suggesting that the body is trying to reestablish energy balance through a negative feedback loop. Since there was no change in endocannabinoid levels or in receptor expression following a 5% weight loss in the obese subjects, it was suggested that the endocannabinoids and their receptor system might be a cause of obesity rather than a consequence (Engeli et al., 2005).

There is thus a strong coupling between highly palatable food, such as high-fat food and the promotion of obesity.

14.9 PALATABILITY-INDUCED FAT FEEDING

Opioids are part of a group of hormones or neurotransmitters promoting high-fat feeding (Welch et al., 1996). They act both in experimental animal models as well as in humans. There is a clear interaction between the host and its diet. Osborne–Mendel rats are known to become obese on high-fat diet, whereas S5B/P1 rats prefer a low-fat diet and are resistant to high-fat diet-induced obesity (Ookuma et al., 1998). In experiments in which Osborne–Mendel rats had a two-choice paradigm between high-fat diet and low-fat diet, it was found that high-fat intake was suppressed by norbinaltorhimine, a kappa-opioid-antagonist, whereas low-fat intake was not affected (Ookuma et al., 1998). This suggests that opioids and opioid receptors are involved in the regulation of fat intake. It was also found that S5B/P1 rats were unaffected by norbinaltorphimine which had no effect on low- or high-fat food intake (Ookuma et al., 1998). Additionally, this study showed that an infusion of U50488, a selective kappa-agonist, into the third cerebroventricle in sated rats, potently stimulated the intake of a high-fat diet in Osborne–Mendel rats but not in S5B/P1-rats. It was concluded that the enhanced preference and consumption of the high-fat diet by the Osborne–Mendel rats is due to an activated or sensitive opioid receptor system specific for kappa-opioids (Ookuma et al., 1998).

Subsequently, Zhang and Kelley showed that high-fat food intake is stimulated not only by kappa-opioids but also by mu-opioid receptor agonists like the enkephalin analogue DAMGO [D-Ala(2), NMe-Phe(4), Gly-ol(5)]-enkephalin acting on the nucleus accumbens (Zhang and Kelley, 2000). A mapping study based on c-fos activation was performed to identify brain regions that were important for the appetite promoting effect of palatable food. In addition to the nucleus accumbens, the lateral and ventral regions of the striatum were found to be activated (Zhang and Kelley, 2000).

Similar results were obtained by Barnes et al. (2003), who fed male Wistar rats either a high-fat diet or a low-fat diet for 12 weeks. At the end of the feeding regimen, the high-fat fed animals were found to have increased body fat, plasma leptin, and plasma insulin levels. Immunohistochemistry and in situ hybridization studies of their brains demonstrated that the high-fat fed rats had increased hypothalamic levels of mu-opioid receptors compared to controls, hence supporting the importance of the mu-opioid system in mediating high-fat diet-induced overeating and body weight gain. The increased mu-opioid receptor expression could also contribute to the higher mean arterial pressure and renal sympathetic nerve activity observed in these animals (Barnes et al., 2003).

14.10 CONTROL OF ENERGY EXPENDITURE THROUGH THERMOGENESIS

Following high-fat intake, diet-induced thermogenesis including the increased expression of uncoupling proteins acts as a defense mechanism against weight gain (Erlanson-Albertsson, 2002). The increased heat produced by uncoupling proteins not only helps increase energy expenditure but also promotes satiety, thereby establishing energy balance. Diet regimens with pure fat, the so-called ketogenic diets, have been introduced and demonstrated to cause rapid weight loss. One reason for

this is the difficulty these patients demonstrate to overeat pure fat without any carbohydrates. The long-term effects of high-fat diets are similar to the low-fat diets in regard to weight loss and metabolic parameters. Sucrose in a mixture with fat is reported to weaken the satiety signals for fat.

14.11 PEPTIDES INVOLVED IN THE REGULATION OF FAT INTAKE

Several peptides are specifically active in the regulation of fat intake. Galanin, ghrelin, and AgRP have been demonstrated to stimulate fat intake (Figure 14.2), whereas enterostatin, Apo A-IV, CCK, PYY, and NPY have been shown to inhibit fat intake (Figure 14.3). Below is a description of the peptides and their properties specific to the regulation of fat intake.

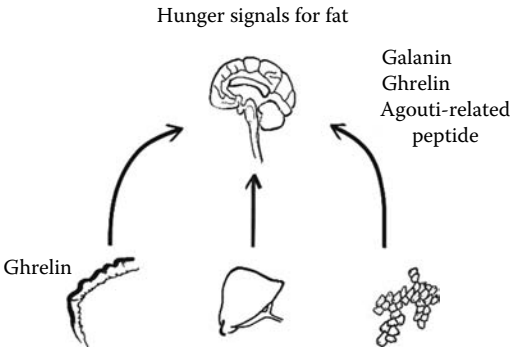

FIGURE 14.2 Hunger signals for fat. Ghrelin is the only signal produced in the periphery; AgRP and Galanin are produced in the brain, where the hunger mechanism is acting. It may be that the centrally produced ghrelin stimulates fat intake. Both ghrelin and galanin have been shown to also stimulate ethanol intake, suggesting a strong reward component.

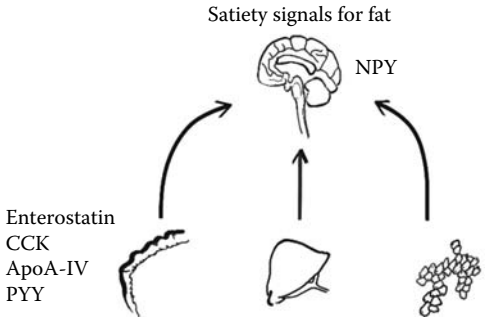

FIGURE 14.3 The satiety signals for fat are mostly situated in the periphery, except for NPY, which is produced in the hypothalamus. CCK and enterostatin are early satiety signals, whereas PYY is a late satiety signal released upon arrival of fat in the ileum and responsible for the "ileal break." Apo A-IV is released during the formation of chylomicrons in the intestine and promotes satiety for fat. The NPY inhibition of fat intake is thought to occur through its anxiety relieving effect.

14.11.1 Galanin

Galanin is a 30 amino acid peptide that has been found to regulate ingestive behavior. Galanin has been described to stimulate fat intake after central administration into the paraventricular nucleus (PVN) of the hypothalamus in rats (Leibowitz, 1994). High-fat diets cause a rapid upregulation of galanin expression in the PVN, suggesting a feed-forward process. It has also been found that the injection of Intralipid into the peritoneum, which causes an increased level of triglycerides, also leads to increased expression of galanin in the PVN (Wortley et al., 2003). This suggests that the postprandial fat by-products induce continued dietary fat intake. The food intake promoting effect of galanin was blocked by the nonselective galanin receptor antagonist M40 (Bartfai et al., 1993).

However, the capacity of galanin to stimulate fat intake has been questioned with arguments emphasizing the possibility that its specificity may be dependent on the endogenous preference of the animal species (Smith et al., 1997).

Central galanin also has anabolic effects, shifting energy utilization from fat to carbohydrates, thereby stimulating the deposition of fat in adipocytes (Yun et al., 2005). In rodents, an increased fat mass was observed with chronic administration of galanin (Gaysinskaya et al., 2007). Galanin decreases energy expenditure and the sympathetic drive to brown adipose tissue (Nagase et al., 1997), which may be one mechanism promoting energy storage.

14.11.1.1 Physiological Role of Galanin in the Regulation of Fat Intake

The physiological role of galanin in the regulation of fat intake has been investigated with the use of various galanin receptor antagonists. Such studies have demonstrated contradictory results, from no effect to a decrease in spontaneous fat intake (Bartfai et al., 1993; Leibowitz, 1994). There are three G-protein–coupled receptors for galanin. In one study, galanin receptor 1 –/– mice were used (Zorrilla et al., 2007); this study demonstrated that the food intake was the same as in control mice. However, the galanin receptor –/– mice lost their ability to adapt to a high-fat diet while the normal mice decreased their food intake. The results are somewhat the opposite of what should be expected, since the galanin receptor knockout mice had a larger consumption of a high-fat diet than the control animals. Moreover, the galanin receptor knockout mice demonstrated an impaired glucose tolerance, suggesting a maladaptation to palatable food.

14.11.1.2 Galanin Expression Is Stimulated by High-Fat Feeding

The increased feeding with a high-fat diet may be explained by an activation of the opioid system triggered by orexigenic peptides like galanin. Gaysinskaya et al. (2007) have found that galanin in the PVN and orexin in the perifornical lateral hypothalamus were increasingly expressed when a high-fat diet was given as a preload to induce overeating. The overeating following the preload of high-fat food was accompanied by elevated circulating triglyceride levels while there was no change in leptin or insulin levels during these short-term experiments. These experiments suggest that high-fat feeding-induced overconsumption occurs rapidly and this is due to an increased expression of orexigenic hormones in the hypothalamus and/or elevated levels of blood lipids rather than changes in leptin and/or insulin levels (Leibowitz et al., 2004). It is as if overfeeding stimulates its own progress.

14.11.1.3 Galanin Stimulates Alcohol Intake

Galanin not only stimulates fat intake but has also been found to stimulate the intake of ethanol. In rats training to drink ethanol, injection of galanin into the PVN increased the intake of ethanol over the next few hours, without affecting consumption of water or food (Schneider et al., 2007). Injection of M40, a galanin antagonist, or of the nonselective opioid receptor antagonist, naloxone methiodide, prevented the intake of ethanol indicating the importance of the galanin signaling pathway and its link to the opioid system in terms of ethanol ingestion. Galanin is thus a mediator of reward, bridging nutrients like fat with rewarding food like ethanol. A similar finding is observed with ghrelin, which also stimulates fat as well as ethanol consumption after central injection in experimental animal models. Transgenic mice overexpressing galanin in noradrenergic neurons showed decreased morphine withdrawal signs, indicating that galanin helps against withdrawal symptoms (Zachariou et al., 2003). In short, galanin has the ability to stimulate fat intake and promote energy storage as well as drive reward.

14.11.2 Agouti-Related Peptide

AgRP is a peptide signaling hunger synthesized in the arcuate nucleus with the property of a hunger signal. AgRP is an endogenous melanocortin receptor antagonist coexpressed with NPY in the arcuate nucleus; both peptides display orexigenic effects (Broberger et al., 1998). Transgenic mice overexpressing AgRP are obese and hyperphagic (Graham et al., 1997). Further evidence supporting AgRP as a physiological hunger signal came from the observation that its expression both at the mRNA and protein level is increased in the hypothalamus following fasting (Hahn et al., 1998; Li et al., 2000). AgRP is colocalized with NPY, (Hahn et al., 1998) and there are several common physiological links between the two peptides. The neurons producing the peptides contain receptors for leptin, which regulate the expression of NPY and AgRP. Since NPY has been suggested as a hunger signal for carbohydrate intake (Wang et al., 1998), the question of whether AgRP would be a hunger signal for fat intake arose.

14.11.2.1 AgRP and Fat Intake

Several lines of evidence support AgRP as a hunger signal specifically stimulating fat intake. In a two-choice paradigm between high-fat and low-fat food, AgRP was found to significantly stimulate high-fat (41 energy percent) food intake during the 4 h following an injection into the third ventricle, whereas low-fat (11 energy percent) food intake was not affected (Hagan et al., 2001). The fact that such a preference for fat also occurs in animals with chronic overexpression of Agouti-protein is supported by the finding that the agouti mice (Ay/a), when given a three-choice diet between fat, protein, and carbohydrates, chose to eat fat, thus inducing a significant weight gain (Koegler et al., 1999). The increased fat preference was immediate and persisted throughout the 7-week long experiment. Agouti-protein interacts with the same receptor as AgRP, the melanocortin receptors 3 and 4. Further support for a specific ability of AgRP to regulate fat intake is the finding that enterostatin, a peptide that inhibits fat intake was found to decrease the expression of AgRP (Lin et al., 2007). Hence, fat intake appears to be regulated through a melanocortin pathway.

14.11.2.2 Mechanism of Increased Fat Intake by AgRP

AgRP interacts with the melanocortin receptors 3 and 4. In an experiment during which MTII, a melanocortin receptor antagonist, was administered (Samama et al., 2003), it was found that rats receiving a three-choice macronutrient of protein-fat-carbohydrate diet decreased their fat intake. The suppression of fat intake did not occur in MC4 (–/–) mice, suggesting that fat intake was regulated through the melanocortin pathway.

The way in which AgRP stimulates fat intake could be related to preingestive factors such as taste and palatability or postingestive factors such as the release of certain gut peptides or even a rapid digestion. In further experiments, it was found that AgRP increased the appetite response of fat, suggesting that the AgRP pathway is important for the anticipation of fat consumption (Tracy et al., 2007). Such an anticipatory response was not observed with sucrose, indicating that AgRP is not a general factor regulating the intake of palatable food, but is specific to fat.

Since high-fat intake has been found to be mediated through activation of opioid pathways, the involvement of opioid receptors in the regulation of fat intake by AgRP was investigated (Hagan et al., 2001). It was found that naloxone when injected intraperitoneally significantly reduced high-fat food intake that had been stimulated with AgRP over 4 h, whereas there was no response with the low-fat food. These experiments thus suggest that fat intake is stimulated by AgRP and that the mechanism occurs through the activation of an opioidergic pathway. The exact site for the interaction of AgRP with opioid receptors is not known. Such a circuit might be important in mediating overeating and obesity that occurs in humans following exposure to high-fat diets.

14.11.2.3 Physiological Implications of AgRP in Obesity

In Western societies, high-fat foods are readily available and the passive consumption of high-fat diets due to their low satiety has been claimed to be one important factor in the ongoing epidemic of overweight and obesity. AgRP has also been associated with high body fat in humans (Argyropoulos et al., 2003). Diets aimed at losing weight are often not working; rather the original body weight and sometimes even a higher body weight is regained in a short time at the end of the dieting period. This process is known as yo-yo dieting. Since AgRP has an increased expression following fasting or restriction of energy intake, one could imagine it to be a signal promoting overeating of high-fat food. It is thus important to find strategies to block this upregulation of AgRP following fasting.

14.11.2.4 AgRP-Deficiency Increases Life Span

AgRP is increased by fasting and when overexpressed in transgenic mice, it leads to hyperphagia and the development of obesity (Redmann and Argyropoulos, 2006). Global AgRP knockout mice, on the other hand, display a relative minor phenotype of age-dependent leanness or no phenotype at all. Redmann and Argyropoulos (2006) found that female –/– mice were lean while consuming a low-fat diet but identical to wild-type animals when fed a high-fat diet. Male –/– mice were heavier on a low-fat diet while similar to control mice on a high-fat diet. Unexpectedly, AgRP deficient mice, both females and males, had a lifespan 10% longer (Redmann and Argyropoulos, 2006). This tells us that fat intake may be an important factor in

shortening lifespan. Experiments aimed at identifying bioactive compounds that will suppress AgRP expression have produced candidates that may assist in regulating fat intake and perhaps increase lifespan.

14.11.3 Ghrelin

Ghrelin is a peptide produced in the stomach (Ariyasu et al., 2001), and was discovered as an endogenous ligand of the growth hormone receptor (Kojima et al., 1999). Ghrelin has been described as a hunger signal, stimulating food intake when administered to rodents or humans (Wren et al., 2001). Ghrelin secretion is increased during fasting and suppressed after the start of food intake. Conditions that promote a negative energy balance like starvation, insulin-induced hypoglycemia, and anorexia nervosa as well as physical activity cause an upregulation of ghrelin secretion (Cummings et al., 2004). Conditions that induce a positive energy balance such as overeating, obesity, and hyperglycemia induce a suppression of ghrelin levels (Tschop et al., 2000; Lindqvist et al., 2005; Cummings, 2006).

14.11.3.1 Ghrelin Is Downregulated after Fat and Carbohydrate Intake

Macronutrients differ in their ability to suppress ghrelin secretion after the start of a meal. Fat or carbohydrate containing meals suppress ghrelin secretion (Nakagawa et al., 2002) whereas proteins are ineffective (Beck et al., 2002). This is surprising considering that proteins are the most satiating macronutrients (Erdmann et al., 2003). Fat's ability to reduce ghrelin levels occurs only after fat digestion suggesting that fatty acids are an important signal suppressing ghrelin secretion. The addition of tetrahydrolipstatin, an inhibitor of gastric lipases, leads to a marginal decrease of ghrelin level (Feinle-Bisset et al., 2005) suggesting that fatty acids released in the gastro-intestinal tract play an important role in this process (Feltrin et al., 2006). The exact mechanism underlying the suppression of ghrelin secretion following fat consumption is not known yet. It has been reported that PYY, a peptide that inhibits fat intake is secreted 15 min after infusion of lipids into the stomach. Thus, PYY is a likely candidate for ghrelin suppression (Batterham et al., 2003).

14.11.3.2 Central Ghrelin Enhances Fat Intake

Most studies on ghrelin have used single-choice diet paradigms. In a two-choice paradigm between high-fat and high-carbohydrate diets, ghrelin, when given centrally, was found to specifically enhance fat intake in rats (Shimbara et al., 2004). This effect was observed 1 h after ghrelin injection and reached maximal effect 2 h after injection. The enhancement of fat intake was in the same order as that of galanin (Shimbara et al., 2004).

Since fat intake was stimulated after central administration of ghrelin, it is suggested that centrally produced ghrelin, i.e., the ghrelin produced in the arcuate nucleus (Lee et al., 2002) regulates fat intake. In animals and humans, the appetite for fat is at its peak at the end of the feeding period, thus suggesting that ghrelin is released during that period. In studies where rats were given sucrose, ghrelin levels continued to rise (Lindqvist et al., 2005) suggesting that ghrelin signals the need for energy in the form of fat rather than carbohydrate. Further studies are needed to understand the regulation of fat intake by ghrelin.

14.11.3.3 Ghrelin Promotes Addiction

It has been demonstrated that in the case of ghrelin, there is a neurochemical overlap between the reward system and the appetite regulating system. Intracerebroventricular administration of ghrelin was found to stimulate the intake of ethanol (Jerlhag et al., 2006) through a mechanism linked with increased dopamine secretion from the nucleus accumbens as well as increased locomotor activity (Jerlhag et al., 2006) in the same way as would occur after ethanol consumption (Jerlhag et al., 2007). Furthermore, ghrelin was found to activate a cholinergic-dopaminergic reward link much as ethanol does. A role ghrelin has on brain rewards could be to increase the incentive values of motivated behavior, e.g., food searching. Alcoholics were also found to have higher levels of ghrelin in their circulation. Ghrelin thus has a role in brain reward in addition to regulating energy balance.

14.11.4 Enterostatin

The first discovery of enterostatin as a feeding-related peptide was in 1988 when during immunization of rabbits with enterostatin the animals lost their appetite. In systematic studies in rat, we found that enterostatin decreased food intake after intraperitoneal administration (Erlanson-Albertsson and Larsson, 1988). Enterostatin is a pentapeptide released from the N-terminal end of pancreatic procolipase by proteolytic cleavage (Figure 14.4). The residual product called pancreatic colipase is a protein cofactor for pancreatic lipase during intraduodenal hydrolysis of fat (Erlanson-Albertsson, 1992). Since enterostatin and colipase are released more specifically during the intake of fat, experiments were performed to elucidate whether enterostatin had any specific effect on fat intake. In studies during which food was given either as a three-choice diet between fat, protein, and carbohydrate (Okada et al., 1991) or as a two-choice paradigm with high-fat and low-fat diets (Erlanson-Albertsson et al., 1991), it was found that enterostatin inhibited fat intake as opposed to carbohydrate or protein intake. Furthermore, it inhibited high-fat intake as opposed to low-fat intake. The effect was observed after both central administration and peripheral administration of enterostatin.

14.11.4.1 Occurrence of Enterostatin

Not surprisingly enterostatin was found to be present in the intestinal contents of rats and humans (Mei et al., 1993; Erlanson-Albertsson and York, 1997). Enterostatin was found to be present at micromolar levels in rats and increased two- to threefold following consumption of a high-fat diet (Mei et al., 1993). This occurred as soon as 24 h after the switch to a high-fat diet, indicating a rather rapid and robust adaptation of procolipase and enterostatin to high-fat feeding. In the gastro-intestinal tract, enterostatin was also found to be present in the gastric mucosa, secreted by chief cells. Cloning of gastric procolipase revealed that this molecule is identical to the pancreatic procolipase (Winzell et al., 1998).

Enterostatin has also since been identified in the brain in the form of procolipase (York et al., 2006; Rippe et al., 2007). Procolipase and enterostatin immunoreactivity was demonstrated in PVN, ARC, supraoptic nuclei, amygdala, and dorsal median thalamus (York et al., 2006). These regions are associated with the regulation of

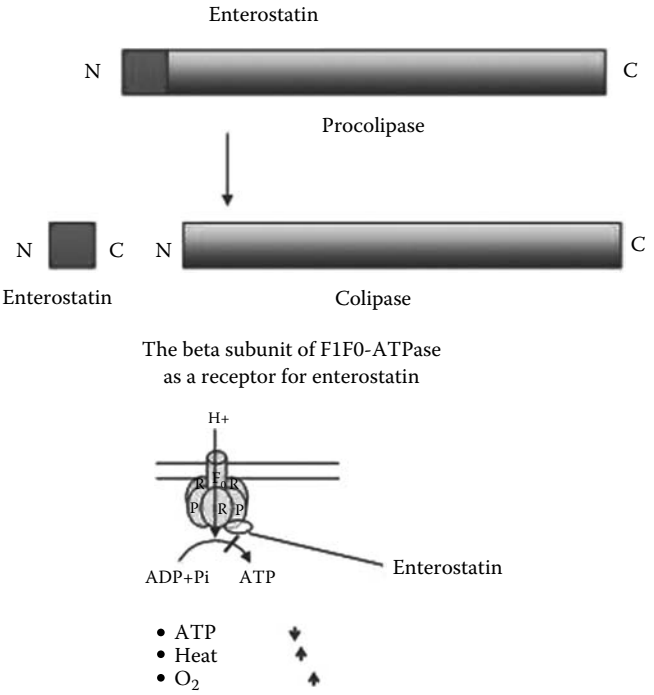

FIGURE 14.4 Enterostatin is formed by the proteolytic cleavage of procolipase. Procolipase is found in the stomach, pancreas, the intestine as well as in the hypothalamus. The receptor for enterostatin is the beta-subunit of the F1-ATPase. The receptor protein is localized on the plasma membrane of many cells e.g., in the amygdala, where it regulates fat intake, in the myocytes, where it triggers fatty acid oxidation, and in INS-1 cells, where it inhibits insulin secretion.

food intake and energy expenditure. Procolipase was seen as dense particles in the cytoplasm, whereas enterostatin immune reactivity was observed in nerve fibers. This suggests that procolipase is produced and processed in the neuronal cell body, whereas enterostatin is transported down the nerve fiber to be released at the nerve terminal (York et al., 2006). Since enterostatin is particularly active when injected into the amygdala, the endogenous production of enterostatin in this nucleus suggests that this might be important for regulation of fat intake. Enterostatin was also present in cells lining the third ventricle, where it could be secreted into the cerebrospinal fluid, as confirmed by Imamura et al. (1998). Thus the procolipase gene is expressed in the brain, translated to protein, and cleaved to release enterostatin.

In further studies, the hypothalamic procolipase was found to be upregulated by a high-fat diet in a similar fashion as the pancreatic procolipase (Rippe et al., 2007). Since diets rich in fat are energy-dense, the upregulation of hypothalamic procolipase seems to be an adequate event to induce satiety and energy balance. Other palatable food like mono- and disaccharides (glucose, fructose, and sucrose) had no significant effect on the expression of hypothalamic procolipase, indicating

that the procolipase expression was unresponsive to sugars. Fasting overnight caused a threefold downregulation of hypothalamic procolipase, which is in agreement with the activity of a satiating agent. There was no expression of classical lipase in the hypothalamus, supporting a role of procolipase in the hypothalamus in the production of enterostatin (Rippe et al., 2007).

The presence of enterostatin and its precursor procolipase in the pancreas, the stomach, and the central nervous system is another example of a peptide regulating food intake present both in the gut and in the brain, much like CCK, ghrelin, and galanin.

14.11.4.2 Mechanism of Action of Enterostatin

Enterostatin acts through both direct and indirect pathways. The direct pathway involves the interaction of enterostatin with its receptor, the β subunit of F1-ATPase (Berger et al., 2002; Park et al., 2004) while the indirect pathways involve serotonin, CCK, and melanocortin.

14.11.4.2.1 The β-Subunit of F1-ATPase as a Receptor for Enterostatin

Using purified rat membranes, Berger et al. (2002) were able to demonstrate specific binding of enterostatin to a 60 kDa protein, which was sequenced through MALDITOF analysis and found to be β-subunit of F1-ATPase. The identification of the β-subunit of F1-ATPase as a receptor for enterostatin was confirmed by Park et al. (2004) (Figure 14.4) who established the binding constant to be 1.7×10^{-7} M using surface plasmon resonance technique. Using an aqueous two-phase system, the binding between enterostatin and its receptor was found to be equal to 1.5×10^{-7} M (Berger et al., 2004). There was no binding using the whole multimeric protein (Park et al., 2004). Strikingly, Park et al. (2004) reported the presence of the β-subunit of F1-ATPase in the plasma membranes of liver cells as well as in amygdala (Park et al., 2004). Lindqvist et al. (2008) later reported the presence of β-subunit of F1-ATPase in the plasma membrane of INS-1 cells which were used originally to demonstrate the enterostatin effect and the activation of the receptor (Berger et al., 2002). It was also found that incubation of the INS-1 cells with enterostatin caused a threefold upregulation of the expression of the β-subunit of F1-ATPase on the plasma membrane (Lindqvist et al., 2008). Likewise, fatty acids were found to stimulate the translocation of F1-ATPase to the plasma membrane (Lindqvist et al., 2008). The fact that fat upregulates the receptor for enterostatin may explain the need to feed animals high-fat diets before they respond to enterostatin (Lin and York, 1998).

14.11.4.2.2 Indirect Pathways Mediating Enterostatin's Action

In the amygdala, enterostatin functions with a serotonin receptor to reduce fat intake (Lin and York, 2004). With a 5HT1b receptor antagonist, the response of enterostatin was abolished. Since enterostatin does not interact directly with a serotonin receptor, the mechanism of action could involve the ability of enterostatin to release serotonin (Koizumi and Kimura, 2002). Serotonin has been described as a signal specifically related to satiety for fat, which seems to be a likely mechanism to promote satiety.

Another component that is important for the enterostatin response is the presence of CCK A receptors (Lin et al., 2003). This is based on studies using the OLEFTA rat which lacks CCK A receptors that were found to be unresponsive to enterostatin.

The third pathway for enterostatin is the melanocortin pathway. In this pathway enterostatin is able to decrease the expression of AgRP in the amygdala and in the hypothalamus (Lin et al., 2007). Since AgRP stimulates fat intake, this inhibition could explain a decreased fat intake by enterostatin. At the same time, enterostatin caused an increased expression of the satiety hormone POMC (Lin et al., 2007). Since enterostatin does not interact with the melanocortin receptor, it is suggested that enterostatin causes the release of melanocyte-stimulating hormone (MSH), a satiety hormone, in the PVN. The involvement of the melanocortin receptor pathway is supported by the fact that enterostatin failed to show any feeding inhibitory effect in MC4R knockout mice. Moreover, an antagonist to the MC4 receptor blocked the effect of enterostatin on the inhibition of fat intake (Lin et al., 2007).

14.11.4.3 Intracellular Mechanisms Mediating Enterostatin's Effect

Intracellular enterostatin has been found to activate different pathways depending on cell type and intracellular events. Regarding the stimulation of fatty acid oxidation in myocytes, enterostatin was demonstrated to act through phosphorylated AMP kinase (Lin et al., 2006). Enterostatin was found to act through cAMP with regard to the regulation of insulin secretion whereas the regulation of AgRP expression seems to be under the control of the cAMP as well as the MAP kinase ERK pathway (Park et al., 2008).

In further studies aimed at understanding enterostatin's mechanism of action, the human hepatoma cell line (HepG2 cells) was used and subjected to glucose deprivation to induce angiogenesis (Park et al., 2008). In this situation, enterostatin was found to inhibit angiogenesis (Park et al., 2008). Phosphyrolated AMP kinase (pAMPK) and vascular endothelial growth factor A (VEGP-A) mRNA were significantly elevated by glucose deprivation, but this activation was inhibited by the presence of enterostatin (Park et al., 2008). These data suggest that enterostatin has an antiangiogenic effect occurring through inhibition of the AMPK activity (Park et al., 2008). The inhibition of angiogenesis by enterostatin also occurred in adipocytes (Park et al., 2008). The blocking effect may be important to induce weight loss. In the ob/ob mouse, the loss of body fat was associated with decreased food intake and increased fatty acid oxidation (Rupnick et al., 2002). In human obesity, a number of angiogenic factors are increased including VEGF (Silha et al., 2005). It is therefore possible that the antiangiogenic effect of enterostatin may contribute to the loss of body weight and body fat during chronic administration of enterostatin.

14.11.4.4 Enterostatin Stimulates Energy Expenditure

Enterostatin is an example of a peptide that regulates both feeding and energy expenditure. Chronic injection of enterostatin either intracerebrovascularly (ICV) or peripherally caused a greater weight loss than could be accounted for by the reduction of food intake (Lin et al., 1997; Berger et al., 2002). Additionally, enterostatin was found to enhance sympathetic activation of brown adipose tissue (Nagase et al., 1997). Enterostatin was also found to enhance the expression of uncoupling protein 1

expression in brown adipose tissue in mice fed a high-fat diet, leading to increased heat production (Rippe et al., 2000). The increased energy expenditure served to achieve energy balance during high-fat feeding.

In INS-1 cells enterostatin was found to increase heat production as well as oxygen consumption (Berger et al., 2002), an effect believed to occur through an interaction with the β-subunit of F1-ATPase. Enterostatin was also found to activate AMP-activated kinase and beta-oxidation of fatty acids (Lin et al., 2006). AMPK is now recognized to have a central role in regulating energy balance between anabolic and catabolic pathways (Ruderman and Prentki, 2004). AMP-kinase's activation leads to increased fatty acid oxidation and glucose transport in muscle as well as decreased fatty acid synthesis and gluconeogenesis. Phosphorylation and subsequent activation of AMPK by enterostatin has been linked to increased energy expenditure in myocytes (Lin et al., 2006).

Enterostatin's stimulation of energy expenditure could occur through a central or a peripheral pathway. The amygdala is believed to be the site of action of enterostatin when inhibiting fat intake, whereas the PVN could be the site of action linked to the stimulation of energy expenditure.

14.11.4.5 Enterostatin as an Endogenous Regulator of Fat Intake

The question of whether enterostatin is an endogenous regulator of fat intake is answered by the fact that the receptor antagonist beta-casomorphin has the ability to increase fat intake (Lin et al., 1998; Berger et al., 2002). There are also studies demonstrating an increased food intake after intracerebroventricular administration of enterostatin antibodies (Unpublished, York, 2007). Voluntary fat intake in rodents was demonstrated to be inversely related to the amount of procolipase/enterostatin (Okada et al., 1992) suggesting that endogenous enterostatin regulates fat intake.

14.11.5 Apolipoprotein A-IV

Apo A-IV is a small protein with a molecular weight of 43 kDa. It is synthesized in both the liver and the intestine. In the intestine Apo A-IV is synthesized by epithelial cells found mostly in the jejunum in the upper portion of the villi (not in the crypts). The intestinal Apo A-IV is stimulated by lipid feeding (Fukagawa et al., 1994) and is released into the circulation. The release of Apo A-IV occurs during the process of assembly and transport of intestinal chylomicrons. Inhibition of chylomicron formation totally abolishes Apo AI-V secretion (Hayashi et al., 1990). Apo A-IV is secreted only upon feeding with fat containing long-chain fatty acids (>C14), since these are taken up into chylomicrons (Kalogeris et al., 1996).

Intravenous infusion of Apo A-IV has been found to decrease food intake in rats in a dose-dependent way (Fujimoto et al., 1992). This suggested that Apo A-IV is a circulating satiety signal released by the small intestine in response to fat ingestion. The mechanism of inhibition of Apo A-IV on feeding has been suggested to occur at a central level. Central infusion of Apo A-IV was 50 times more potent than peripheral administration (Fujimoto et al., 1993). Apo A-IV was also detected by RT-PCR in the arcuate nucleus of the hypothalamus (Liu et al., 2001). A specific effect of Apo A-IV may be to inhibit the onset of feeding, rather than promote the end of feeding.

14.11.5.1 Regulation of Apo A-IV Synthesis

In the intestine, the level of Apo A-IV is increased with accrued consumption of fat. In the hypothalamus, the level of Apo A-IV was found to decrease upon fasting, whereas it was increased upon lipid feeding as could be expected for a satiety factor responsible for fat intake (Liu et al., 2001; Tso et al., 2004). Thus, the regulation of Apo A-IV in the intestine and in the brain follows the same pattern.

Somewhat surprisingly, central infusions of NPY increased the expression of Apo A-IV (Liu et al., 2003). One possible explanation for this phenomenon could be that since NPY relieves anxiety by increasing food intake and fat is in itself anxiety relieving, preventing fat intake with Apo A-IV would not alter NPY's anxiety relieving effect.

The synthesis of Apo A-IV was reported to be downregulated by leptin (Doi et al., 2001). Leptin is a peptide that is synthesized and secreted by the adipocyte. Plasma leptin levels are elevated when animals are maintained on a high-fat diet (Frederich et al., 1995). It could be speculated that obesity, a condition with high leptin levels, is self-filling, because several hunger hormones are promoted, like galanin and NPY, whereas some satiety signals are suppressed or made inefficient. In this context, Apo A-IV would fit in the pattern of a candidate hormone promoting obesity by being downregulated by leptin.

In conclusion, Apo A-IV is a peptide that is released into the circulation following uptake of lipids from the intestine. The exact role of Apo A-IV in the regulation of fat intake is not known, and so are the target protein and receptor for Apo A-IV.

14.11.6 Peptide YY

"Ileal break" is a phenomenon described as an inhibition of food intake through infusion of nutrients into the ileum. Various peptides produced in the distal ileum have been proposed to mediate this effect. However, the most likely candidate is PYY (Pappas et al., 1986), which causes an inhibition of intestinal motility (Savage et al., 1987). The "ileal break" phenomenon was formerly considered operative only during malabsorption states when undigested nutrients reach the distal gut. However, it is now recognized that nutrients reach the distal gut even under normal conditions (i.e., when gastric emptying occurs rapidly). It has been documented that 10%–15% of a given amount of lipid reaching the upper small intestine is recovered in the distal ileum (Tso and Liu, 2004b).

PYY is synthesized by endocrine cells located in the ileum and colon (Adrian et al., 1987) and released by nutrients such as long-chain fatty acids. In the presence of tetrahydrolipstatin, an irreversible blocker of pancreatic lipase the release of PYY is completely abolished (Feinle-Bisset et al., 2005). The use of PYY is being tested as a drug to reduce fat intake.

14.11.7 Cholecystokinin

It is well established that CCK functions as a satiety signal (Ritter, 2004; Woods, 2004). CCK is released from enteroendocrine cells in the presence of lipids and proteins (Sayegh and Ritter, 2003). Proteins are stronger releasers of CCK than amino

acids. In contrast, fatty acids, especially long-chain fatty acids, are better at releasing CCK than triacylglycerol (Lieverse et al., 1994; Ledeboer et al., 1998). CCK works by activating a specific receptor, the CCK1 receptor (or CCKA receptor), present on vagal afferents innervating the intestinal mucosa (Moran et al., 1997). The vagal afferent fibers project to the nucleus of the solitary tract and are involved in feeding termination. CCK inhibits food intake, both in rodents and in humans (Lieverse et al., 1994). Administration of devacepide, a CCK receptor antagonist, was reported to increase food intake (Reidelberger and O'Rourke, 1989). The CCK1 receptor –/– mice had, however, the same body weight as normal mice, although their eating pattern was different (Bi et al., 2004).

The CCK1 receptor seems to be responsible for the size and duration of a meal, especially during a high-fat meal (Lo et al., 2007). When given a high-fat diet, CCK1-receptor –/– mice were unable to decrease meal size and therefore adapt their feeding, whereas wild-type mice were able to (Whited et al., 2006). The normal mice decreased their meal size when receiving high-fat food, whereas the –/– mice continued to eat the same meal size as with the low-fat diet. Thus, the CCK1 receptor plays an important role in the adaptation of meal size when fed energy-dense meals (Savastano and Covasa, 2007). Mechanisms underlying this effect involve activation of the 5-HT3 receptor at the central level (Savastano and Covasa, 2007) as well as inhibition of gastric emptying, a feature important for satiety (Reidelberger et al., 2001). Donovan et al. (2007) found that the CCK effect was most pronounced for the actual meal rather than for the subsequent meal.

14.11.7.1 Role of Fat Digestion in Satiety

There are several pieces of evidence indicating that a proper digestion of fat is needed for optimal satiety (Figure 14.5). When measuring the release of hunger and satiety hormones during fat digestion, it was reported that addition of tetrahydrolipstatin, a lipase inhibitor, completely abolished the release of CCK (Goedecke et al., 2003; Beglinger and Degen, 2004) suggesting that CCK is released only upon complete fat digestion. This is also true for the satiety hormone GLP-1 which is released only after proper fat digestion and not after administration of tetrahydrolipstatin (Feinle et al., 2003; Sahin et al., 2007). In these experiments carried out in healthy humans, there was also a lower degree of satiety when the lipase inhibitor was added to the lipids consumed.

14.11.7.2 Interaction of Enterostatin and CCK

Since CCK and enterostatin are both involved in the suppression of fat intake, an experiment was carried out to investigate whether there was an interaction between the two systems. In OLETF rats lacking the CCK-A receptor, enterostatin failed to inhibit high-fat food intake (Covasa and Ritter, 2005). Addition of enterostatin failed to inhibit high-fat food intake whereas the control LETO rats still were responsive (Lin et al., 2003). Thus, CCK receptor expression was necessary for enterostatin action. There was, however, no direct synergism between CCK and enterostatin (Lin et al., 2003). Since enterostatin does not interact directly with the CCK-A receptor, it is possible that there is a cross-talk downstream of receptor activation.

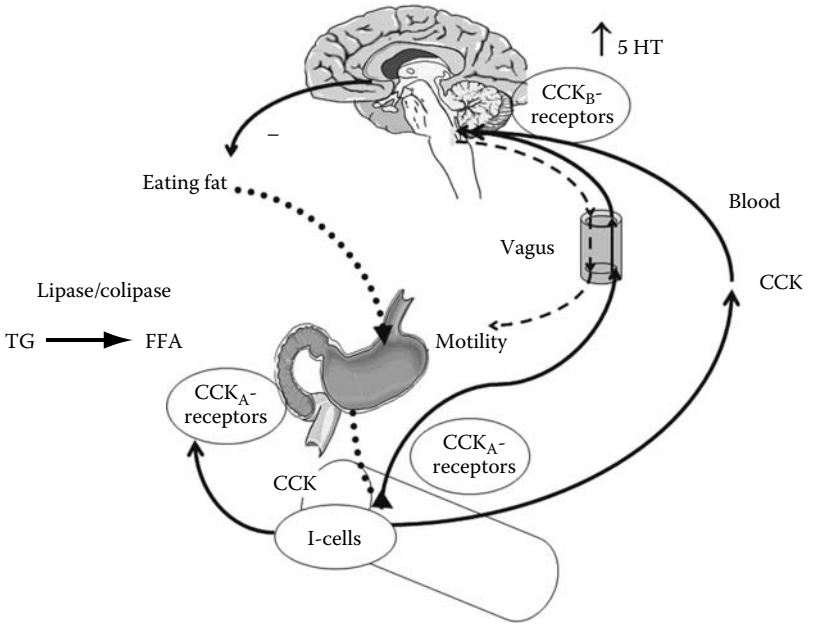

FIGURE 14.5 Mechanism by which CCK induces satiety for fat. This mechanism works through CCK-A receptors acting in the gastrointestinal tract to inhibit gastric emptying as well as through CCK-B receptors acting centrally to release serotonin (5-HT). The fatty acids are potent releasers of CCK, thus the promotion of satiety is optimal after complete fat digestion.

14.11.8 Neuropeptide Y

NPY is an important orexigenic peptide in the central nervous system (Beck et al., 2002). The peptide is found in several places in the brain especially in the neurons projecting from the arcuate nucleus to the PVN. When NPY is injected ICV, food intake is increased (Beck et al., 2002). Following administration of NPY, rats tested with a three-choice paradigm between fat, carbohydrates, and proteins presented a preference for carbohydrates (Stanley et al., 1985; Jhanwar-Uniyal et al., 1993). If carbohydrates were excluded and rats had to choose between fat and proteins, the animals chose proteins. When rats were under continuous NPY administration and had to choose between a high-fat and a high-carbohydrate diet, both diets were eaten (Beck et al., 2002). The preference for the high-fat diet lasted only 2 days; thereafter, the animals consumed the sweet tasting high-carbohydrate diet. In fully sated rats, NPY injected animals chose the diet with the highest sucrose or artificial sweetener concentration (Lynch et al., 1993). NPY has also been shown to stimulate the intake of milk over water in newborn rats (Capuano et al., 1993). The specific role of NPY on the stimulation of carbohydrate intake is related to the original preference of the rat for either carbohydrate or fat (Welch et al., 1994; Smith et al., 1997).

The endogenous role of NPY has been demonstrated through the use of antibodies and antisense oligodeoxynucleotides (Beck, 2000). Destruction of NPY neurons

by neurotoxic agents such as monosodium glutamate (MSG) lead to a decreased food intake (Stricker-Krongrad and Beck, 2004). NPY acts by reducing the lag time before eating, thus in the –/– NPY mice, a pronounced delay in feeding was observed (Sindelar et al., 2005). NPY also increases the motivation to eat, thus stimulating the animals to work harder to get food (Jewett et al., 1995). Furthermore, NPY is involved in the consumption of palatable food as demonstrated by the fact that the endocannabinoid receptor antagonist Rimonabant prevented the release of NPY (Gamber et al., 2005). Blocking of the opioid system also prevented the NPY-induced feeding (Israel et al., 2005).

14.11.8.1 Factors Regulating NPY Expression

Food deprivation is an important regulatory factor for NPY. When rats were deprived of food, the expression of NPY was markedly increased and refeeding returned NPY expression to a normal level (Beck, 2006). Fasting leads to a drop in blood and brain glucose which, through glucose sensing neurons present in the arcuate nucleus, could be a factor stimulating the expression of NPY. In fact, it has been reported that NPY is reactive to moderate hypoglycemia to induce feeding (Akabayashi et al., 1993) and its secretion is inhibited by hyperglycemia (Rowland, 1988). This phenomenon was not seen if fructose was injected, the reason being that fructose is not metabolized by the brain. Other explanations put forward implicate a decrease in leptin receptors upon fasting which could be an explanation for an upregulation of NPY expression (Van Vugt et al., 2006) as well as glucocorticoids (Larsen et al., 1994). There is also a diurnal variation of NPY. In rats, hypothalamic NPY peaks 1 h before dark onset, i.e., 1 h before the natural feeding period (Jhanwar-Uniyal et al., 1990). NPY decreased 1 h after lights were turned-off. Hence, food intake in the beginning of the dark period is associated with high NPY release. NPY, thus, has the general properties of an appetite stimulating peptide that promotes the intake of carbohydrates, particularly sweet tasting carbohydrates. It may be a surprise that NPY, when injected centrally, could inhibit fat intake as discussed below.

14.11.8.2 NPY Is Anorexogenic and Decreases Fat Consumption

NPY's anorexogenic activity has been brought to light in experiments in which rats were presented with either high-fat (56% fat, 20% carbohydrate) or low-fat food (10% fat, 66% carbohydrate) and NPY was injected in the amygdala. As a result, a significant decrease of high-fat food intake was reported after 24 h whereas the low-fat food intake was unaffected (Primeaux et al., 2005). One potential explanation for the observed effect is a decrease in anxiety by NPY, substituting the anxiety relief normally provided by palatable food. NPY has been shown to display an important anxiolytic effect mediated through NPY1 receptors in the amygdala, which plays a critical role in anxiety related situations (Primeaux et al., 2005).

It is important to note that this decrease in fat food intake occurred after 24 h as opposed to the early effect observed when NPY is injected in other parts of the brain. The slow onset of response suggests that indirect mechanisms are acting to suppress fat intake. The decrease in fat intake, however, was substantial and not a consequence of malaise. Since NPY itself reduces anxiety and fat is known

to relieve stress, it could be hypothesized that the decrease in fat intake is due to NPY's anxiety releasing property normally attributed to high-fat food (Primeaux et al., 2005).

There are also other factors determining the effects of NPY on food intake. For instance, NPY was found to stimulate (Benoit et al., 2005), show no effect (Seeley et al., 1995), or inhibit consumption of sucrose solutions (Ammar et al., 2000) depending on the training the animals received.

The biological action of NPY is mediated through eight receptors subtypes, Y1–Y8. The different NPY receptors activate different pathways. Y5 receptor activation leads to consumption of food and is also important for the reduction of energy expenditure during diet restriction (Widdowson, 1997). The Y1 receptor has been linked to inhibition of food intake (Day et al., 2005) and could hence be involved in situations where NPY inhibits feeding, e.g., decrease fat intake. Thus, the regulation of palatable food intake by NPY is complex and dependent on the situation, the site of action and which receptors are activated.

14.12 STRATEGIES TO PROMOTE THE CONTROL OF FAT INTAKE

Strategies aiming to block peptides stimulating fat intake are currently being tested. Antibodies against ghrelin are being tried in animal experiments, which would be an elegant way of controlling appetite for fat. Other strategies include the use of

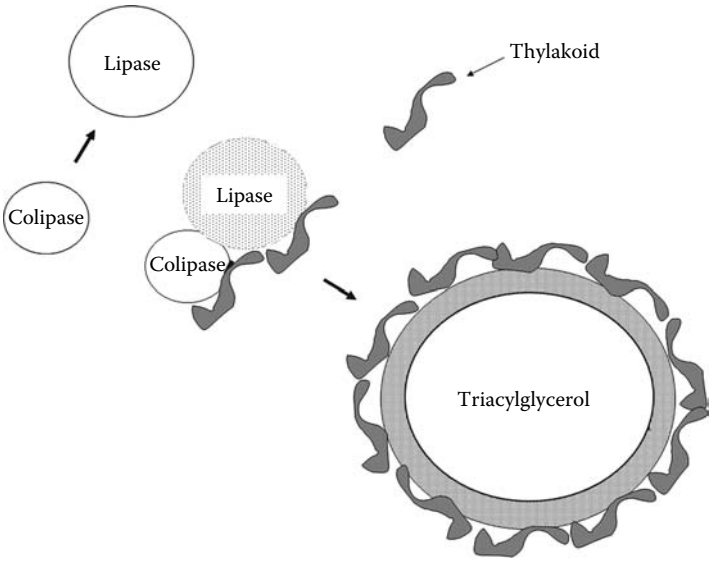

FIGURE 14.6 Thylakoids are membranes from plant cells containing galactolipids and membrane proteins. They have been found to reduce the rate of fat digestion by pancreatic lipase and colipase by covering the lipid droplet as well as interfering with the lipase–colipase interaction. As a result they promote satiety for fat by stimulating the release of CCK both in animal and human studies.

lipase inhibitors which will actually inhibit uptake of fat in the intestine. Although tetrahydrolipstatin promotes body weight loss, it has the drawback of delivering fatty stools and accelerating gastric emptying (O'Donovan et al., 2004) which lead to a reduced production of satiety hormone such as CCK and GLP-1 (O'Donovan et al., 2004). Another approach developed in our laboratory is adding components that retard fat digestion without actually inhibiting fat digestion (Albertsson et al., 2007). Thylakoids are natural components isolated from green leaves constituted of galactolipids and proteins (Albertsson et al., 2007). They have been shown to prolong fat digestion (Figure 14.6) and at the same time cause an upregulation of satiety hormones like CCK and enterostatin (Albertsson et al., 2007). Their efficiency has been proven both in animal studies and human studies (Köhnke et al., 2009). Long-term studies in humans are currently underway since this strategy seems to be very promising.

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15 Fats and Satiety

Rania Abou Samra

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15.1 INTRODUCTION

Dietary fat has frequently been blamed for the increase in prevalence of obesity (Bray et al., 2004). Epidemiological studies have demonstrated a positive relationship between high-fat diets and excess energy intake due to their high energy density and palatability (Prentice and Poppitt, 1996). However, this association is confounded by differences in physical activity, smoking, and food availability and variety (Willett, 1998; Bray et al., 2004). Furthermore, epidemiological studies investigating the association between high fat intake and obesity have been inconsistent (Seidell, 1998; Willett, 1998).

Preload studies have shown that fat exerts the weakest effect on satiety compared to carbohydrate and protein, suggesting that fat may lead to "passive overconsumption" (Blundell et al., 1993). But when preloads were matched for energy density

and palatability, differences in satiety were not obvious (Geliebter, 1979; Stubbs and Harbron, 1996; McCrory et al., 2000), pointing to energy density as the key driver of satiety under experimental conditions. Furthermore, lipids suppress later food intake when present in the small intestine of both humans and animals (Welch et al., 1988; Greenberg et al., 1990; Drewe et al., 1992; Woltman and Reidelberger, 1995; Castiglione et al., 1998; Van Wymwlbeke et al., 1998).

Relatively few studies have investigated the responses of specific fats and fatty acids on food intake. Furthermore, studies have used different fats and fatty acids making it almost impossible to draw conclusions. However, it is clear that not all fats are equal in their effect on appetite and associated biological processes.

15.2 DIETARY FATS AND SATIETY: FAT STRUCTURE

The effect of fats on satiety has been investigated in four areas associated with fat structure: chain length, degree of saturation, degree of esterification, and functionality of specific fat molecules, particularly conjugated linoleic acid (CLA) and Olibra® (Lipid Technologies Provider AB, Karishamn, Sweden).

15.2.1 Chain Length

Studies on the effect of fatty acid chain length on satiety have shown that medium-chain triacylglycerols (MCT, 8–12 C) are more satiating than long-chain triacylglycerols (LCT) in animals (Friedman et al., 1983) and humans (Stubbs and Harbron, 1996; Rolls et al., 1988; Van Wymelbeke et al., 1998, 2001; St-Onge et al., 2003). MCT consumed as a preload resulted in lower energy intake 30 min later compared to LCT in healthy individuals (Rolls et al., 1988) (Figure 15.1). A breakfast high in

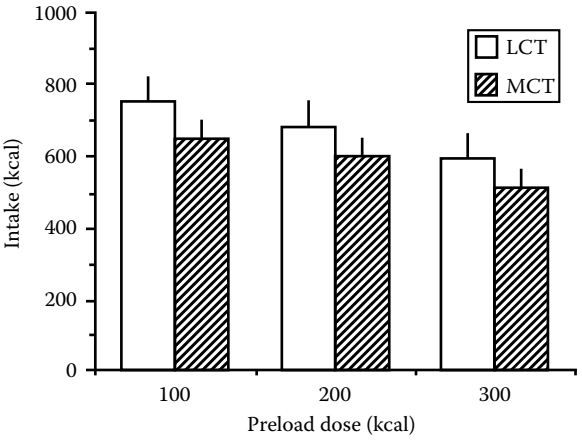

FIGURE 15.1 Mean energy intake (kcal) from an *ad libitum* lunch in 12 individuals. Lunch was offered 30 min after a preload of 100 (10 g), 200 (20 g), and 300 (30 g) kcal of MCT or LCT. There was a significant reduction in calories (14%–15% fewer calories) after the MCT compared to the LCT ($P < 0.01$) with a significant dose response after both MCT and LCT ($P < 0.001$). (Adapted from Rolls, B.J. et al., *Am. J. Clin. Nutr.*, 48, 66, 1988. With permission.)

MCT (30%) resulted in lower energy intake (220 kcal) at lunch 4 h later compared to a high oleic acid breakfast (30%) in healthy individuals (St-Onge et al., 2003). A similar study also found that food intake at lunch was lower after a high MCT breakfast (43 g) compared to high oleic or high saturated fat breakfast in men (Van Wymelbeke et al., 1998). The same authors found lower intake at dinner when a high MCT lunch was consumed (Van Wymelbeke et al., 2001).

Studies on weight loss have shown that adding MCT to a very low calorie diet improved satiety and resulted in a higher rate of weight loss without affecting fat-free mass (FFM) compared to LCT in the first 2 weeks of the diet in obese women (Krotkiewski, 2001). As well, consumption of 18–24 g/day of MCT with a weight reduction diet resulted in lower endpoint body weight and a trend toward greater fat mass loss after 16 weeks compared to LCT in overweight subjects (St-Onge and Bosarge, 2008).

Mode of intake (oral vs. gastrointestinal infusions) plays an important role on the effect of chain length on appetite. MCT is more satiating compared to LCT when taken orally in humans (Stubbs and Harbron, 1996; Rolls et al., 1988; Van Wymelbeke et al., 1998, 2001; St-Onge et al., 2003). However, when infused in the stomach, fatty acids with different chain lengths did not show different effects on satiety in rats (Maggio and Koopmans, 1982). On the other hand, intraduodenal infusion of long-chain fatty acids (sodium oleate) inhibited food intake, whereas infusion of medium-chain fatty acids (sodium caprylate) had no effect on food intake in humans (Matzinger et al., 2000).

The time between fat ingestion and subsequent meal has been shown to be an important variable on the effect of chain length on appetite. In diabetic rats, 1.5 mL of MCT suppressed subsequent food intake in the first 2 h after the preload, whereas the reduction in intake with 1.5 mL LCT occurred after 2–4 h compared to a no preload control (Friedman et al., 1983). The difference is proposed to be due to a differential rate of delivery of the ingested lipid to the liver (Friedman et al., 1983). MCT have an advantage over triacylglycerols in getting hydrolyzed and absorbed. Furthermore, MCT are absorbed into the portal system and are rapidly taken up and oxidized by the liver, whereas, LCT are packed into chylomicrons that bypass the liver via the lymphatic system, favoring uptake of LCT into the adipose tissue and muscle. In the mitochondria, MCT do not require acylcarnitine transferase to cross the inner mitochondrial membrane, and therefore, it is not a rate-limiting step in MCT oxidation as it is for LCT (Bremer, 1983). As a result, plasma ketone bodies are increased, which is an indication of enhanced hepatic acid oxidation (Krotkiewski, 2001; Van Wymelbeke et al., 2001). Satiety has been associated with increased fatty acid oxidation in the liver (Langhans, 1996).

Fatty acid chain length seems to be a determinant of gut hormone secretion. Only fatty acids with a chain length greater than C12 are able to stimulate the secretion of Cholecystokinin (CCK), Gastric Inhibitory Peptide (GIP), neurotensin, and pancreatic polypeptide (PP) (McLaughlin et al., 1999; Barbera et al., 2000; Drewe et al., 2008). MCT (<C12) ingested or given intraduodenally did not stimulate CCK or neurotensin release in humans (Matzinger et al., 2000; Drewe et al., 2008). Peptide YY (PYY) secretion is stimulated by both MCT and LCT, but the magnitude and concentration was greater after LCT compared to MCT (Maas et al., 1998).

Overall, when compared to LCT, MCT suppress energy intake when consumed in high amounts. However, intake of high quantities of MCT has been linked to adverse events such as nausea, vomiting, gastrointestinal discomfort, and abdominal discomfort, which limits the amount of MCT that can be incorporated in the diet.

15.2.2 Saturation

Within the same chain length, a greater degree of unsaturation is associated with enhanced satiety, but studies have been inconsistent. In the C-18 fatty acids, linoleic acid resulted in lower appetite and short-term food intake compared to oleic and stearic acids when administered intraduodenally in human subjects (French et al., 2000) (Figure 15.2) or incorporated in foods (Lawton et al., 2000). In weanling Zucker rats, a daily gavage of 100 μL of γ-linolenic acid suppressed food intake and weight gain compared to soy oil (Phinney et al., 1993; Thurmond et al., 1993). γ-Linolenic acid (890 mg/day) has been shown to reduce weight regain in formerly obese humans compared to oleic acid after 1 year of supplementation (Schirmer and Phinney, 2007). On the other hand, a 2-week study of ingestion of oils with different degrees of saturation has reported no difference in satiety scores, energy intake, or weight in overweight subjects consuming a diet high in oleic or γ-linoleic or α-linolenic acid (45 mL oil) (Kamphuis et al., 2001). In overweight subjects,

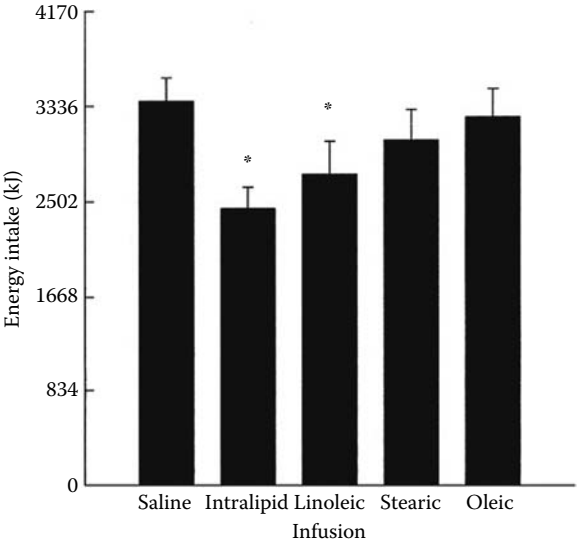

FIGURE 15.2 The effect of upper intestine infusion of C-18 fatty acid-enriched oils on food intake. Infusions were given for 100 min at a rate of 1 mL (8.3 kJ)/min (200 mL/L emulsions). At 90 min after the start of the infusion, subjects were given a liquid meal (strawberry drinking yogurt) and were instructed to eat to comfortable fullness; the infusion continued throughout ingestion of the meal. Values are means + SEM represented by vertical bars. Mean values were significantly different from those for saline (9 g NaCl/L) infusion (*) $P < 0.05$. (Adapted from French, S.J. et al., *Gastroenterology*, 119, 943, 2000. With permission.)

polyunsaturated fatty acid (PUFA) and monounsaturated fatty acid (MUFA) in a preload (63–87 g) suppressed appetite and short-term food intake to the same extent (Flint et al., 2003). Similarly, muffins rich in MUFA (40 g) decreased food intake and appetite in a similar manner to muffins rich in saturated fatty acids (40 g) in lean subjects (Alfenas and Mattes, 2003). However, a recent study found that in rats, MUFA (600 mg/kg/12 h) were able to suppress food intake and decrease body weight to a larger extent compared to PUFA (Vogler et al., 2008).

Several mechanisms have been suggested for the association between the degree of unsaturation of fatty acids and satiety. These mechanisms include both peripheral and central pathways for appetite regulation. In the periphery, plasma CCK in humans (Beardshall et al., 1989) and apolipoprotein A-IV (ApoA-IV) in rats (Kalogeris et al., 1996) have been shown to be more potently released following ingestion of linoleic acid-containing oils compared to other less unsaturated fatty acids. Diunsaturated oil resulted in the highest release of CCK followed by monounsaturated oil with saturated fat showing no effect on plasma CCK release (Beardshall et al., 1989). However, other studies have failed to show a relationship between plasma CCK concentrations and degree of unsaturation (French et al., 2000). Another gut hormone, GLP-1, is increased with α-linolenic acid, an unsaturated long-chain free fatty acid (FFA), in a dose-dependent manner *in vitro*, whereas medium-chain and saturated long-chain fatty acids did not have any effect (Hirasawa et al., 2005). In the same study but in rats, α-linolenic acid resulted in the highest plasma GLP-1 secretion compared to other unsaturated and saturated fatty acids of same chain length (Hirasawa et al., 2005). Centrally, serotonin (5HT), a neurotransmitter, has also been implicated in the association between appetite and unsaturation (Friedman et al., 1986; Mullen and Martin, 1992). In diet-induced obese mice, Neuropeptide Y (NPY) mRNA in the arcuate nucleus was decreased and pro-opiomelanocortin mRNA was increased with the administration of $n-3$ PUFA (Huang et al., 2004). Furthermore, it was suggested that $n-3$ PUFA (α-linolenic, eicosapentaenoic acid [EPA], docosahexaenoic acid [DHA]) can exert anorexigenic effects in the peripheral endocannabinoid system by acting as antagonists to $n-6$ PUFA (Oda, 2007). $n-3$ PUFA competes with the $n-6$ PUFA derivatives, anandamide and 2-arachidonoyl glycerol, which are major agonists for the endocannabinoid system (CB1) (Oda, 2007). In mice, the concentration of $n-3$ PUFA in the brain of mice is inversely related to 2-arachidonoyl glycerol concentrations (Watanabe et al., 2003).

In conclusion, a greater number of double bonds seems to be associated with enhanced satiety when given in high amounts, but large amounts of fat cannot be recommended for human nutrition. Whether moderate amounts of PUFA can affect food intake remains to be established.

15.2.3 Esterification

The degree of esterification of dietary fats seems to play a role in satiety, but only a limited number of studies address it. One-monoglycerides infused into the duodenum of pigs suppressed subsequent intake in excess of its energy content compared to oleic acid. CCK was shown to be the mediator of this effect by administering a CCK antagonist and abolishing the reported suppression of intake by

monoglycerides (Gregory and Rayner, 1987; Gregory et al., 1989). However, in humans, one-monoglycerides, given at 25% of daily energy intake, behaved in a similar manner to triglycerides with regard to appetite and energy intake in the short term and on the subsequent day (Johnstone et al., 1998a,b).

Diacylglycerols (DG), more specifically the 1,3-diacylglycerol isomer, have been reported to lower hunger, appetite, and desire-to-eat over 12 h compared to triacylglycerols when incorporated in foods (~26 g) (Kamphuis et al., 2003b). In terms of weight control, DG consumption is associated with decreased total and visceral body fat accumulation in both rats (Murase et al., 2001) and humans (Nagao et al., 2000; Maki et al., 2002). Suggested mechanisms include enhanced β-oxidation and greater energy expenditure due to the availability of free fatty acids in the portal circulation (Murata et al., 1997). DG have a similar energy value to triacylglycerols (9 kcal/g) (Taguchi et al., 2001). Similar to MCT, DG are incorporated into the portal vein and transported to the liver where they are mostly oxidized (Breckenridge and Kuksis, 1975). However, the potential effect of DG on satiety still requires further investigation.

15.2.4 Functional Fats

15.2.4.1 Conjugated Linoleic Acid

CLAs are a group of geometric and positional isomers of linoleic acid that occur naturally in food (e.g., dairy products, beef). CLA intake from dietary sources is generally <600 mg/day (Kovacs and Mela, 2006). CLA has been implicated with a number of potential health benefits including weight control. Most of the physiologic effects of CLA rest with the *trans*-10, *cis*-12 and *cis*-9, *trans*-11 isomers, linked to weight control effects; and *trans*-10, *cis*-12, associated with body composition changes (Pariza et al., 2001).

The few studies assessing the effect of long-term CLA supplementation on appetite have found inconsistent results in human subjects. Two studies found no effect (Blankson et al., 2000; Medina et al., 2000), while another study found that 13 weeks of mixed isomer CLA intake (1.8 and 3.6 g) increased fullness and satiety compared to baseline (Kamphuis et al., 2003a).

Studies on CLA supplementation and weight control in human subjects have shown that daily intake of 3.4 g of CLA (equal parts of both isomers) reduced total body fat (Blankson et al., 2000) and abdominal fat (Riserus et al., 2001). Furthermore, two other studies have also shown significant reductions in plasma leptin concentrations with daily intake of 3 g of CLA (isomer mix) for 64 days (Medina et al., 2000) and an inverse association with plasma leptin concentrations after 8 weeks of CLA intake (isomer mix) (Belury et al., 2003). Increased β-oxidation has been suggested as a mechanism. For example, infusion of 1% CLA mixture into perfused livers of rats for 2 weeks produced more ketone bodies than 1% linoleic acid-fed rats (Sakano et al., 1999), suggesting that fatty acid oxidation was increased.

While further explorations are required into the effect of CLA on appetite regulation, it is worthwhile noting that CLA *trans*-10, *cis*-12 has been shown to induce inflammation and hyperinsulinemia in animal models (Poirier et al., 2006) and impair insulin sensitivity in a few clinical trials (Riserus et al., 2002, 2004; Moloney

et al., 2004), which raises concerns regarding supplementation with CLA *trans*-10, *cis*-12. Further research is needed in order to asses the health benefits or risks of CLA on the long term.

15.2.4.2 Olibra

Olibra is a novel fat emulsion consisting of a mixture of fractionated palm oil (40%) and fractionated oat oil (2.5%) in water. Olibra has been associated with increased satiety and decreased energy intake (~1 MJ difference) at a meal 4 h later when added to yogurt (5 g) in nonobese, overweight, and obese subjects. The decrease in intake was maintained for the rest of the day (Burns et al., 2000, 2001, 2002). However, a recent study has failed to find an effect on food intake and appetite (Logan et al., 2006). Speculated mechanisms include the physiochemical stability of the emulsion resulting in delayed digestion and stimulation of distal small intestine receptors and gut hormone secretion, but there is no supporting evidence in the literature. One study reported an increase in plasma GLP-1 response but only at 180 min after 25 weeks consumption of 10 g of Olibra (Diepvens et al., 2007). However, the mechanism of action requires further clarification.

15.3 FAT IN RELATION TO OTHER MACRONUTRIENTS AND SATIETY

15.3.1 Fiber

Combining fat with fiber has been shown to increase the satiating potential of fat (French and Read, 1994; Burton-Freeman, 2000; Burton-Freeman et al., 2002). Fiber intake is associated with enhanced satiety and reduced food intake (Howarth et al., 2001; Samra and Anderson, 2007). The properties of some fibers that prolong the contact of dietary fats with the intestinal mucosa and retard fat digestion may contribute to enhanced satiety. Partially hydrolyzed guar gum (PHGG) (6 g) added to yogurt has been shown to decrease bioaccessibility of fat in a dynamic model of the gastrointestinal tract (Minekus et al., 2005). Accordingly, PHGG was able to suppress postprandial serum lipid concentrations in healthy volunteers with moderate hypertriglyceridemia after a high-fat high-cholesterol meal (Kondo et al., 2004). Viscous fiber (12 g guar gum) added to high-fat treatment prolonged satiety and slowed gastric emptying compared to a high-fat treatment without fiber (French and Read, 1994). This effect may be partly mediated through enhanced release of cholecystokinin. Adding fiber (20 g) to a low-fat treatment (16–23 g) increased plasma CCK secretion and subjective satiety to a similar extent as a high-fat treatment (31–40 g) without fiber in healthy individuals (Burton-Freeman et al., 2002).

Combining fat with fiber is an interesting venue to enhance the satiating potential of fat-containing products and therefore merits further exploration.

15.3.2 Carbohydrate

Short-term studies investigating satiety after meals varying in fat-to-CHO ratios have been inconsistent (Fryer et al., 1955; Driver, 1988; van Amelsvoort et al., 1989; Foltin

et al., 1990). Meals with a high-fat–carbohydrate ratio either show a weaker suppression of hunger (van Amelsvoort et al., 1989; Cotton et al., 2007) or have the same effect (van Amelsvoort et al., 1989; Foltin et al., 1990; de et al., 1992; Stubbs et al., 1995) compared to meals with a low-fat–carbohydrate ratio. Such inconsistencies could result from diverse experimental parameters and not upon actual differences in the metabolic properties of the macronutrients themselves. For example, variations in the type of fat and carbohydrate used, palatability and energy density of the test meal, population studied (gender, age, dietary restraint, body mass index, etc.), and the mode of test food delivery could result in different outcomes between studies.

15.4 FACTORS BEHIND THE IMPACT OF FAT ON SATIETY

15.4.1 Physical Properties

The chemical composition of fatty acids affects the physical properties of the triacylglycerol molecule. For example, the melting point of the fatty acid is inversely related to its degree of unsaturation (Table 15.1). Therefore, the degree of unsaturation is likely to affect the ease of emulsification of the triacylglycerol in the digestive tract, which is predicted to markedly affect the ease of digestion and absorption of fatty acids. The result is modulation of the rate of interaction between fatty acids and satiety signals on the intestinal wall (Small, 1991).

15.4.2 Cephalic Modulation

Cephalic stimulation with fat is associated with modulation of digestive processes and appetite. Gilbertson et al. have shown that different fatty acids can be discriminated by taste receptors on the tongue of rats (Gilbertson et al., 1997). K^+ currents on the tongue were shown to be markedly inhibited by linoleic and linolenic acids but not by stearic or oleic acid. These findings seem to parallel the human food intake data (Figure 15.1). K^+ currents are identified in other tissues including the duodenum (Gilbertson, 1998), suggesting that similar signaling from the small intestine in response to fatty acids may occur. In general, presence of food in the mouth is associated with modulation of digestive processes (Helman, 1988; Teff and

TABLE 15.1
Effect of Increasing Unsaturation of Component Fatty Acids on the Melting Point of Pure Long-Chain Triacylglycerols

Fatty Acid	Structure	Melting Point (°C)
Stearic acid	18:0	67.9
Oleic acid	18:1	16.0
Linoleic acid	18:2	−5.0
Linolenic acid	18:3	−11.0

Engelman, 1996). Cephalic stimulation with fats, particularly long-chain unsaturated fatty acids, elicit several digestive processes including gastric lipase (Wojdemann et al., 1997) and pancreatic digestive enzymes secretion (Hiraoka et al., 2003), CCK release (Wisen et al., 1992), and pancreatic polypeptide secretion (Crystal and Teff, 2006). Stimulation of the human tongue with full-fat soft cheese increases the serum lipid response to an intragastric load of triacylglycerol when compared with nonfat soft cheese or no stimulation (Mattes, 1996). Subjects were blinded on the nature of the cheese and were not able to distinguish between the cheese samples in sensory tests suggesting that a specific chemosensory or tactile mechanism from the mouth mediated this change.

Few studies have investigated the association between oral stimulation of fat and satiety. A study has shown that oral stimulation with different fats by modified-sham-feeding (MSF) resulted in increased feelings of satiety compared with water in human subjects, with linoleic acid showing the strongest response (Smeets and Westerterp-Plantenga, 2006). On the other hand, sham feeding a high-fat cake increased food intake at the next meal compared to nonfat cake in restrained eaters (Crystal and Teff, 2006).

15.4.3 Oxidative Qualities

Fatty acid oxidation in the liver seems to be associated with appetite and food intake (Friedman and Tordoff, 1986; Friedman et al., 1986; Langhans and Scharrer, 1987; Stubbs et al., 1995). Studies on the effect of fat oxidation on food intake suggest that fat that is oxidized generates a satiety signal and, in contrast, fat that is stored is less satiating (Friedman, 1998). In rats, feeding is stimulated when the oxidation of long-chain fatty acids is inhibited by methyl palmoxirate, which blocks carnitine palmitoyltransferase-1 and decreases transport of long-chain fatty acids to the mitochondria (Friedman and Tordoff, 1986; Friedman et al., 1990). Perhaps because MCT do not require carnitine palmitoyltransferase-1 for their transport into the mitochondria, they are more readily oxidized in the mitochondria compared to LCT, which need carnitine palmitoyltransferase-1 (Williams et al., 1968). The fast oxidation rate may partly explain the reported reduced feeding response after MCT intake compared to LCT (Friedman et al., 1990).

15.4.4 Gut Hormones

Regulation of appetite upon fat consumption has been shown to be mediated by a number of gut hormones. Administration of the CCK-A receptor antagonist loxiglumide suppressed the reduction of food intake resulting from intraduodenal fat administration; suggesting that CCK is a candidate mediator of this interaction (Matzinger et al., 2000). Duodenal fat administration in animals has been shown to result in elevated concentrations of plasma CCK (Schwartz et al., 1999). Another candidate peptide is enterostatin, which is produced from pancreatic colipase in equimolar amounts to colipase in the intestine (Erlanson-Albertsson and York, 1997). Furthermore, long-chain triglycerides have been shown to suppress plasma ghrelin (Heath et al., 2004) and stimulate secretion of CCK, GIP, neurotensin, PP,

and PYY in humans (Spiller et al., 1984; Maas et al., 1998; Barbera et al., 2000) and ApoA-IV (Liu et al., 2003) in rats. PUFA stimulated the release of plasma GLP-1 in mice (Hirasawa et al., 2005).

15.4.5 Delayed Fat Digestion

Dietary fat is usually digested and absorbed in the duodenum, but if digestion and absorption of fat occurs in the distal sections of the small intestine, it stimulates a strong feedback signal associated with slowing of gastrointestinal transit and release of various satiety hormones (Read et al., 1984; Spiller et al., 1984; Welch et al., 1985; Van Citters and Lin, 1999). Infusion of corn oil into the jejunum induced early satiety and reduced energy intake in healthy volunteers compared to infusion in the duodenum (Welch et al., 1985, 1988). Furthermore, administration of a compound that retards fat digestion, by inhibiting the lipase–colipase-mediated fat hydrolysis, was associated with reduced food intake and elevated concentrations of plasma CCK and enterostatin in rats (Mei et al., 2006). Therefore, delaying fat digestion might be a promising method to enhance the satiating effect of fats and deserves further research.

15.4.6 Inhibited Fat Digestion

Products of fat digestion seem to be essential for a fat-induced satiety response. Inhibition of fat digestion by administration of a lipase inhibitor (tetrahydrolipstatin) has been shown to decrease proximal gastric relaxation (Feinle et al., 2001) and antropyloroduodenal motility (Feinle et al., 2003) resulting from intraduodenal fat administration. Furthermore, the suppression of fat breakdown modulates gut hormone release by decreasing the secretion of CCK, GLP-1, PP, and PYY and the suppression of ghrelin (Feinle et al., 2001, 2003; Feinle-Bisset et al., 2005) resulting from intraduodenal infusion of triacylglycerol. When Orlistat, a lipase inhibitor, was administered with a high-fat preload (70% fat), suppression of food intake at the next meal was prevented (Feinle et al., 2003) and the fat-induced increase in circulating neurotensin and CCK was blocked in healthy subjects (Drewe et al., 2008). These observations suggest that digestion of fats with the consequent release of free fatty acids into the small intestine is important for the effects of fat on gastrointestinal function and energy intake (Little et al., 2007).

15.4.7 Length of Small Intestine Exposed to Fat

Gastric emptying rate and intestinal transit time seem to be dependent on the length and region of small intestine exposed to dietary fats. Studies on dogs implanted with intestinal fistulae have demonstrated that regardless of the region exposed, gastric emptying was inhibited only when more than 15 cm of the small intestine was exposed to fat (sodium oleate) with the highest degree of inhibition achieved after exposure of more than 150 cm (Lin et al., 1989, 1990). However, exposure of the proximal small intestine to fat produced a stronger inhibition of gastric emptying compared to the distal small intestine (Lin et al., 1990).

Intestinal transit is inhibited by fat in both the proximal (jejunal brake) (Lin et al., 1996a) and distal gut (ileal brake) (Read et al., 1984; Spiller et al., 1984). However, contrary to gastric emptying, intestinal transit was more potently inhibited by fat in the distal than in the proximal small intestine in dogs implanted with intestinal fistulae (Lin et al., 1997). PYY has been suggested as the primary mediator of fat-induced ileal brake (Lin et al., 1996b). PYY releasing cells are located in the ileum and colon (Adrian et al., 1985; Taylor, 1985); therefore, fat in the proximal gut can release PYY indirectly via CCK (McFadden et al., 1992) or by direct stimulation of these endocrine cells in the distal small intestine (Aponte et al., 1988).

Suppression of energy intake has also been reported to be dependent on the length of small intestine exposed to fat. In rats, energy intake was decreased only when the entire small intestine was exposed to fat. Exposing only 35 cm of the jejunum to fat did not affect energy intake (Meyer et al., 1998).

15.5 CONCLUSION

In the present chapter, we tried to answer the question: Is dietary fat satiating? Within a controlled environment, yes, fats do have an effect on satiety and appear to regulate appetite through several mechanisms including the release of appetite hormones and inhibition of gastric emptying and intestinal transit. Certain types of fats are more satiating than others. However, in free-living conditions, the situation is complicated. Several genetic, psychological, and behavioral factors interact with physiological and metabolic systems on their effect on food behavior in free-living individuals. For instance, it has been found that certain individuals are genetically "immune" to the effects of a high-fat diet, suggesting that the consumption of a high-fat diet does not universally lead to weight gain. These individuals habitually consume a high-fat diet and remain lean and are therefore labeled as having "high-fat phenotypes" while others gain weight and are labeled as having "low-fat phenotypes" (Cooling and Blundell, 2001). Furthermore, in a free-living environment, the presence of highly palatable foods, a characteristic of high-fat foods, could chronically activate the hedonic system which would promote higher appetite and more energy intake (Lowe and Levine, 2005). The hedonic system is regarded as pleasure associated with eating palatable foods. In an environment with unlimited availability of highly palatable foods, there is a concern of how much the homeostatic appetite regulatory mechanisms can override the hedonic components and hyperresponsiveness to palatable foods (Blundell et al., 2005).

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Part VI

Genetic Factors Influencing Fat Preference and Metabolism

16 Heritable Variation in Fat Preference

Danielle R. Reed

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16.1 INTRODUCTION

For humans, eating is often a group rather than a solitary activity, and it is inevitable when eating with others that individual differences in food preferences become obvious. These food preferences form early in life (Mennella et al., 2001) and persist into adulthood (Nicklaus et al., 2004). People like to eat familiar foods that are safe and avoid foods associated, even indirectly, with illness. However, pleasant experiences and time also help to form food preferences. For instance, the ability to tolerate and even like bitterness increases as children grow to adulthood, and the liking for sweet and sour decreases (Desor and Beauchamp, 1987; Liem and Mennella, 2003). Over a lifetime, new foods are tried, rejected, or incorporated into the diet. Against this backdrop of development and environment, there are inborn differences in food likes and dislikes which may be due to genetic constitution. There is a genetic basis to bitter detection in humans (Bufe et al., 2005) and given that fat intake is moderately to highly heritable, it is likely that genotype contributes to food selection and, by extension, to fat preference.

The focus here is on how individual differences in fat preference are formed and, in particular, the evidence that the liking for fat is influenced by genotype. The interest in dietary fat arises because its intake is tied to metabolic syndrome, a constellation of disorders that feature obesity, diabetes, and hypertension. An adage is that everything that tastes good is bad for you, and the liking for fat fits well into this viewpoint: fat is desirable and when people are given the opportunity to do so, many will adopt a high-fat diet. Two aspects of fat make it attractive, its sensory qualities (Reed et al., 1992b) and postingestive consequences (i.e., feelings of satiety). Fat is sensed in the mouth and although the texture is a key feature of its sensory properties, fat itself may be a legitimate taste stimulus. The evidence for this assertion is recent and reviewed below, but it is useful to know that fat has been considered a taste by some through the ages. For instance, Fernel wrote "There are nine classes of flavors, and the sense of taste recognizes no others: acrid, tart, fatty; salty, sour, and sweet; bitter, pungent, and insipid" (Fernelius, 1581). While controversies arise when applying the term "taste" to fat, and the issue is dealt with elsewhere in this book, "umami" as a taste was equally controversial but it was readily adopted as a fifth basic taste once the receptor(s) was identified (Chaudhari et al., 2000; Nelson et al., 2002). Likewise, when the oral receptors for fat are unequivocally identified, its place as a basic taste will probably become equally well accepted. What is known about fat as a taste is outlined below.

Taste is both the gatekeeper and advance messenger of ingestion, keeping out bad food and warning the gastrointestinal system about the impending rush of nutrients. One of the effects of fat stimuli in the mouth is to prepare the body for calories, setting off a cephalic phase response. This cascade of events may be a general response to incoming dietary fat in mammals because it is found in rats (Ramirez, 1985) as well as people (Mattes, 2001; Crystal and Teff, 2006). Under normal circumstances, once fat is ingested, it is briefly held in the stomach and then absorbed in the intestines. From here it is either oxidized for energy or stored, primarily in adipose tissue. In some abnormal states, such as untreated diabetes, fat is more easily oxidized than carbohydrate and is thus preferred, at least in experiments using animal models (Tordoff et al., 1987). In addition to the other benefits of fat, it contains pharmacologically active substances, for instance, olive oil has an anti-inflammatory agent (Beauchamp et al., 2005). These compounds may also contribute to the human liking for fat.

16.2 FAT AS A TASTE QUALITY

The details of how fat is sensed in the mouth are not well understood but it is worth reviewing what is currently known to help put the potential for genetic influences into perspective. The chemical properties of fat (as opposed to its texture) are probably sensed after it is hydrolyzed to free fatty acids rather than sensed as triglycerides. This conclusion is drawn from a study in rats which demonstrated that when lingual lipase (the oral enzyme responsible for breaking down triglyceride to free fatty acids) is reduced, triglycerides become less preferred while the preference for free fatty acids remain unchanged (Kawai and Fushiki, 2003). There is a belief that humans do not have as much lingual lipase as rats (Pritchard et al., 1967) and therefore probably

do not rely on fat "taste" to the same extent, but this widely held belief is due for reconsideration, perhaps using other methods to measure lingual lipase, such as proteomics. From rat studies, we know that lingual lipase acts quickly on the tongue to liberate free fatty acids but if oral fat perception requires an enzymatic step, it may explain why fat-containing foods are often savored. Perhaps, keeping the fat-containing food in the mouth for a few extra seconds enhances its enjoyment. Lingual lipase is also most highly concentrated around the circumvallate papillae (in the rear of the mouth) and so the path to fat perception may start there (Hamosh and Scow, 1973). Although the majority of studies on oral free fatty acid detection are done using rats and mice as experimental subjects, humans can detect them in solution, and people differ markedly in their threshold and perceptions of intensity for free fatty acids (Chale-Rush et al., 2007a).

How are free fatty acids sensed in the mouth so that information about their presence can be relayed to the brain? The current hypothesis is that they are sensed in the same way that some other tastes are sensed in the mouth, i.e., they are detected by G-protein-coupled receptors (GPCRs) in taste receptor cells on the tongue, which in turn signal through a common second-messenger cascade involving G-proteins, phospholipase C beta2, and the transient receptor potential M5 (TRPM5) ion channel. This signal is then relayed to the brain by gustatory nerves and decoded.

Many lines of evidence support this model of fat perception. Investigators have recorded from or cut the gustatory nerves (as opposed to trigeminal sensory nerves) and demonstrated that information about the presence of fat in the mouth is conveyed to the brain (Stratford et al., 2006; Gaillard et al., 2007; Stratford et al., 2008). Furthermore, free fatty acids elicit an increase in intracellular calcium in about 30% of rat taste receptor cells (Liu et al., 2008a) and this event is associated with cell signaling through the release of neurotransmitters. In addition, several GPCRs have been suggested to be the "fat taste receptor": one such is GPR120, which is expressed in taste tissue but not nonsensory epithelium (Cartoni et al., 2007; Tsuzuki, 2007; Matsumura et al., 2008). When introduced into a cell-based assay, GPR120 causes the cell to respond to fatty acids by increasing intracellular calcium (Tsuzuki, 2007; Eguchi, 2008), similar to the response seen by other GPCRs specific to classic taste stimuli (Adler et al., 2000; Chandrashekar et al., 2000; Nelson et al., 2001, 2002). GPR40 has also been implicated in fat taste but this finding is controversial; some investigators find that receptor is expressed in taste receptor cells (Cartoni et al., 2007) while others do not (Matsumura et al., 2008). However, mice genetically engineered to be null for this receptor (GPR40) have reduced preferences for fats like corn oil, which supports the role of this receptor in oral fat perception (although this effect could also come from other tissues since GPR40 is also expressed in pancreatic β cells) (Itoh et al., 2003).

Another membrane-bound protein that is related to fat taste is CD36. Several lines of evidence support its role in taste: (a) it is detected at the apical surface of taste receptor cells (where chemical stimuli in the mouth and receptors would interact), (b) it colocalizes in taste papillae with known signaling molecules (Laugerette et al., 2005), (c) the normal increase in intracellular calcium of taste receptor cells in response to free fatty acids is blocked in CD36 knockout mice, and (d) the activation of brain areas associated with fatty acid stimulation is eliminated in these knockout

mice (Gaillard et al., 2007). Furthermore, (e) CD36 knockout mice are indifferent to fat solutions that mice with intact alleles prefer (Fushiki and Kawai, 2005; Laugerette et al., 2005; Sclafani et al., 2007a). However, CD36 and GPR120 are not often found in the same taste receptor cells (Matsumura et al., 2008), so whether they reflect two mechanisms of fat sensing or work together through cell-to-cell signaling is not known. There are a few more available pieces of information about the signaling cascade involved in fatty acid perception. Gustducin (a G-protein found in taste receptor cells) does not seem to be involved in fat taste because knockout of this gene does not affect fat preference in mice (Sclafani et al., 2007a,b). However, the transient receptor potential cation channel found in taste cells (TRPM5) is important in fat perception because knockout of this gene abolishes fat intake (Sclafani et al., 2007a,b). Taken together, free fatty acids are detected in taste receptor cells, and this information is conveyed through gustatory nerves where the information is interpreted by the brain. Although several genes and their associated proteins involved in the transduction pathway have been identified, the details are not well understood. However, the genes that are known to be involved are polymorphic in humans (see "Electronic Resources"), and therefore, alleles of these receptors and other signaling molecules would be a natural target of genetic investigation.

16.3 ANIMAL MODELS OF FAT PREFERENCE

A brief review of the advances in knowledge through animal research will be helpful in interpreting the human studies. Research on fat preferences has focused on mice and rats, mostly because they are commensal with humans and have similar food preferences. In fact, one way to make mice and rats fat is to feed them human "junk-food" (Sclafani and Springer, 1976), which is typically high in fat. However, research in the area of taste and food preferences is developing using flies and worms as model systems and these approaches will also add to our knowledge of the molecular aspects of fat preference (Gordesky-Gold et al., 2008). One of the important messages from animal research is the power of both genetics and experience to change fat preference. Rats given a sample of pure fat to eat for several days later select a diet very high in fat when offered a choice between fat, protein, and carbohydrate (Reed et al., 1992a,b). Rats offered only a high-fat diet become avid fat consumers and will drink large amounts of pure oil (Reed et al., 1991). Although these experiments demonstrate the power of experience on fat preference, the results of genetic studies have also reinforced the point that fat preference is inborn. Inbred strains of rats and mice treated similarly differ markedly in their willingness to ingest fat and they also differ in how much weight they gain when they do (Schemmel et al., 1970; West et al., 1992; Lewis et al., 2007; Svenson et al., 2007). Specific strains have been identified and studied because of their disparate fat preference or response to a high-fat diet (West et al., 1995; Levin et al., 1997; Smith Richards et al., 2002; Almind and Kahn, 2004; Collins et al., 2004; Ehrich et al., 2005; Kumar et al., 2007; Svenson et al., 2007; Tordoff et al., 2008).

With the advent of new methods in molecular genetics, it is possible to identify the specific genes that contribute to individual differences among mice (and humans,

see below) in fat preference. One such study was conducted by crossing inbred strains of mice that had either a high- or low-fat preference, and evaluating the offspring for macronutrient preference. By comparing the parents, grandparents, and offspring from three generations, the genetic effects on fat preference could be established ($h^2 = 0.19$) (Smith Richards et al., 2002), an estimate strikingly similar to the heritability of fat preference in humans (see below). The investigators conducted a genome-wide scan and found three chromosomal regions where fat-preferring mice shared DNA in common more often than could be expected by chance. These loci were on chromosomes 8 and 18, and also on the X-chromosome. The genes that contribute to the fat preference within these regions are not known, but this landmark study demonstrates that specific genetic regions are associated with fat preference and that it may be possible soon to identify the specific genes involved. This work is likely to advance further, because one of the benefits to studying model systems for fat preference is the tighter control over experience and learning and the wealth of molecular resources, e.g., genetic engineering, microarray, proteomics, and breeding techniques available.

16.4 HUMAN FAT PREFERENCE

Animal models are useful because they may point the way to understand human behavior toward high-fat foods. The study of fat preference in animal models is simpler than for humans because it is assumed that when animals are offered a choice, the item selected is the one preferred. However, this is not the case in humans, who are motivated by complex thoughts and constraints such as health beliefs, price, advertising, and social embarrassment. To make the problem even harder, humans are faced with multiple choices and rarely encounter the simple two-food choice offered to rats or mice. Therefore, to understand fat preference in humans, it is important to explicitly consider how it is measured and the limitations of these methods. This issue of measurement is also of particular concern for genetic studies because accurate estimates of heritability and genotype–phenotype associations depend upon the ability to assess a large number of people using methods that detect stable individual differences, and thus, even the most rudimentary measures in a controlled laboratory study are often out of reach.

16.5 METHODS OF MEASURING FAT PREFERENCE

Because of the impracticality of testing thousands of people in the lab, most genetic studies have relied on self-reported fat intake as a proxy measure of fat preference, with the expectation that in the broadest terms, people eat more of the food they prefer. A common way to collect data is through food diaries, in which the subject tracks food intake. Advantages of this method are that it is readily available, the subject is in their habitual environment and eating as usual—or nearly so, because there will be effects of recordkeeping on food intake. The limitation is that even for subjects who are motivated and honest in their reports, errors are introduced because of estimates of food composition or portion size, and there is also the risk

that subjects may try to mislead the investigator by failing to record foods that are undesirable. The underreporting of food intake by the diary method is a well-known and often studied phenomenon, and to make matters worse in the realm of fat preference, these types of foods are sometimes selectively underreported (Goris et al., 2000). To circumvent this bias, creative investigators have tried to reduce recording errors, by asking subjects to photograph their meals immediately before they are eaten (de Castro, 2000). Another pencil and paper method to assess food intake is through food frequency questionnaires, which require that subjects recall their food intake and give information about how many times they have consumed a certain food during the past. The benefit of this method is that the time spent in collecting data is reduced and in contrast to food diaries, it does not rely on a subject's sustained attention and continuous participation. The disadvantage is that this method is a less sensitive way to measure food intake.

Another way to measure human food intake is to have subjects live in structured environments in which their food choices and intake can be monitored more precisely—these types of studies can be conducted in a cafeteria where subjects come for meal time (Levitsky and Youn, 2004), a restaurant (Wansink et al., 2007), or in environments where subjects come to live (for short periods of time) so that their food intake can be closely monitored (Larson et al., 1995). Another method to measure fat intake and preference is to invite subjects into the laboratory and give them an opportunity to select food for a single meal or to ask subjects to taste and rate foods that differ in fat content (Mela and Sacchetti, 1991). The advantage of these types of laboratory studies is that the amount and type of food consumed can be more accurately monitored, but the limitation is that the subjects' eating behavior is unlikely to be the same as it would be were the subjects living in their normal environment. The benefit of these short-term laboratory-based methods is that they can be tailored to ask specific research questions, but there are limitations because short-term tests are less likely to generalize to food preferences and intake outside of the laboratory (Pangborn and Giovanni, 1984). Furthermore, subjects are not adept at discerning the fat content of food in short-term tests (Mela and Christensen, 1987).

16.6 FAT PREFERENCE AS A HERITABLE HUMAN TRAIT

With these limitations in mind, the liking for a diet high or low in fat may be a stable trait (phenotype) of human subjects (Geiselman et al., 1998; Blundell and Cooling, 1999) and identifying a stable trait is the first step in establishing heritability. However, it is surprisingly rare for investigators to include reliability data in food preference studies with a genetic focus. Therefore, the genetic studies reviewed below need to be evaluated in this context: although fat preference may be a stable individual trait, methods to assess it are imperfect and thus, true heritability may be higher than reported due to measurement errors in the methods used to assess fat preference. It is also important to remember that exposure, experience, learning, and culture shape fat preference. Studies in rats suggest that maternal exposure to a high-fat diet results in offspring or even grandchildren that have an increased fat preference (Wu and Suzuki, 2006; Bayol et al., 2007). Other studies have suggested that maternal diet can affect the methylation of gene promoters which can in turn

change gene regulation in the offspring (Waterland and Jirtle, 2003; Burdge et al., 2007). Given these observations, it is a short step to speculate that women who eat a high-fat diet during pregnancy and lactation might pass this trait on to their offspring through epigenetic mechanisms. In addition to exposure during development, fat preference may be due in part to cultural learning and the most obvious example is the changes that occur when people immigrate. However, something as simple as an enforced change of routine can change preference too, for instance, subjects asked to eat a low-fat diet report less liking for some high-fat foods (Mattes, 1993; Ledikwe et al., 2007), indicating that fat preference is at least partly affected by recent dietary experience.

Since learning and experience affect fat preference, it is hard to parse the inborn effects of genotype from these closely allied influences. There is cross cultural research using populations that differ in both genotype and culture and there have been some attempts to measure fat preference in different groups. One such study was focused on the fat preference of Pima Indians and Caucasians. Pima Indians are a native population in the United States with a high prevalence of obesity. They traditionally lived in a frugal desert climate and were of normal weight but as circumstances changed they have adopted a diet higher in calories and fat, and have become one of the most obese populations in the world. Although obesity is often associated with elevated fat preferences (Drewnowski et al., 1985), on average, the Pima Indians have lower preferences than do Caucasians (Salbe et al., 2004). Although genetics and environment probably both contribute to this group difference, the design of the experiment does not allow us to estimate the contribution of each factor.

16.7 GENETIC COMPONENT TO HUMAN FAT PREFERENCE

To try to untangle the effects of environment, learning, and culture, the study of twins provides a useful methodology. Monozygotic (MZ) twins are genetically identical (although recent studies challenge this notion (Fraga et al., 2005); see below) whereas dizygotic (DZ) twins are no more alike genetically than siblings. Thus, the behavior of MZ and DZ twins can be compared and heritability assessed. An alternative to twin studies are family studies, which follow a similar principle. The degree of genetic sharing between family members is compared with the degree of phenotypic similarity and the contribution of genotype to the trait is evaluated. The scientific literature about twins and fat preference was reviewed several years ago and the heritability estimates ranged widely from study to study (Reed et al., 1997). This conclusion has not changed when data from more recent studies are considered (Stafleu et al., 1994; Feunekes et al., 1997; Yeo et al., 1997; Vachon et al., 1998).

16.8 A NEW LOOK AT TWIN STUDIES OF HERITABILITY

As mentioned above, although twins are thought to be genetically identical, several lines of evidence suggest that this is not strictly accurate: the mutation rate for DNA replication, while low, is not zero, and so for a given cell lineage (including the germ cells), twins can differ in genotype based on spontaneous mutation. In addition, the

results of recent studies suggest two other sources of differences in the genomes of twins. First, one of the surprises about the human genome is the extent to which small patches of the genome are duplicated, giving rise to multiple copies of genes (Sharp et al., 2005; Wong et al., 2007). While this type of duplication was known and appreciated as a part of a sequence of events (duplication and diversification), giving birth to new genes, the degree of duplication, especially among sensory genes, was unexpected (Nozawa et al., 2007). Even more surprising is that studies of MZ twins have revealed instances where genetically "identical" twins differ in gene copy number (Bruder et al., 2008). Second, the other source of differences in the genome of MZ twins is the degree of epigenetic modification, as measured by the methylation of particular parts of the genome. This methylation is thought to affect gene expression, and so twins that differ in methylation status at a particular location will presumably differ in the rate of transcription of a given gene (Fraga et al., 2005).

These observations about the genome of human twins have implications for fat preference. First, MZ twins that differ in body weight have been described and a key difference between these twins who are discordant for body weight is their preference for dietary fat (Rissanen et al., 2002), which raises the possibility that mutation, copy number variation, or epigenetic events might have a detectable effect on fat preference. The second implication is more general, which is that heritability estimates depend upon the assumption that MZ twins are genetically identical—if they are not, current estimates of heritability for the fat preference phenotype (as well as other traits) are underestimates.

16.9 FAMILY SIMILARITY AND HERITABILITY OF FAT PREFERENCE

Family and twin studies suggest fat intake and preference are heritable traits but individual studies differ in the strength of this effect. Table 16.1 contains a summary of the relevant statistics from studies that measured the percent of calories ingested as fat, which we will refer to as fat preference for simplicity. Some studies compute the percentage of the phenotype that can be accounted for by the additive effect of genes (heritability, h^2) and some studies report the correlation among relative pairs, e.g., similarity among siblings. No attempt was made in these family correlation studies to estimate the additive effect of genes relative to household or unshared environmental effects. When these data are considered as a whole, the most obvious point is that the values range from no heritability at all to strikingly high values for a behavioral trait ($>h^2 = 0.48$). Likewise, family similarity ranged from none ($r = 0.0$) to strong ($r > 0.6$). Other points also emerge from these studies. There was one study of twins reared apart, an experimental genetic design often considered the most informative (since twins are not reared in the same environment and the degree of similarity is thought to be genetic in origin). For this study, the heritability (h^2) of fat intake was 0.35. This study makes the useful point that living in the same household is not a necessary prerequisite for family members to resemble each other in fat intake (Hur et al., 1998).

TABLE 16.1
Family Correlations or Heritability Estimates of Percent of Calories as Fat

Subjects	*N* Relative Pairs	Method	Corr (*r*) or h^2	References
Brother–brother	431	2-day food diary	$r = 0.67$	Feunekes et al. (1997)
Father–daughter	889	2-day food diary	$r = 0.39$	Feunekes et al. (1997)
Father–son	914	2-day food diary	$r = 0.40$	Feunekes et al. (1997)
Mother–daughter	998	2-day food diary	$r = 0.44$	Feunekes et al. (1997)
Mother–father	940	2-day food diary	$r = 0.54$	Feunekes et al. (1997)
Mother–son	994	2-day food diary	$r = 0.37$	Feunekes et al. (1997)
Sibling–sibling	1541	2-day food diary	$r = 0.65$	Feunekes et al. (1997)
Sister–sister	467	2-day food diary	$r = 0.69$	Feunekes et al. (1997)
Parent–child	198	24-h recall	$r = 0.22$[a]	Laskarzewski et al. (1980)
Brother–brother	93	3-day food diary	$r = 0.47$	Vauthier et al. (1996)
Brother–sister	179	3-day food diary	$r = 0.43$	Vauthier et al. (1996)
Father–daughter	409	3-day food diary	$r = 0.28$	Vauthier et al. (1996)
Father–son	365	3-day food diary	$r = 0.39$	Vauthier et al. (1996)
Mother–daughter	409	3-day food diary	$r = 0.40$	Vauthier et al. (1996)
Mother–father	387	3-day food diary	$r = 0.39$	Vauthier et al. (1996)
Mother–son	365	3-day food diary	$r = 0.31$	Vauthier et al. (1996)
Sister–sister	115	3-day food diary	$r = 0.52$	Vauthier et al. (1996)
Families	375	3-day food diary	$h^2 = 0.19$	Perusse et al. (1988)
Mother–father	339	3-day food diary	$r = 0.45$	Perusse et al. (1988)
Parent–child	1212	3-day food diary	$r = 0.31$	Perusse et al. (1988)
Siblings	361	3-day food diary	$r = 0.36$	Perusse et al. (1988)
DZ twins	60	3-day food diary	$r = 0.59$	Perusse et al. (1988)
MZ twins	59	3-day food diary	$r = 0.61$	Perusse et al. (1988)
Uncle/aunt–niece/nephew	88	3-day food diary	$r = 0.21$	Perusse et al. (1988)
First degree cousins	95	3-day food diary	$r = 0.42$	Perusse et al. (1988)
Unrelated siblings	115	3-day food diary	$r = 0.04$	Perusse et al. (1988)
Foster parent-adopted child	314	3-day food diary	$r = 0.18$	Perusse et al. (1988)
Mother–father	83	3-day food diary	$r = 0.39$	Oliveria et al. (1992)
Father–child	83	3-day food diary	$r = 0.15$	Oliveria et al. (1992)
Mother–child	87	3-day food diary	$r = 0.46$	Oliveria et al. (1992)
Father–son	50	3-day food diary	$r = 0.15$	Oliveria et al. (1992)
Father–daughter	33	3-day food diary	$r = 0.12$	Oliveria et al. (1992)
Mother–son	54	3-day food diary	$r = 0.40$	Oliveria et al. (1992)
Mother–daughter	33	3-day food diary	$r = 0.49$	Oliveria et al. (1992)
Mother–father (A)	87 and 58	3-day food diary	$r = 0.44$	Patterson et al. (1988)
Mother–father (MA)	101 and 42	3-day food diary	$r = 0.14$	Patterson et al. (1988)
Father–older child (A)	58 and 37	3-day food diary	$r = -0.23$	Patterson et al. (1988)
Father–older child (MA)	42 and 63	3-day food diary	$r = -0.12$	Patterson et al. (1988)

(*continued*)

TABLE 16.1 (continued)
Family Correlations or Heritability Estimates of Percent of Calories as Fat

Subjects	*N* Relative Pairs	Method	Corr (*r*) or h^2	References
Father–younger child (A)	58 and 95	3-day food diary	$r = 0.31$	Patterson et al. (1988)
Father–younger child (MA)	42 and 106	3-day food diary	$r = -0.09$	Patterson et al. (1988)
Mother–older child (A)	87 and 37	3-day food diary	$r = 0.23$	Patterson et al. (1988)
Mother–older child (MA)	101 and 63	3-day food diary	$r = 0.44$	Patterson et al. (1988)
Mother–younger child (A)	87 and 95	3-day food diary	$r = 0.24$	Patterson et al. (1988)
Mother–younger child (MA)	101 and 106	3-day food diary	$r = 0.20$	Patterson et al. (1988)
Sibling–sibling (A)	132	3-day food diary	$r = 0.06$	Patterson et al. (1988)
Sibling–sibling (MA)	169	3-day food diary	$r = 0.23$	Patterson et al. (1988)
Twins	13MZ/10DZ	3-day food diary	$h^2 = 0.48$	Wade et al. (1981)
Families	1431 people/42 families	FFQ	$h^2 = 0.19$	Cai et al. (2004)
Grandmother–granddaughter	97	FFQ	$r = 0.12$	Stafleu et al. (1994)
Mother–daughter	97	FFQ	$r = 0.19$	Stafleu et al. (1994)
Mother–grandmother	97	FFQ	$r = -0.02$	Stafleu et al. (1994)
Sister–sister	3515 sisters/1701 families	FFQ	$r = 0.08$–0.13[b]	Vachon et al. (1998)
Twins	662MZ/750DZ	FFQ	$h^2 = 0.32$	Yeo et al. (1997)
Twins reared apart	66MZ/51DZ	FFQ	$h^2 = 0.35$[c]	Hur et al. (1998)
Twins-DZ	223DZ	FFQ	$r = 0.14$[c]	Fabsitz et al. (1978)
Twins-MZ	232MZ	FFQ	$r = 0.42$[c]	Fabsitz et al. (1978)
Twins	36MZ/18DZ	Meal in lab	$h^2 = 0.0$	Faith et al. (1999)
Twins	106MZ/94DZ	Weighed food diary	$h^2 = 0.24$[c]	Heller et al. (1988)

"Subjects" refers to the biological relative pair when family correlations were computed or the type of experimental design (families or twin) for studies which estimated heritability. MZ, monozygotic; DZ, dizygotic twin; MA, Mexican-American; A, Anglo (Caucasian). When the number of relative pairs was not provided, the number of each relative type is given, e.g., 87 and 58. "Method" refers to the type of procedure used to collected fat intake data. FFQ, food frequency questionnaire. Family similarity was assessed by inter- or intraclass correlations (r) for % of calories from fat unless otherwise indicated. In some cases, heritability was estimated (h^2). See individual references for the models used to calculate heritability.

[a] Saturated fat. Ref., reference. The average value for heritability (h^2) = 0.25.

[b] Range of fat types, e.g., animal or vegetable.

[c] Value refers to fat intake in grams.

Examining the pattern of family correlations for fat intake revealed several other results. First, the method of data collection affected the strength of the family correlations. Values from food diary methods were generally higher (mean = 0.33, range = −0.23 to 0.69) than from food frequency questionnaires (mean = 0.16, range = −0.02 to 0.42). Age-related effects were also apparent because family correlations among people of the same generation, e.g., siblings, were higher (mean = 0.40, range = 0.04–0.69) than for people of different generations, e.g., parent–offspring (mean = 0.24, range = −0.23 to 0.49). Sex effects were not apparent. Family correlations computed for a same-sex pairing, e.g., mothers and daughters, were similar to those computed for opposite sex pairings, e.g., mothers and sons ($mean_{same\text{-}sex}$ = 0.38, range = −0.02 to 0.69 versus $mean_{opposite\text{-}sex}$ = 0.36, range = 0.12–0.54). The twin studies typically reported heritability (rather than family correlations) but the same trend as for the family correlations was observed: the food diary method associated with higher heritability (h^2 = 0.48) than other methods of measuring fat intake (h^2 = 0.0–0.42). In the twin studies, generation effects do not apply because all twins are of the same generation and sex effects could not be evaluated (because the studies did not typically report heritability separately for opposite sex DZ twin pairs). Overall the highest family correlations or heritability estimates of fat intake were obtained using food diaries from people of the same generation.

16.10 HERITABILITY OF PREFERENCE FOR SPECIFIC FOODS OR TYPES OF FOOD

When studying genetics of fat preference, sometimes investigators choose to focus on the intake of individual high-fat foods, e.g., cheese or ice cream rather than the total amount of fat consumed or the percent of calories from fat. A recent study of food preferences of twins reports a high heritability for foods in the category of meat and fish (Breen et al., 2006) and another found similarly high heritability for specific foods like hamburgers (Falciglia and Norton, 1994). Other investigators have used factor analysis to group foods into categories, finding that additive genetic effects accounted for about 44% of the variance in the intake of "high-fat" foods (Keskitalo et al., 2008a), or have asked questions about liking and food use frequency for sweet high-fat foods (e.g., ice cream) and salty high-fat foods (French fries), finding that depending on the twins samples, heritability estimates ranged from 0% to 71% (Keskitalo et al., 2008a,b). There is a heritable component to high-fat food consumption, but like the analysis of total fat intake, the degree of genetic contribution varies widely based on the population studied and how the phenotype is measured.

16.11 ASSOCIATION AND LINKAGE STUDIES OF FAT PREFERENCE

Twin and family studies are a classic method to study human heritability for a given trait but the genetic contribution to fat preference can be studied with other types of experimental designs. In the case of the studies above, the focus is on trying to assess the relative contribution of genes and environment whereas with the genetic

methods described below, the focus is on identifying which genes might be particularly influential. To identify these genes, there are several types of approaches, the most common of which is an association study. In this type of design, biologically unrelated subjects are grouped by genotype and their intake or preference for dietary fat is compared. There are two types of association studies, a "candidate gene" approach in which only genes selected for some prior association with the trait are genotyped, or a "genome-wide association" approach in which alleles are densely genotyped throughout the genome. Both types of studies are called "association studies" because the association between genotype and phenotype is evaluated, but the designs differ in the scope of the genes considered.

In contrast to association studies which involve unrelated individuals, linkage studies incorporate people who are biologically related. In this type of experimental design, the degree of genetic sharing between relatives as well as their similarity or lack of similarly in fat preference and intake is calculated and compared. Linkage methods do not precisely localize the effect to a particular gene but rather identify a large chunk of DNA (that would contain many genes) which is shared by family members who are similar in phenotype. In the next section, the relationship of specific genotypes to fat intake in humans will be reviewed from both candidate gene association and linkage studies. As of now, no genome-wide association (GWA) studies of fat preference have been completed although their potential utility is discussed at the end of this chapter.

Candidate gene association studies focus on alleles from only one or a few genes and their association with fat intake or preference. An example of this type was a study of variation in the agouti-related protein (AgRP) gene and fat intake. The motivation to examine this particular gene comes from the study of obese mice. In the early days of mouse genetics, obese yellow mice were discovered (Danforth, 1927). The obesity was later determined to be due to the inappropriate expression of a gene involved in coat color (agouti) (Bultman et al., 1992). Investigators reasoned that the coat color gene was not likely to be normally involved in obesity but that it could be related to a different gene that did have a natural role in obesity. The discovery of agouti "related" protein confirmed this hypothesis (Shutter et al., 1997). This second gene is expressed in brain regions that regulate feeding and obesity and has several alleles in the human population, one of which is more common in Caucasians and one of which is more common in African-Americans. Each of these "most-common" alleles is associated with reduced fat intake (as a percent of total calories) in their respective racial population (Loos et al., 2005).

A potential target for candidate gene studies is CD36 because of its role in the sensory transduction of fatty acid perception (see above). If the CD36 gene is involved in fat sensing in humans as it is in mice, these differences could partially account for differences among people in oral fat perception. The human homologue of the mouse CD36 gene is located in the middle of human chromosome 7, and during the last several years, many alleles of CD36 have been discovered (Ma et al., 2004) (see Table 16.2). The CD36 gene and its alleles in humans have been studied by investigators who noticed its relationship with diet-induced disease such as diabetes. One hypothesis to explain these associations is that CD36 alleles might change fat

TABLE 16.2
Alleles of CD36 in Humans and Known Health Relationships

Allele	Function	Outcome	Reference	Frequency
rs1527479	Promoter	Increased diabetes	Corpeleijn et al. (2006)	High
rs1049654	5′ UTR	No effect on arthritis	Valdes et al. (2006)	High
rs13306228	Intron	Increased meat eating and colon cancer	Kuriki et al. (2005)	High in Japanese
rs13306227	L5R	Not studied	NA	Very rare
rs4645507	M405L	Not studied	NA	Very rare
rs3211938	Y325Ter	Not studied	NA	High in Africans
rs2272350	A137V	Not studied	NA	Very rare
rs1803256	G436Ter	Not studied	NA	Very rare
rs5957	F154V	Not studied	NA	Very rare

The known genotypes of CD36 are listed by rs number, and are catalogued in dbSNP (see "Electronic Resources"). The important aspects of the allele (when known) are listed in the "Function" column. All genotypes are single nucleotide polymorphisms (SNPs). For SNPs that do not change a predicted amino acid, their function is given by location in the gene, e.g., intron. For SNPs that do change a predicted amino acid, they are denoted by the most common amino acid first, the position changed in the protein, and the substituted amino acid, e.g., L5R means that leucine is changed to arginine (R) at amino acid position 5. Two SNPs create a stop (termination; Ter) codon and predict the gene (in the alternate form) is a pseudogene. When associations between SNPs and human traits are known, they are listed in the "Outcome" column but this list is not exhaustive. The allele frequency is listed in the rightmost column. UTR, untranslated region.

perception and intake and thereby contribute to metabolic diseases. The discovery of a putative CD36 pseudogene allele (Table 16.2), which is analogous to a natural human "gene-knockout," suggests that if this gene indeed codes for an oral fat receptor, there might be large differences among people with working and nonworking copies of the gene.

There are two linkage studies that have examined fat intake as a phenotype, and both studies used food frequency questionnaires to assess the amount of fat habitually consumed by the subject (Cai et al., 2004; Collaku et al., 2004). One study reported linkage to fat intake on chromosome 2, a region which was also associated with body fatness. The other study used Caucasian and African-American families recruited at five locations in the United States. The results from this study were different: the main linkage finding for fat intake (irrespective of total calories) was on chromosome 12. These two studies were similar in design and methodology but the regions of the genome implicated were different and it is worth noting that neither of these regions overlapped genetic regions associated with fat preference in the mouse (Smith Richards et al., 2002). The individual results may be valid and the difference may be due to the genetic background of the populations studied, or the disparate results may be due to the relatively low heritability or low power (which led to noise and false positive results in one or both studies).

16.12 GENOME-WIDE ASSOCIATION AND FAT PREFERENCE

Candidate gene association studies such as the one described above are undertaken because there is evidence that a particular gene may be involved in a particular biological pathway associated with a genetic trait, but these types of studies have limitations. The results are often difficult to replicate and they do not assess every gene in the genome. However, there has been a recent change in the direction of human genetics of complex traits, with the introduction of GWA studies, which use a dense or ultradense mapping panel (so that nearly every gene can be included in the analysis). Most genes are tested for their association with the trait and therefore the design gives investigators the ability to discover new effects of genes that were not previously understood. This approach is done at a more fine-grained resolution than a linkage study, which then requires substantial followup to find a particular gene (within the broadly linked region). Given the advantages of GWA studies, progress in the study of other complex genetic traits has been swift. For instance, GWA studies suggest that alleles of the fat mass and obesity-associated (FTO) gene are related to obesity (Frayling et al., 2007), a finding that is replicable across many human populations (Frayling et al., 2007; Scott et al., 2007; Scuteri et al., 2007; Andreasen et al., 2008; Chang et al., 2008; Do et al., 2008; Freathy et al., 2008; Liu et al., 2008a,b; Marvelle et al., 2008; Ng et al., 2008; Qi et al., 2008; Tan et al., 2008). GWA studies have started to encompass traits similar to fat preference, like sweet taste-related traits (Hansen et al., submitted) and studies of fat intake and fat preference may be just around the corner.

16.13 SUMMARY AND CONCLUSIONS

Human food intake is subject to all manner of vicissitudes and this is equally true for particular aspects, like fat preference. Although fat preference has normally been studied from a metabolic or neuroscience perspective, the recent understanding that fat may be a taste quality in the same way as sweet or bitter has led to renewed interested in how and why fat is liked and consumed. Several lines of evidence point to genetic influences on fat preference. In mice and rats, inbred strains differ markedly in preference and since their environment is precisely controlled, these influences are ascribed mostly to genotype and certain regions have already been identified as harboring fat preference genes. Genetic studies in humans also suggest a strong streak of genetic influence but the manner in which fat preference is measured (food diaries versus food frequency questionnaires) influences the outcome of twin and family studies. Several investigators have begun the long march to discover genes that influence fat preference. Although the initial studies are few and inconsistent, new GWA methods providing greater stability are on the horizon. If these methods can be used to study fat preference in thousands (or tens of thousands) of individuals, progress might be quick in understanding the contribution of genotype to the preference for a high-fat diet.

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Electronic Resources
dbSNP
http://www.ncbi.nlm.nih.gov/projects/SNP/

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17 Dietary, Physiological, and Genetic Impacts on Postprandial Lipid Metabolism

José Lopez-Miranda and Carmen Marin

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17.1 INTRODUCTION

Much of our knowledge about the relationship between lipid, lipoprotein metabolism, and the development of atherosclerosis and cardiovascular disease is based on measurements in the fasting state. Although such measurements remain the foundation of clinical assessment and an important basis for decisions regarding hypolipidemic interventions, it should be acknowledged that we spend a considerable amount of time in a nonfasting, postprandial state. Based on typical Western eating patterns, most people consume three or more meals a day, with each containing 20–70 g of fat (Cohn et al., 1988). Aside from breakfast, each of these meals is most likely consumed before plasma triacylglycerols (TGs) have returned to baseline levels from the lipemic conditions resulting from the previous intake. Thus, humans spend the majority of their day in a postprandial (fed) state, with a continual fluctuation in the degree of lipemia throughout the day. The postprandial state is a dynamic, nonsteady-state condition, with rapid remodeling of lipoproteins compared with the relatively stable fasting condition. Determination of the postprandial response is complex, and it is, therefore, more challenging to assess the cardiovascular risk associated with postprandial lipemia than during fasting conditions. In spite of this, it is becoming increasingly evident that future efforts to study and treat lipids related to atherogenesis should include postprandial parameters. The aim of this chapter is to consider the regulatory pathways of postprandial lipoproteins and the major factors including nutrition, life style, pathophysiology, and genetics which may contribute to interindividual variability in postprandial lipaemia, and thereby, susceptibility to atherosclerosis.

17.2 METABOLISM OF POSTPRANDIAL TRIACYLGLYCEROL-RICH LIPOPROTEINS

The metabolism of postprandial triacylglycerol-rich lipoproteins (TRLs) is described in Figure 17.1.

17.2.1 Chylomicron Production and Secretion

Upon digestion and absorption of dietary fat, short- and medium-chain fatty acids are albumin-bound and transported directly to the liver. Long-chain fatty acids are reesterified into TG and "packaged" into large chylomicron particles in the Golgi complex of intestinal cells. Assembly and secretion of chylomicrons is dependent upon the presence of apolipoprotein (apo) B-48, which in humans is synthesized

only in the intestine (Cohn et al., 1988). The fasting level of apo B-48 is very low or barely detectable in most individuals (Havel, 1995; Parks et al., 1999).

Upon entering the circulation, chylomicrons interact with lipoprotein lipase (LPL) to hydrolyze TG into monoglycerides and fatty acids on the surface of endothelial cells, primarily in adipose and muscle tissue (Havel, 1994). These hydrolytic products are either bound to albumin or rapidly taken up by muscle for oxidation or by adipose tissue for storage (Havel, 1994).

After approximately 90% of the hydrolysis is complete the chylomicrons, now designated as chylomicron remnants, are released back into the circulation (Havel, 1995). Apo E is the predominant protein remaining with the chylomicron remnants and is important in mediating the hepatic uptake of these particles (Karpe, 1999). Upon binding to the receptors, chylomicron remnants are rapidly internalized via coated pits on the cell and the particles are subsequently degraded in the lysosomes (Havel, 1995).

17.2.2 Very Low-Density Lipoprotein Metabolism

At least two apparent populations of very low-density lipoprotein (VLDL) particles, ranging in size from 300 to 700 Å in diameter, are secreted by the liver (Griffin, 1999). Secretion of the larger population, the postprandial VLDL particles, appears to be regulated by insulin-sensitive mechanisms in the fed state (Karpe, 1999). The regulation of the second, TG-poorer population of VLDL is less well characterized. In the postprandial state, insulin stimulates endothelial LPL to hydrolyze chylomicrons, generating a source of long-chain fatty acids to be shunted to the liver (Frayn et al., 1994). De novo synthesis of fatty acids as a source of hepatic TG for VLDL is not likely a significant source of fatty acids in humans under normal conditions (Parks et al., 1999; Parks and Hellerstein, 2000). Newly synthesized VLDL contains apo B-100, as well as some apo E and apo C (Havel, 1994; Karpe, 1999). It has been suggested that apo E plays a role in the synthesis and secretion pathways for VLDL (Huang et al., 1999; Mensenkamp et al., 1999; Maugeais et al., 2000). Upon release into the circulation, VLDL acquires additional apo E and apo C from circulating high-density lipoproteins (HDLs) (Havel, 1994; Karpe, 1999). The subsequent metabolism of VLDL follows a path similar to that of chylomicrons, where VLDL competes with chylomicrons and chylomicron remnants for LPL. However, because VLDL are smaller than chylomicrons, each particle presumably interacts with fewer LPL molecules and is hydrolyzed more slowly. The ongoing TG depletion and concurrent loss of apo E and apo C result in progressively smaller VLDL (referred to as VLDL remnants) or intermediate-density lipoproteins (IDLs), and ultimately low-density lipoprotein (LDL) particles. Apo B-100 remains with the particle until the final degradation occurs in the hepatocytes.

The competition between intestinal and hepatic TRL for the same lipolytic and receptor-mediated uptake pathways accounts, in part, for the accumulation of these particles during the postprandial period (Bjorkegren et al., 1996). Primarily, endogenous or hepatic TRL accumulate in the plasma after fat intake, presumably due to less efficient hydrolysis of VLDL by LPL (Cohn et al., 1993; Schneeman et al., 1993; Havel, 1994). At the peak of postprandial lipemia, the increase in apo B-100-containing

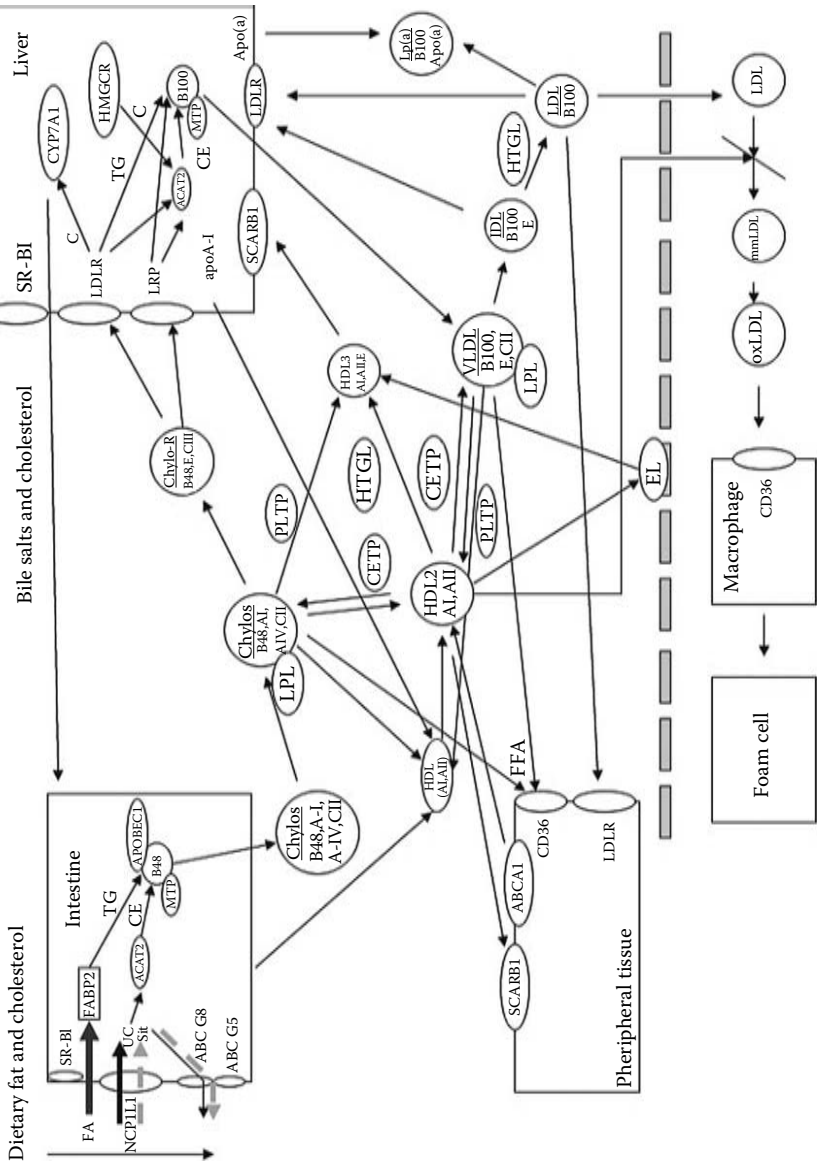

FIGURE 17.1 Human lipoprotein metabolism. Dietary fatty acids (FA) are absorbed from the gut and converted to triglycerides to be incorporated into chylomicrons in the intestinal epithelial cells.

FIGURE 17.1 (continued) The triglyceride-rich apolipoprotein (apo) B48 containing chylomicrons enter the plasma via the intestinal lymph. LPL hydrolyzes the triglyceride in chylomicrons to fatty acids, which are taken up by muscle cells for oxidation or adipocytes for storage. The remaining particles, the chylomicron remnants, are removed from the circulation by the liver through binding of their surface apo E to the LDL receptor or LDL receptor-related protein. VLDL particles are triglyceride-rich apo B100 containing particles, synthesized by the liver. As with chylomicrons, VLDL triglycerides are hydrolyzed by LPL. VLDL remnants or intermediate density lipoproteins (IDL) are taken up by liver receptors via apo E or converted to LDL. Chylomicrons, VLDL, and their respective remnants (RLP, remnant lipoproteins) are termed triglyceride-rich lipoproteins (TRL). Under physiological conditions, insulin, which is raised in the postprandial state, suppresses lipolysis from adipose tissue and hepatic VLDL production, however, this insulin action is inappropriate in insulin resistance and type 2 diabetes, resulting in high TRL concentrations. The large amount of TRL and their prolonged residence time in the circulation increase the exchange of esterified cholesterol from HDL and LDL to TRL and of triglycerides to LDL and HDL particles, which is mediated by cholesterol-ester transfer protein (CETP). Triglyceride enrichment of LDL particles renders them better substrates for HL, which hydrolyses triglycerides from the core of LDL and turns them into smaller and denser particles. Small dense LDL are more atherogenic as they readily enter the subendothelial space and become oxidized. Triglyceride enriched HDL particles are smaller and are more rapidly catabolized, which may explain the observed low plasma HDL in insulin resistance and type 2 diabetes.

particles is much greater than that of apo B-48-containing particles, accounting for up to 80% of the increase in particle number (Schneeman et al., 1993). However, due to the large size of chylomicrons and chylomicron remnants, approximately 80% of the postprandial rise in TG is accounted for by apo B-48-containing particles (i.e., large quantities of TG are transported in a relatively few number of large chylomicrons particles). However, the half-life of chylomicron particles and remnants is variable, and for smaller size particles this half-life may be as long as that of circulating VLDL of corresponding size (Lichtenstein et al., 1992).

During the postprandial period there is an active exchange of lipids between circulating lipoproteins (Karpe et al., 1993). The outcome of the lipid exchange is cholesteryl ester enrichment of TRL at the expense of cholesteryl ester in LDL and HDL. The lipid exchange between lipoproteins during prolonged postprandial lipemia is of interest because of the relationship between lipoprotein size, composition, and potential atherogenicity (Krauss, 1994; Griffin, 1999).

17.3 EXPERIMENTAL EVIDENCE LINKING POSTPRANDIAL LIPEMIA WITH ATHEROSCLEROSIS

The potential atherogenicity of postprandial TG levels and TRL did not gain widespread attention until the idea was put forth in a widely quoted paper by Zilversmit (1979), who proposed that hydrolysis of chylomicrons by LPL resulted in the subsequent internalization of cholesterol esters-enriched chylomicron remnants by arterial smooth muscle cells. A confirmation of this hypothesis has been complicated by the multiple factors impacting the postprandial response, the lack of standardized methodology, and the considerable heterogeneity among postprandial TRL species. Evidence supporting an association between postprandial lipemia and atherosclerosis has been provided by clinical trials and mechanistic studies of both direct and indirect effects of TRL using animal models and cell culture.

17.3.1 Clinical Trials

Several clinical studies have shown that delayed elimination of postprandial TRL is associated with atherosclerosis (Tables 17.1 and 17.2). There are also reports of an association between postprandial lipemic response and subsequent progression of atherosclerosis in patients with preexisting coronary heart disease (CHD).

In men, the presence of CHD is associated with higher postprandial TG concentrations in plasma compared with healthy controls, even after correction for higher levels of fasting TG in the CHD group (Groot et al., 1991; Patsch et al., 1992; Karpe et al., 1993; Meyer et al., 1996). The data are less clear for women, although recent studies have shown that nonfasting triglyceride levels were associated with incident cardiovascular events independent of traditional cardiac risk factors (Bansal et al., 2007; Nordestgaard et al., 2007). One smaller study reported elevated postprandial TG and apo B-48 concentrations in women with CHD (Meyer et al., 1996). However, a larger study showed no significant relationship between prolonged postprandial lipemia and CHD in middle-aged women (Ginsberg et al., 1995). In a number of studies, carotid IMT is used as a surrogate marker for atherosclerosis (Ryu et al.,

TABLE 17.1
Clinical Trials Summarizing the Effect of Postprandial Lipoprotein Metabolism on Carotid Artery Atherosclerosis

Study	Population	Conclusion
Boquist et al. (1999)	96 healthy 50-year-old men with an apolipoprotein (apo) E3/E3 genotype underwent an oral fat tolerance test and B-mode carotid ultrasound examination	In the postprandial state, plasma triglycerides at 1–4 h, total triglyceride AUC, incremental triglyceride AUC, and the large VLDL (Sf 60–400 apo B-100) concentration at 3 h were significantly related to carotid artery intima-media thickness (IMT). Multivariate analyses showed that plasma triglycerides at 2 h, LDL-cholesterol, and basal proinsulin were consistently and independently related to IMT
Karpe et al. (1998)	IMT and postprandial TRL were quantified during a standardized oral fat tolerance test in 30 healthy normo- and hypertriglyceridemic middle-aged men	Postprandial plasma triglycerides, in particular those measured late (6 h) after intake of the test meal, correlated positively with the IMT ($r = 0.44$, $P < .05$). In a multivariate analysis, 39% of the total variability for the common carotid IMT was explained by age, LDL-cholesterol, and the postprandial triglyceride levels
Ryu et al. (1992)	47 middle-aged, moderately hypercholesterolemic individuals were recruited	The only variable that showed a unvaried correlation with B-mode score was peak triglyceride response. Forward-selection stepwise regression resulted in the inclusion of only peak triglyceride response and smoking history as important predictors of carotid wall thickness in a linear model
Hamsten et al. (2005)	72 healthy men with an apo E3/E3 genotype who had undergone an oral fat load test and B-mode ultrasound examination of IMT	Multivariate analysis revealed that the apo CI content of TRL at 6 h, plasma triglyceride concentrations at 2 h, and fasting plasma cholesterol concentration independently predicted IMT

1992; Karpe et al., 1998; Boquist et al., 1999). Several studies have confirmed a positive association between carotid IMT and postprandial lipemia (Ryu et al., 1992; Karpe et al., 1998; Boquist et al., 1999; Hamsten et al., 2005). However, these data do not address the issue of whether prolonged postprandial lipemia predicts risk of developing CHD or whether the presence of CHD results in subsequent impairment of postprandial TRL.

In order to address this question, one cross-sectional study examined postprandial TG levels after consumption of a high-fat liquid drink in healthy sons of men with angiographic evidence of severe CHD compared with sons of control subjects without CHD (Uiterwaal et al., 1994). In spite of comparable fasting lipids between

TABLE 17.2
Recent Genetic Association Studies on Postprandial Lipoprotein Response

Gene	SNP	Study	Results
Apo A5	T-1131C	Jang et al. (2004)	The C allele was found to be associated with greater increases in total chylomicron and VLDL TG than did subjects with the TT genotype
Apo E	E2/E3/E4	Cardona et al. (2005)	Patients with the metabolic syndrome who do not have the E3/3 genotype have a greater risk of hyperuricemia and postprandial hypertriglyceridemia after a fat overload
FABP-2	A54T	Dworatzek et al. (2004)	In this study in which subjects were given three oral fat tolerance tests (butter, safflower oil, and olive oil) the T54 group had increased chylomicron cholesterol only after the olive oil test
LPL	Hind III, and S447X	Lopez-Miranda et al. (2004)	Carriers of the "H1" allele (H1S447 and H1X447 genotypes) presented a lower postprandial lipemic response than subjects with the H2S447 genotype
Hepatic Lipase	−514C/T	Gomez et al. (2004)	Homozygotes for the T allele showed a lower postprandial response of TRL particles with a decrease in both total triglyceride levels and in small and large TRL-TG
Microsomal triglyceride transfer protein	−164 T/C	Phillips et al. (2004)	No association was found between the −164 T/C polymorphism and the postprandial lipid profile
Scavenger receptor class B type I	Exon 1	Perez-Martinez et al. (2004)	The presence of the two allele at the SR-BI polymorphism in exon 1 was associated with faster clearance of small-TRL
CETP	TaqIB	Kolovou et al. (2007)	At higher TG concentrations, the B2 allele may protect against exaggerated postprandial TG increases and subsequent lowering of HDL-C concentrations

groups, sons of patients with CHD had significantly higher plasma TG levels after 8, 10, and 12 h postprandially, indicative of delayed clearance of TG.

These data were some of the first to suggest that altered postprandial lipid metabolism might be associated with familial risk for CHD. In another study in offspring of patients with CHD, young males with (case subjects) or without (control subjects) a paternal history of CHD underwent a postprandial study. Although no difference in postprandial TG was found in the groups as a whole, subgroup analysis revealed an increased postprandial response among cases with a moderate elevation of fasting TG levels (Tiret et al., 2000). There is evidence that higher levels of TRL or their

remnants predict progression of disease in subjects with established CHD. In The Montreal Heart Study, undertaken on 335 men and women with moderate to extensive CHD, the concentration of hepatic TRL remnants predicted progression of atherosclerosis (Phillips et al., 1993). In a summary of clinical studies of postprandial lipemia and atherosclerosis, Karpe (1999) suggested that elevated plasma TG levels measured at late postprandial time points after fat intake "might reveal a state of fat intolerance linked to an elevated risk of CHD that is under genetic control and cannot be detected by simple measurement of fasting plasma TG." However, additional studies are needed to determine the effect of specific TRL fractions and underlying mechanisms for a link between postprandial lipemia and atherosclerosis.

17.3.2 Mechanistic Evidence

The pathogenesis of the relationship between postprandial TRL and CHD remains unclear, but experimental evidence has provided several plausible mechanisms. Atherogenic effects may be mediated directly by TRL particles or components of the particles. In addition, indirect mechanisms of TRL atherogenicity may be due to metabolic changes associated with the presence of postprandial TRL.

17.3.2.1 Direct Effects of TG-Rich Lipoproteins

Studies designed to assess the direct atherogenicity of postprandial TRL have focused on characterizing their interaction with the arterial endothelium, determining the ability of postprandial TRL to penetrate the endothelial layer to the subintimal space, and assessing TRL interaction with monocyte macrophages and other components of the developing atherosclerotic lesion.

A variety of in vitro and clinical studies suggest that postprandial chylomicrons and VLDL are associated with adverse effects on arterial endothelium (Figure 17.2). In cell culture studies TRL, particularly postprandial remnants, are directly cytotoxic to endothelial cells (Speidel et al., 1990). Notably, HDL protected against the injury mediated by these particles. Further, TRL lipolysis products, including NEFA, may impair endothelial function. Cell culture studies in a porcine pulmonary artery model showed that presence of NEFA enhanced LDL uptake, suggestive of increased permeability (Hennig et al., 1985). Increased permeability was also seen during perfusion of murine arteries with TG-rich emulsions in the presence of LPL (Rutledge et al., 1997). Thus, arterial endothelial cells may be exposed to high levels of TRL products, particularly NEFA, in the postprandial state, although in vivo the cytotoxicity of NEFA may be attenuated by circulating albumin (Arbogast, 1988). However, in cultured endothelial cells a high NEFA to albumin ratio correlated with an augmented VLDL toxicity, suggesting that a high NEFA concentration may reduce the protective effect of albumin (Arbogast, 1988). Collectively, these in vitro studies suggest that TRL and products of TRL hydrolysis have the potential to promote endothelial dysfunction, which is thought to be important in the initiation of atherosclerosis.

Clinical evidence also demonstrates that postprandial TRL adversely affect the endothelium by mediating changes in vascular tone. After consumption of a high-fat meal, a reduction in flow-induced dilation of the brachial artery correlated with

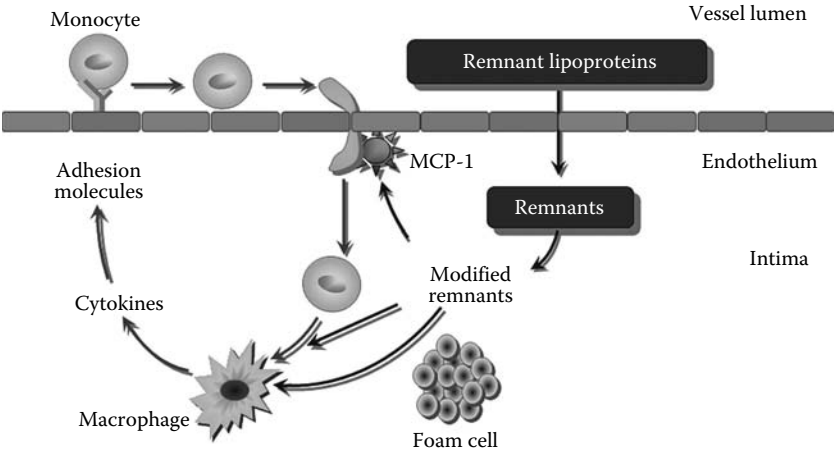

FIGURE 17.2 (See color insert following page 166.) The effects of postprandial chylomicrons and VLDL on arterial endothelium. VLDL remnants and chylomicron remnants behave in much the same way as LDL. They enter the subendothelial space, where they become modified. The modified remnants stimulate MCP-1 thereby promoting the differentiation of monocytes into macrophages which are taken up by the macrophages to form foam cells. Like LDL, the remnant lipoproteins are proinflammatory and proatherogenic. (From Lopez-Miranda, J. et al., *Br. J. Nutr*., 98(03), 458, 2007. With permission.)

postprandial plasma TG concentration in healthy subjects (Vogel et al., 1997). A similar response was seen after infusion of a lipid emulsion in seven healthy male subjects (Lundman et al., 1997). Further, TRL remnant concentration in healthy subjects correlated with an impaired epicardial coronary vasomotor response, and was inversely related to coronary blood flow under fasting conditions (Kugiyama et al., 1998). It has been proposed that TRL effects on endothelial tone are mediated in part by reduced nitric oxide production, either because of the TRL particles themselves or due to oxidized LDL associated with the postprandial period (Kugiyama et al., 1998; Karpe, 1999). Consumption of L-arginine attenuates endothelial impairment at 4 and 6 h postprandially, presumably due to increased availability of nitric oxide (Marchesi et al., 2001).

Moreover, blocking of functional cell-surface glycoproteins with heparin and lactoferrin did not affect remnant like particle induced impairment of vasorelaxation. These results suggest that remnant lipoproteins exert a direct effect on endothelial function without binding to glycoproteins that are located at the apical surface of the endothelium. Endothelial function is markedly impaired by a high-fat meal that causes an acute hypertriglyceridemia, this impairment is evident in dyslipidemic patients with baseline hypertriglyceridemia but not in normotriglyceridemic controls (Giannattasio et al., 2005). Endothelial microparticles may be an indirect marker of endothelial dysfunction or injury induced by postprandial TRL. Ferreira et al. evaluated a possible relationship between levels of endothelial microparticles, known to be a sensitive indicator of endothelial disturbance, and changes in postprandial lipid levels in healthy volunteers after a low- or high-fat meal. The high-fat meal

led to a significant elevation of plasma endothelial microparticles levels in healthy, normolipidemic subjects and correlated with a postprandial elevation of serum TG (Ferreira et al., 2004).

Incubation with remnant lipoproteins, but not VLDL or LDL, induced an elevation in expression of intercellular adhesion molecule-1, vascular cell adhesion molecule-1, and tissue factor in a human umbilical vein endothelial cell model in part through a redox-sensitive mechanism (Doi et al., 2000). Incubation with the antioxidant α-tocopherol dose dependently suppressed the remnant lipoprotein-induced mRNA expression of these factors. In addition, treatment of subjects with elevated plasma remnant lipoprotein-C levels (RLP-C > 5.1 mg/dL) for 4 weeks with α-tocopherol (300 mg/dL) prevented the rise in plasma levels of adhesion molecules such as soluble intercellular adhesion molecule-1 and soluble vascular cell adhesion molecule-1 (Doi et al., 2000).

Incubation of remnant lipoproteins with human umbilical vein endothelial cells and a mononuclear cell fraction in a flow-conditioned model resulted in enhanced expression of CD11a, CD18, CD49d, and interleukin-1β (Kawakami et al., 2002), which indicates a role of remnant lipoproteins in the initiation of vascular inflammation. Activation of leukocytes is obligatory for inflammation and atherogenesis by adhering to the endothelium via specific ligands.

Postprandially there is a TG-specific increase of neutrophil counts and increased activation of monocytes and neutrophils (van Oostrom et al., 2004). Furthermore, a recent study showed that acute hypertriglyceridemia act as a leukocyte activator most likely due to a direct interaction between TRL, leukocytes, and the uptake of fatty acids. TG-mediated leukocyte activation is an alternative proinflammatory and proatherogenic mechanism of hypertriglyceridemia in part associated with the generation of oxidative stress (Alipour et al., 2008).

Considering the presence of endothelial dysfunction as an early event in atherosclerosis (Ross, 1999), evidence from clinical and basic science studies suggests that TRL may play a role in the initiation of atherosclerosis. However, in order to perpetuate the progression of the disease, TRL or their contents need to penetrate into and remain in the intimal space of the arterial wall. Lipoprotein flux into the endothelium increases in direct proportion to TRL concentration in plasma and decreases as particle sizes become larger (Nordestgaard et al., 1995). This process involves formation of vesicles on the luminal surface of arterial endothelial cells, which migrate to the basolateral surface of arterial endothelial cells, where their contents are expelled by exocytosis into the subendothelial space (Simionescu and Simionescu, 1991). These transcytotic vesicles have been shown to accommodate lipoproteins up to 70 nm in diameter, which excludes the possibility of transporting unhydrolyzed TRL, such as chylomicrons (75–1200 nm), by this route. However, smaller chylomicron and VLDL remnants (40–70 nm) might enter the arterial wall by this mechanism. In addition, activation of endothelial cells and paracellular pathways may allow entry of larger sized particles.

Animal studies provide evidence for influx and selective retention of chylomicron remnants and VLDL (Proctor and Mamo, 1998). Further, arterial efflux of chylomicron remnants was incomplete, with focal accumulation of these lipoproteins in the subintimal space. Several lines of evidence suggest that TRL particles smaller than

chylomicrons such as VLDL and VLDL remnants can access the arterial intima. A dual isotope method was used to demonstrate influx of radiolabeled VLDL, IDL, and LDL into healthy and lesioned arterial intima of hypercholesterolemic rabbits (Shaikh et al., 1991). There was an inverse relationship between lipoprotein diameter and fractional loss from the arterial intima (i.e., a combination of efflux back into the arterial lumen, degradation of the particles, or irreversible attachment of lipoproteins to arterial wall components) (Shaikh et al., 1991). VLDL, VLDL remnants, IDL, and also LDL were more likely to be "trapped" in the arterial intimal and inner medial areas than smaller particles such as HDL.

It is intriguing that the presence of VLDL and IDL-sized particles has been reported in human intima as well as in atherosclerotic plaque (Rapp et al., 1994). The plaque samples were found to contain VLDL and IDL with a lipid composition similar to the corresponding plasma lipoproteins, suggesting that TRL can enter and be retained in atherosclerotic plaque.

The assessment of a direct effect of postprandial TRL on atherogenic mechanisms also relates to the nature of their interaction with components in the intimal space. Incubation of macrophages with chylomicrons and large VLDL from hypertriglyceridemic subjects resulted in their conversion into cells resembling foam cells in atherosclerotic lesions (Gianturco and Bradley, 1999). Furthermore, significant increases in macrophage TG and lipoprotein-C contents have been observed after a 4 h incubation with TRL. In addition, surface remnants of TRL hydrolysis were shown to be cytotoxic to macrophages, although this effect could be inhibited by the addition of HDL to the media (Cohn, 1998).

17.3.2.2 Indirect Effects of TG-Rich Lipoproteins

In addition to the possibility of direct effects of postprandial TRL, there is mounting evidence that metabolic changes occurring during prolonged or elevated postprandial lipemia may be proatherogenic. Of particular interest is the TRL-mediated modification of LDL composition and size.

17.3.2.2.1 Lipoprotein Modification

In the postprandial period, there is an enhanced exchange of core lipids between circulating lipoproteins. In this state, similar to that of hypertriglyceridemic subjects, it is postulated that the extent of this exchange may be determined by particle residence time in the circulation (Mann et al., 1991). This implies an enhanced exchange in patients with prolonged postprandial lipemia. The resultant TG-enriched LDL and HDL particles are subject to lipolysis by hepatic lipase (HL), thus forming small, dense particles. Although chylomicron remnants are likely to play a role in this process, the main driver in this process is the increase in VLDL secretion from endogenously synthesized lipids rather than for exogenous lipids (Kwiterovich, 2002), possibly due to a prolonged residence time and opportunity for lipid exchange (Schneeman et al., 1993). This is an important point because very high carbohydrate diets increase basal rates of VLDL synthesis, whereas higher fat diets result in greater postprandial increases in plasma TG. It has not been demonstrated that a reduction in postprandial lipemia per se leads to an increase in HDL-C or a reduction in the proportion of dense LDL. Neither

does there appear to be any effect of diets high in monounsaturated fatty acids (MUFAs) compared with diets high in saturated fatty acids (SFAs) on LDL size (Minihane et al., 2000; Rivellese et al., 2003). The increase in LDL size following the consumption of large amounts of long-chain omega(n) − 3 fatty acids in hypertriglyceridemic subjects (Minihane et al., 2000) can be attributed to decreased VLDL synthesis (Sanders et al., 1985).

Karpe et al. (1993) showed that postprandial TRL levels and LPL activity accounted for about 50% of the variability in LDL particle size. The size of circulating LDL is not acutely affected by the ingestion of a fat-rich meal (Cohn, 1998), but there is a consistent relationship between increased fasting TG levels, increased postprandial TG, and the presence of atherogenic small, dense LDL (Krauss, 1994). A detailed analysis of the correlation between fasting plasma TG levels and LDL subclasses suggests that small dense LDL is more common above a fasting TG level of 132 mg/dL (1.5 mmol/L) (Griffin, 1999). The relationship between small, dense LDL and plasma TG has been defined mainly in the fasting state, although metabolic processes in the postprandial state are important in the generation of small dense LDL (Griffin, 1999).

Small, dense LDL particles appear to be highly atherogenic by several related mechanisms (Austin et al., 1990; Griffin, 1999). Prospective studies confirm that small, dense LDL are highly predictive of CHD and are present in 40%50% of all patients with CHD in spite of normal fasting LDL-C levels (Griffin, 1999). In a group of young, postinfarction men, apo B-48 levels correlated with plasma levels of small dense LDL (Karpe et al., 1993). Another study in diabetic subjects found a higher oxidative susceptibility of postprandial LDL particles (Diwadkar et al., 1999). Postprandial LDL promoted a significantly higher degree of cholesterol esters accumulation and were more susceptible to copper-induced oxidation (Diwadkar et al., 1999).

17.3.2.2.2 High-Density Lipoprotein Effects

The composition and cholesterol concentration of HDL is inversely related to the magnitude of postprandial lipemia and the plasma concentration of TG. Lipolysis of TRL affects the rate of formation of HDL particles (Cohn, 1998). Another mechanism for this association may be a postprandial increase of cholesterol esters. Cholesterol esters transfer from HDL to TRL (Karpe et al., 1993), this is proposed to be one of the atherogenic changes mediated by prolonged postprandial lipemia (Patsch et al., 1992).

17.3.2.2.3 Hemostatic Changes

Postprandial lipemia has been shown to be associated with changes in hemostatic variables known to promote risk for thrombotic events (Miller, 1998). Epidemiologic data have shown that the coagulation activity of factor VII predicts CHD (Meade et al., 1986). Following intake of a fat-rich meal, factor VIIc is transiently increased due to an increase in plasma concentration of factor VIIa, increasing the possibility of initiating a thrombotic response that may increase the likelihood of a clinically significant thrombosis (Miller, 1998). It also appears that lipolysis of chylomicrons is required to stimulate the postprandial increase in factor VIIc (Krauss, 1994). Factor VIIa is associated with plasma phospholipid concentrations, and it is known that factor VII can be activated by synthetic phospholipid particles containing negatively

charged phospholipids. Membrane microparticles can be shed from activated platelet and leukocytes, and this may well occur during the postprandial period when lipid transfer reactions are active and lymph secretion is increased. The postprandial increase in factor VIIa may be related to the activation of the ATP-binding cassette transporter A1, which may be stimulated by the production of nascent HDL of intestinal origin (Miller et al., 2002). Although the postprandial increase in factor VIIa is not associated with downstream markers of thrombin generation (Bladbjerg et al., 2000), its elevation could enhance the risk of major thrombosis triggered by plaque rupture. A postprandial decline in plasminogen activator inhibitor type-1 activity and increase in tissue plasminogen activator activity have been observed in various studies (Sanders et al., 2001; Tholstrup et al., 2003). These changes probably reflect the well-known diurnal variations in fibrinolytic activity. Although plasminogen activator inhibitor type-1 activity is strongly correlated with fasting plasma TG and factor VIIc, it is not associated with changes in the postprandial TG concentrations. This would imply that the association between fasting plasma TG and plasminogen activator inhibitor type-1 activity is not causal and more likely to be related to insulin resistance.

Apart from the effect on thrombogenic factors, circulating postprandial lipoproteins may also impact platelet reactivity (Nordoy et al., 1974). Although a variety of platelet activation markers have been studied to determine if platelets are indeed affected by postprandial lipemia, results are conflicting (Jakubowski et al., 1985; Fuhrman et al., 1986). Recent advances in flow cytometry have provided the opportunity to more accurately assess platelet activation by the expression of surface and intracellular proteins. Using this technology, postprandial lipemia was associated with a mild increase in platelet reactivity that increased the expression of cell-surface markers in healthy men (Broijersen et al., 1998; Hyson et al., 2002). Furthermore, platelet-monocyte aggregation and monocyte intracellular cytokine expression were elevated during the postprandial period and remained elevated after plasma TG levels returned to baseline (Hyson et al., 2002).

17.4 FACTORS AFFECTING THE POSTPRANDIAL RESPONSE

17.4.1 Meal Size and Composition

Postprandial lipemia is influenced by the amount and type of dietary fat present in the test meal, as well as other dietary components including fiber, glucose, starch, and alcohol (Cohen and Berger, 1990; van Tol et al., 1998; Williams, 1998). The amount and type of dietary fat consumed modulate postprandial lipemia. Intake of long-chain omega(n) – 3 polyunsaturated fatty acids (PUFAs) (predominantly fish oil), results in lower TG levels and attenuates postprandial lipemia (Tinker et al., 1999).

17.4.1.1 Fat

17.4.1.1.1 Amount of Fat

The amount of fat required to significantly elevate plasma TG concentration is in the order of 30–50 g. Some studies have been performed with increasing doses of dietary fat (Cohen et al., 1988; Dubois et al., 1994; Murphy et al., 1995; Dubois et al.,

1998). In these studies a very low dose (5 g) or low dose (15 g) of dietary fat does not significantly increase triglyceridemia postprandially; moderate doses (30–50 g) dose-dependently increase postprandial triglyceridemia; and finally, very high doses (80 g and above) exaggerate postprandial triglyceridemia without dose-dependence. On the other hand, consecutive meals containing fat appear to enhance the lipemia (Jackson et al., 2002). Most meals contain 20–40 g of fat, so that when two or three such meals are eaten consecutively, along with snacks eaten between meals, circulating TG levels will be maintained well above fasting concentrations for much of the day. Studies which have monitored TG responses overnight following a fat-containing evening meal have shown values to be elevated for 7–8 h after the meal, only starting to return towards fasting values between 4 AM and 6 AM (Williams et al., 1992).

17.4.1.1.2 Fatty Acid Composition

Acute Meal Studies A relatively large number of studies have attempted to compare the effects of meals with different types of fat on postprandial lipemia (reviewed by Williams, 1998) (Roche et al., 1998; Williams, 1998; Thomsen et al., 1999; Mekki et al., 2002). A number of potentially confounding factors, such as the amount of fat, or the type and amount of carbohydrate, and the physicochemical composition of the meal (Sakr et al., 1997; Armand et al., 1999) are variable between many of the studies, which make comparisons difficult, hence clear conclusions cannot always be drawn. The most common measurement is the postprandial plasma TG response. In this respect one should remember that short or medium dietary fatty acids have a limited effect on postprandial plasma TG response because they enter the portal route instead of through chylomicron secretion into the general circulation. Dairy fats contain significant amounts of short- and medium-chain fatty acids, studies that have used dairy fats as the source of SFA generally report, as expected, a lower postprandial TG response than other SFA or other types of fats. Taking account of these considerations, most studies have shown that meals enriched with either SFA, MUFA, and $n-6$ PUFA do not generally elicit markedly different postprandial lipid responses. However, some studies have reported exacerbated (Thomsen et al., 1999) or reduced (Mekki et al., 2002) responses after the intake of saturated butter fat.

Comparisons of the effect of $n-6$ PUFA-rich oils with olive oil (rich in oleic acid) or MUFA (canola oil) showed a lower (de Bruin et al., 1993) or comparable magnitude of postprandial lipemia (Lichtenstein et al., 1993; Tholstrup et al., 2001; Mekki et al., 2002). $n-3$ PUFA (fish oil) can lower the postprandial TG response if a sufficient amount of it is present within the test meal (Zampelas et al., 1994), but the levels used were far greater than those which would be consumed by most populations. Furthermore, several studies have shown that differences in single meal fatty acid composition exert little or no effect on postprandial changes in plasma lipids (de Bruin et al., 1993; Jackson et al., 1999; Pedersen et al., 1999; Burdge et al., 2006). The influence of the positional distribution of fatty acids within the dietary TG moieties has been investigated with some studies showing some influence (Jensen et al., 1994) but others showing no effect (Zampelas et al., 1994) on postprandial lipemia.

Several studies have found striking findings with regard to the effect of stearic acid-rich fats compared with other SFA on postprandial lipemia. Two independent studies have found that a stearic acid-rich TG prepared from a randomized blend of

hydrogenated and unhydrogenated high-oleic sunflower oil decreased postprandial lipemia compared to unhydrogenated high-oleic acid sunflower oil (Sanders et al., 2000; Tholstrup et al., 2003). However, a stearic acid-rich meal using cocoa butter resulted in similar postprandial lipemia compared with a meal rich in oleate provided by high-oleic sunflower (Sanders et al., 2001). Yet in the same study, a synthetic randomized stearic-rich TG was found to decrease postprandial lipemia. It was hypothesized that the unique symmetrical TG structure of cocoa butter, where almost all of the stearic acid is present as 1,3-di-stearyl, 2-mono-oleylglycerol, was responsible for this difference. In order to test this hypothesis, the postprandial effects of randomized cocoa butter were compared with the effects of unrandomized cocoa butter (Sanders et al., 2003). It currently remains uncertain whether these effects are solely due to TG structure or are related to changes in the physicochemical properties of the TG mixture.

Measurement of postprandial TG response may only provide a limited evaluation of the true impact of meal fat type on postprandial lipoprotein metabolism. More recently, studies that have measured particle number (evaluated by apo B-48 response) and responses in different lipoprotein subfractions have revealed important differences in lipid, apo particle size, and number in response to meals of different fatty acid composition. The studies showed lipemic responses to be in the order SFA > MUFA > PUFA.

This suggests that findings from studies that have employed plasma TG analysis alone may have underestimated the impact of the fatty acid composition of a meal on postprandial lipoprotein metabolism (Jackson et al., 2002, 2005). Meals containing olive oil (oleic acid), result in a greater apo B-48 response compared with palm oil, safflower oil, or a mixture of fish and safflower oil, and stimulated the formation of both small ($S[f] = 60–400$) and large ($S[f] > 400$) apo B-48 containing lipoproteins (Jackson et al., 2002). This finding is consistent with data from Caco-2 cell culture studies (Black et al., 2002), which demonstrated that oleic acid is a potent stimulator of TG secretion, it is also consistent with other test meal studies that have found that meals high in oleic acid-rich oils (e.g., high-oleic sunflower oil) result in a more pronounced and sharper postprandial rise in plasma TG compared with SFA-rich meals (Sanders et al., 2000), although the overall TG response measured as area under the curve (AUC) does not differ from other fat type meals. Because it is still unclear exactly how postprandial lipemia impacts atherosclerosis and CHD risk, the relevance of reported differences in patterns and timings of TG response, as well as in particle number and particle size, elicited by meal fat type, is not yet fully understood. However, it is generally agreed that an elevated TG response that continues into the late postprandial phase (5–8 h) is unfavorable (Patsch et al., 1992); such a response is most commonly observed when nondairy SFA meals are consumed (Roche et al., 1998; Sanders et al., 2000).

Chronic or Habitual Diet The habitual diet of an individual may also influence the postprandial response (Williams, 1998). However, much less data have been published on this aspect (Weintraub et al., 1988; Williams et al., 1992; Roche et al., 1998; Zampelas et al., 1998). Background diets rich in MUFA or $n-6$ PUFA tend to lower the postprandial lipid response as compared to SFA (Weintraub et al., 1988;

Zampelas et al., 1994, 1998). Compared with a SFA-rich diet an increase in chronic MUFA intake led to a marked reduction in apo B-48 production following the test meal, but postprandial lipemia did not differ, which indicated that MUFA diets result in the production of chylomicrons of a larger size (Silva et al., 2003), which is suggested to be an advantage in the postprandial processing of dietary TG.

However, Rivellese et al. could find no difference in postprandial lipemia after the administration of a diet high in MUFA compared with diets high in SFA (Rivellese et al., 2003). On the other hand, postprandial lipemia was shown to be greater on high-oleic acid and trans 18:1 diets compared with a high-carbohydrate diet (Sanders et al., 2003). Comparisons of the effect of $n-6$ PUFA-rich oils with olive oil (rich in $n-9$ MUFA) or MUFA showed lower or comparable magnitude of postprandial lipemia (Lichtenstein et al., 1993; Tholstrup et al., 2001; Mekki et al., 2002). In addition, Rivellese et al. recently showed that a MUFA-rich diet reduces postprandial small VLDL triglycerides in type 2 diabetic patients compared to a SAFA-rich diet, and it also modifies lipolytic enzymes in adipose tissue with an increase of LPL and hormone-sensitive lipase following the MUFA diet (Rivellese et al., 2008).

It is well documented that diets rich in long-chain $n-3$ PUFA (2.7–4 g/day) can lower the postprandial TG response (Williams et al., 1992; Kelley et al., 2007) but some opposing effects have also been reported (Roche and Gibney, 1996). In several studies LPL activity is increased by supplementation with 3–4 g/day of long-chain $n-3$ PUFA (Khan et al., 2002; Park and Harris, 2003; Rivellese et al., 2003). In contrast, the consumption of a diet rich in α-linolenic acid (18:3$n-3$), containing intakes of between 4.5 and 9.5 g/day, taken as margarine for 6 months had no effect on postprandial lipemia (Finnegan et al., 2003). There is abundant evidence indicating that the reduction in postprandial lipemia following $n-3$ PUFA supplementation is due to a decrease in chylomicron (Harris and Muzio, 1993; Westphal et al., 2000) and VLDL-TG (Harris et al., 1990; Nozaki et al., 1991; Westphal et al., 2000) synthesis/secretion. On the other hand, there is also limited evidence supporting the hypothesis that the reduction in postprandial lipemia following $n-3$ PUFA supplementation is due to an increased rate of TG clearance associated with increased endogenous (measured in nonheparinized plasma) LPL activity (Harris et al., 1997; Park and Harris, 2003). A logical conclusion from the above studies would be that both a decrease in chylomicron and VLDL-TG secretion/synthesis along with an increased clearance rate was responsible for the postprandial lipemia reductions following $n-3$ PUFA supplementation.

Overall, studies that have evaluated the impact of habitual fat type on postprandial response to acute fat ingestion have shown that in terms of postprandial TG response effects are in the following order: SFA > MUFA = $n-6$ PUFA > $n-3$ PUFA.

17.4.1.2 Carbohydrate

Clinical studies support the concept that diets rich in highly digestible carbohydrate can lead to elevation of fasting plasma TG as a result of hepatic VLDL and chylomicron remnants accumulation due to altered lipoprotein secretion and/or clearance as reviewed (Parks et al., 1999; Roche, 1999).

Additionally, several studies have shown that the amount or nature of carbohydrate in a meal alters postprandial lipid metabolism. Data obtained after addition of glucose (50, 100 g) to fatty test meals have not shown consistent findings in healthy

subjects (Cohen and Berger, 1990) whereas addition of sucrose or fructose have consistently been shown to increase postprandial triglyceridemia (Grant et al., 1994). In healthy subjects, physiological ranges of postprandial hyperglycemia and hyperinsulinemia as generated by starchy foods (white bread, pasta, and beans) do not induce noticeable alterations in the overall postprandial TG response (Harbis et al., 2001). Furthermore, the data obtained in this study showed that portal and peripheral hyperinsulinism (modulated using different test meals) delays and exacerbates postprandial accumulation of intestinally derived chylomicrons in plasma (Harbis et al., 2001).

Moreover, in subjects with insulin resistance, ingestion of a high-glycemic index mixed meal, as compared to a low-glycemic index one, increases the postprandial rise in insulinemia and accumulation of apo B100 and apo B48 containing TRL in those subjects, thus increasing postprandial triglyceridemia as well as modifying kinetics of peak occurrence (Harbis et al., 2004). Adding various digestible carbohydrates to a test meal can elicit a biphasic response of postprandial lipemia (Harbis et al., 2004). This indicates clearly that the amount as well as the nature of carbohydrate can influence postprandial lipid responses.

In small doses, dietary fructose appears to be beneficial in enhancing glucose tolerance.

However, when consumed in large amounts, fructose has been shown to lead to hypertriacylglycerolemia. In a recent study Chong et al (Chong et al., 2007) showed that the lower insulin excursion after fructose ingestion may result in a lower activation of adipose tissue LPL, which led to impaired TG clearance. The contribution of de novo lipogenesis to fructose-induced hypertriacylglycerolemia is small, but its effect on altering the partitioning of fatty acids toward esterification may be considerable.

17.4.1.3 Fiber

Addition of some dietary fiber sources into mixed test meals (Cara et al., 1992; Lia et al., 1997) at the level of 4–10 g/meal can moderately reduce postprandial triglyceridemia or chylomicron lipids as generated by a mixed meal. Sources of soluble viscous fibers (i.e., oat bran) or with hypotriglyceridemic properties (i.e., wheat germ) were shown to display a delay in the micellar lipid solubilization process and a consequent reduction in the secretion of chylomicrons into the circulation (Lia et al., 1997). These data suggest that soluble fibers reduce the rate of digestion of dietary fats and thereby attenuate the postprandial lipemic response.

17.4.1.4 Protein

Very little information is available so far regarding the influence of the amount or nature of dietary proteins on postprandial lipid responses. However there is evidence indicating that a diet of 20 g of soy protein isolate for 3 weeks reduces baseline plasma remnant-like particles (RPL) cholesterol levels by 9.8% (Higashi et al., 2001). Recent studies showed that postprandial lipemia can be acutely mitigated when proteins are added to the fatty meal, and the neutralization of the lipemia induced by proteins in endothelial dysfunction is caused by direct and indirect effects of the proteins insulinotropy and by an increased supply of L-arginine (Westphal et al., 2005).

By contrast, a low-protein diet exacerbates postprandial chylomicron concentration in moderately dyslipidaemic subjects in comparison to a lean red meat protein-enriched diet (Mamo et al., 2005). Casein added to a fatty meal lowers nonesterified fatty acids (NEFAs) markedly in the postprandial and postabsorption phases, probably via its insulinotropic activity and it also moderately reduces postprandial lipemia (Westphal et al., 2004).

17.4.1.5 Micronutrients

Epidemiological surveys suggest that a higher intake of tea may be associated with a lower risk of CHD (Fielding et al., 2000). Tea catechins attenuated the postprandial increase in plasma TG levels following a fat load in humans (Unno et al., 2005) and delayed lymphatic transport of dietary fat in rats (Ikeda et al., 2005). Furthermore, a study with mice fed a commercial diet (CE-2, Clea, Japan) found that oolong tea suppressed lymphatic absorption of triglycerides more than green tea, and that polymerized polyphenols, a major component in oolong tea, can exhibit this effect in rats and mice (Toyoda-Ono et al., 2007).

17.4.2 Lifestyle Conditions

17.4.2.1 Physical Activity

An acute bout of aerobic exercise has been shown to significantly reduce postprandial lipemia by 24%–35% (Hardman and Aldred, 1995; Tsetsonis and Hardman, 1996; Tsetsonis and Hardman, 1996; Hardman, 1998; Thomas et al., 2001) and significantly increase LPL activity (Ferguson et al., 1998; Zhang et al., 2002). The mitigation of the lipemic response to a meal high in fat and carbohydrate is related to the intensity and/or the energy expenditure of the preceding exercise (Tsetsonis and Hardman, 1996). Physical activity within the 24 h preceding a high-fat meal greatly improves the rate at which lipids are removed from the circulation. In a meta-analysis of data from interventions involving exercise, it was estimated that there was 0.5 standard deviation (SD) reduction in the postprandial TG response in groups in which exercise had occurred before meal ingestion (Petitt and Cureton, 2003). Furthermore, a recent study showed that an acute exercise session, regardless of the time point chosen (i.e., 16 or 4 h before the meal), reduced the total and incremental lipemic responses to a similar extent compared with the nonexercise condition (Silvestre et al., 2008).

Increases in LPL activity following an acute bout of aerobic exercise have been associated with increased rates of TG clearance (Sady et al., 1986) as well as reductions in postprandial lipemia response and fasting TG concentration (Thomas et al., 2001). Furthermore, the postprandial response to an oral fat load is lower and clearance rates of TRL are higher in endurance trained individuals compared with untrained control subjects, although this may not be applicable to moderate exercise (Gill et al., 2001). In a recent article, the combination of exercise and $n-3$ PUFA supplementation was reported to reduce postprandial lipemia response as measured using the incremental area under the postprandial curve of TG to a greater degree in recreationally active males when compared with the individual treatments (Smith et al., 2004).

17.4.2.2 Smoking

Postprandial TG levels were evaluated in previous studies: Axelson et al. (1995) showed a 50% greater TG postprandial increase in habitual smokers without changes in fasting TG. Mero et al. (1997) showed that smoking raised retinyl esters and apo B-48 levels, but not apo B-100 levels. Data obtained in a large sample of men and women support the interpretation of Axelson et al. that smoking affects postprandial TG metabolism. This effect presumably primarily due to a raise in levels of lipoproteins of intestinal origin because cigarette smokers had substantially greater postprandial retinyl palmitate and apo B-48 responses than did nonsmokers, adjusted for fasting triglycerides (Sharrett et al., 2001).

17.4.2.3 Alcohol

The effect of alcohol on postprandial lipids has drawn continued attention over the past 10 years. Ethanol consumed with a meal clearly elevates total plasma and VLDL-TG. In a recent study, the addition of 47.5 g of alcohol to a high-fat meal (54% of energy) was associated with approximately 60% increase in the peak plasma TG concentration as compared with a meal consumed without alcohol (Fielding et al., 2000). The authors attributed this increase to a stimulation of large VLDL secretion. Ethanol has also been shown to increase fatty acid synthesis (Siler et al., 1998), and also to reduce TG clearance from plasma (Pownall et al., 1999).

17.4.3 Physiological Factors

17.4.3.1 Age

In general, tolerance to oral fat intake decreases with age, although the magnitude of postprandial triglyceridemia was inversely correlated with HDL-cholesterol levels and positively correlated with age and fasting levels of plasma TG (Cohn et al., 1988). Information on postprandial lipemia in children is sparse, although in a recent study fasting TG and HDL-cholesterol, but not LDL-cholesterol levels, predicted the postprandial response. Interestingly, there was a significant difference in postprandial response between children and their mothers in spite of similar baseline TG levels (Couch et al., 2000). There also appears to be an age-related change in postprandial lipemia and LPL activity (Jackson et al., 2003), which may in part be attributable to weight gain.

17.4.3.2 Gender, Menopausal Status

A number of studies have demonstrated significant differences between fasting and postprandial TG levels in men and women, with higher levels in men (Cohn et al., 1988). Additional evidence for the presence of increased levels of fasting TG and exaggerated postprandial lipemia in postmenopausal women has been reported after adjusting to fasting TG levels (van Beek et al., 1999). There are several possible mechanisms that might promote the uptake of fat in women. Estradiol probably promotes rapid clearance of chylomicron remnants through its effects on the LDL receptor, it may also promote more effective fatty acid trapping by subcutaneous adipose stores. Estradiol is known to increase the production of nitric oxide, which is a potent vasodilator with direct vasoactive effects (Walker et al., 2001), which in

turn would increase the surface area of endothelium able to express LPL in the postprandial phase. It is noteworthy that for a given meal, the postprandial lipid response is lower in women than men, due to a higher clearance capacity linked to an increase in LPL activity.

On the other hand, hormone replacement therapy is associated with an increase in TG levels which parallels a decrease in remnant lipoprotein–cholesterol levels (Ossewaarde et al., 2003). These results suggest that estrogen might induce a shift in the distribution pattern of TRL, with a decrease of the more atherogenic fractions.

17.4.3.3 Baseline Lipoprotein Levels

Fasting levels of plasma TG tend to be correlated with the magnitude of postprandial lipemic response (Cohn et al., 1988). In addition, previous studies have noted the presence of an early postprandial peak in plasma TG concentration, particularly when successive meals have been consumed and the fat from a previous meal contributes to this early postprandial lipemia (Fielding et al., 1996; Evans et al., 1998; Burdge et al., 2003).

17.4.4 Genetic Background

17.4.4.1 Ethnicity

Various studies have demonstrated that there are differences in postprandial response between different ethnic groups. Young adults of Vietnamese (i.e., SE Asian) and Chinese origin displayed marked postprandial hyperglycemia and hyperinsulinemia compared with age-matched Caucasian subjects (Dickinson et al., 2002). In South Asians, postprandial glucose and insulin concentrations were greater than in Northern Europeans and Latin Americans, although there were no differences in postprandial TG concentrations or in insulin sensitivity as assessed with the insulin tolerance test (Cruz et al., 2001). However, remnant-like particle cholesterol levels in Japanese subjects have consistently been reported to be lower than those in Caucasians (Twickler et al., 2004). In women, plasma TG levels in lean African-Americans were lower than those of lean Caucasians, both during fasting and the postprandial period (Bower et al., 2002). These results may be partly due to enhanced expression, activity, and intravascular availability of LPL. Furthermore, it appears that the racial differences in expression and function of LPL are attenuated in the presence of obesity (Bower et al., 2002). These ethnic differences indicate that genetic heterogeneity plays a part in determining interindividual differences in metabolite and hormone responses to meal ingestion. So far, several candidate genes in which common genetic polymorphisms may be involved in determining the postprandial responses have been isolated. These include genes coding for apos, enzymes such as lipases or those involved in TG synthesis, as well as receptors that regulate uptake of lipoprotein particles (Perez-Martinez et al., 2008).

17.4.4.2 Common Genetic Polymorphisms and Postprandial Lipemia

The effect of several polymorphisms on postprandial lipoprotein metabolism have been studied. However, comprehensive analyses involving haplotypes and multiple genes is often lacking. A list of the most recent studies is presented in Table 17.3.

TABLE 17.3
Clinical Trials Summarizing the Effect of Postprandial Lipoprotein Metabolism on Coronary Artery Disease (CAD)

Study/Design	Population	Conclusion
Patsch et al. (1992) Case–control	61 male subjects with severe CAD and 40 control subjects without CAD as verified by angiography	Single postprandial triglyceride levels 6 and 8 h after the meal were highly discriminatory ($P < .001$), and by logistic-regression analysis displayed an accuracy of 68% in predicting the presence or absence of CAD
Groot et al. (1991) Case–control	Two groups of 20 normolipidemic men, a group of CAD patients, and a matched control group with documented minimal coronary atherosclerosis	CAD patients showed a marked delay in the clearance of retinyl esters as well as in the normalization of plasma TG concentrations. Postheparin plasma HL activity was significantly lower in the CAD group
Meyer et al. (1996) Case–control	12 normocholesterolemic, normotriglyceridemic women, with angiographically proven coronary artery disease and in 12 individually matched controls, without angiographical abnormalities	A greater absolute and incremental apo B-48 response in the IDL fraction ($d = 1.006–1.019$ g/mL) was observed in CAD+ cases [incremental area under the curve (delta-AUC): 0.40 ± 0.12] than CAD-controls (0.01 ± 0.06; $P = 0.01$). The results provide evidence that the metabolism of intestinal TRLP is significantly different in normolipidemic women with angiographically proven CAD compared with individually matched controls without coronary disease
Ginsberg et al. (1995) Case–control	92 and 113 women were recruited from populations undergoing diagnostic exercise electrocardiographic or thallium stress tests. 26 men and 24 women had positive tests	The authors chose exercise-induced myocardial ischemia (EIMI) as the criterion for defining case and control subjects because they wanted participants who did not have a prior diagnosis of CAD. Among men but not women postprandial TG and RP responses were associated with EIMI independent of age, race, and smoking status. In the male group, the odds ratio (OR) for an increase in postprandial TG response of approximately 1 SD was 1.69 ($P = .007$); the OR for an increase in RP response of 1 SD was 2.47 ($P = .011$)
Tiret et al. (2000) Case–control	Male subjects with a paternal history of CHD (cases, $n = 407$) and age-matched male controls ($n = 415$) were recruited from 14 European universities	In the sample as a whole, the postprandial triglyceride responses did not significantly differ between the two groups. However, in the upper tertile of fasting triglycerides, cases displayed a higher AUC (5.71 vs. 4.49 mmol h/L, $P < .001$), a higher peak (1.76 vs. 1.43 mmol/L, $P < .001$) and a more delayed time to peak (3.15 vs. 2.91 h, $P < .05$) than controls

TABLE 17.3 (continued)
Clinical Trials Summarizing the Effect of Postprandial Lipoprotein Metabolism on Coronary Artery Disease (CAD)

Study/Design	Population	Conclusion
Uiterwaal et al. (1994) Case–control	80 sons of men with severe coronary artery disease and 55 sons of controls	Healthy young adult sons, whose fathers have established coronary artery disease, have prolonged postprandial hypertriglyceridemia (difference, 0.35 mmol/L; 95% CI, 0.07–0.62 mmol/L) at 8 h
Nordestgaard et al. (2007) Cohort	7,587 women and 6,394 men from the general population of Copenhangen, aged 20–93 years, followed up from baseline (1976–1978) until 2004	In this general population cohort, elevated nonfasting triglyceride levels were associated with increased risk of myocardial infarction (MI), ischemic heart disease (IHD), and death in men and women
Bansal et al. (2007) Cohort	26,509 initially healthy U.S. women (20,118 fasting and 6,391 nonfasting) participating in the Women's Health Study, enrolled between November 1992 and July 1995 and undergoing follow-up from a median of 11.4 years	Nonfasting triglyceride levels were associated with cardiovascular events, independent of traditional cardiac risk factor, levels of other lipids, and markers of insulin resistance. On the other hand, the fasting triglyceride levels showed little independent relationship

17.4.4.2.1 Apolipoprotein Polymorphisms

Apolipoprotein A-I Apo A-I is the main HDL protein and plays a crucial role in lipid metabolism. It is an in vivo activator of the lecithin-cholesterol acyltransferase (LCAT) enzyme (Fielding et al., 1972), and is an essential element of reverse cholesterol transport (Reichl and Miller, 1989); these facts may be relevant to postprandial metabolism. Calabresi et al. (1993), showed that carriers of the rare apo A-I Milano mutation have threefold higher postprandial lipemia. However, if a correction for the different baseline TG levels is applied, A-I Milano mutation carriers have similar TG levels as control subjects. In another study, carriers of the A allele in the promoter region of apo A-I (–76 base pairs G/A genotype), which occurs at a frequency of 0.15–0.20 in Caucasians, have a greater postprandial increase in large TRL (35%) and a smaller decrease in LDL-cholesterol (10%) and apo B (8%) after the consumption of a fatty meal than do those with the G/G genotype (Marin et al., 2002). These responses are accompanied by a greater postprandial decrease in the concentration of apo A-IV carried in large TRL. The different postprandial responses observed could be due to changes in fat and cholesterol absorption from the diet or could also be the result of a decreased clearance of

TRL particles of intestinal origin, as indicated by the greater increase of apo B-48 (twofold) and large postprandial TRL-TG concentrations, independently of baseline plasma TG levels.

Apolipoprotein A-II Genetic studies have associated apo A-II with plasma TG levels as a result of accumulation of large, chylomicron-like VLDLs. This phenomenon is probably due to inhibition of LPL and HL by human apo A-II present on VLDLs (Boisfer et al., 1999; Pastier et al., 2001). A common functional polymorphism in the promoter region, a T to C substitution at position –265 in element D, which occurs at a frequency of 0.35 in Caucasians, was associated in healthy men with decreased plasma apo A-II concentration and enhanced postprandial metabolism of large-VLDL (20%) after an oral fat tolerance test (van't Hooft et al., 2001).

Apolipoprotein A-IV Apo A-IV influences dietary fat absorption and chylomicron synthesis (Ordovas et al., 1989), modulates the activation of LPL by apo C-II (Goldberg et al., 1990), activates LCAT (Steinmetz and Utermann, 1985), and participates in reverse cholesterol transport (Stein et al., 1986; Steinmetz et al., 1990; Weinberg and Patton, 1990; Kronenberg et al., 2000). The most common variants detected are Gln360His and Thr347Ser (Menzel et al., 1990; de Knijff et al., 1992). Subjects with the His360 allele, which occurs at a frequency of 0.08–0.10 in Caucasians, had a higher postprandial increase in small TRL-cholesterol, small TRL-TG ($P < .01$), and large TRL-TG than 360 Gln/Gln subjects (Ostos et al., 2000), this is probably due to a delayed hepatic clearance of chylomicron remnants. The Thr347Ser polymorphism, which occurs at a frequency of 0.18–0.22 in Caucasians, also conditions the postprandial lipemic response so that carriers of Ser347 allele presented a lower postprandial response (–26%) in the TG levels of chylomicrons remnants particles associated to a higher postprandial response in the plasma levels of Apo A-IV than subjects homozygous for the Thr347 allele (Ostos et al., 1998). The Thr347Ser polymorphism induces changes in the secondary structure and slightly increases the hydrophilic profile at this position, which could decrease its affinity for the lipids on TRL particles; this could facilitate the exchange with apo C-II and thus increase LPL activity on the particles themselves, which would in turn accelerate the clearance of remnants.

Apolipoprotein A-V Apo A-V plays an important role in lipid metabolism by modulating hepatic VLDL synthesis and/or secretion, as well as TRL catabolism at the level of LPL (Weinberg et al., 2003). In, humans T-1131C (originally referred to as SNP3) and Ser19Trp polymorphisms have been detected (Pennacchio et al., 2001; Pennacchio et al., 2002; Talmud et al., 2002). Associations between these polymorphisms and TG concentrations have been found in healthy, nonsmoking subjects not receiving lipid lowering medication as well as in different population samples (Ribalta et al., 2002; Vrablik et al., 2003). In addition, the C allele of the T-1131C single nucleotide polymorphism (SNP) located in the promoter region, which occurs at a frequency of 0.20–0.25 in Caucasians, was found to be associated with higher concentrations of plasma TG (Masana et al., 2003) and higher postprandial TG levels (30%) (Jang et al., 2004; Moreno et al., 2005) during the postprandial state. However,

the effect of this polymorphism on postprandial lipoprotein response may be mediated, at less in part, by its effect on fasting TG levels, since multiple regression analysis showed an effect of this SNP on both fasting and postprandial TG levels (Jang et al., 2004).

Apolipoprotein B Apo B is required for the assembly and secretion of chylomicrons in the intestine and VLDL in liver, and it also acts as the ligand for the recognition of LDL by the LDL receptor. Since apo B is the main protein of LDL and a major component of VLDL, it is to be expected that genetic variations at this locus could influence plasma cholesterol and/or TG levels in both fasting and postprandial states. The XbaI polymorphism, a silent mutation (ACC → ACT) in exon 26 (Carlsson et al., 1986), was related to the interindividual variability observed during postprandial lipemia. Thus, the frequent X-allele is associated with a significantly increased postprandial response of retinyl palmitate (50%) in all TRL fractions, independently of baseline TG levels (Lopez-Miranda et al., 1997). This mutation does not lead to an amino acid change at the affected codon and cannot have a direct functional effect. However, it is in strong linkage disequilibrium with the apo B Val591Ala polymorphism (Ag a1/d), which may be the functional sequence change. The D allele at the three-codon (leu-ala-leu) I/D polymorphism within the apo B signal peptide (SP) (Boerwinkle and Chan, 1989) was associated with reduced postprandial lipid response in comparison with individuals homozygous for the I allele, thus suggesting that this SP mutation may affect apo B secretion during the postprandial state. More recently, the association between postprandial NEFA concentrations and TRL has been reported to be influenced by this common deletion polymorphism (Byrne et al., 1996), which is also involved in postprandial response (Regis-Bailly et al., 1995).

Apolipoprotein C-I Apo C-I is a constituent of TRL and has been shown to displace apo E from TG-rich emulsions and interfere with their hepatic clearance. Apo C-I also interferes with the binding of VLDL to the LDL-related protein receptor (Weisgraber et al., 1990) and of VLDL/IDL to LDL receptors (Sehayek and Eisenberg, 1991). The presence of the apo C-I 317-321ins allele, which occurs at a frequency of 0.30 in Caucasians, has been shown in vitro to increase expression of apo C-I by 50%. Elevated apo C-I levels have been shown to inhibit both apo E mediated uptake of TG-rich emulsions in perfused rat liver and remnant clearance in apo C-I transgenic mice (Jong et al., 1999). Thus, a direct inhibitory mechanism would most likely explain the high levels (40%) of remnant lipoprotein-TG and remnant lipoprotein-C observed in apo C-I 317-321 ins/ins subjects (Waterworth et al., 2000). This effect appeared to be recessive, with no obvious effect in heterozygous carriers. However, as expected from their strong genetic relationship, the effect of the apo E and apo C-I variants were not independent of each other.

Apolipoprotein C-III Plasma apo C-III inhibits LPL and the binding of apo E containing lipoproteins to its receptors. Five polymorphisms (−641C/A, −630G/A, −625T/deletion, −482C/T, and −455T/C) have been identified in the promoter region of this gene, all of which are in linkage disequilibrium with the SstI site in the 3′

untranslated region. The SstI polymorphism, which arises from a cytosine to guanine substitution in the 3′ untranslated region of the apo C-III gene, distinguishes the S1 and S2 alleles. Recently, the raising effect of the −482C/T variant on plasma remnant particles was confined to homozygous carriers of the −482T allele rather than *Sstl* polymorphic site (Waterworth et al., 2000). It should be noted that a second variant, −455T/C, which was not evaluated in that study, is also present in the insulin response element, and would also be likely to show an association with remnant lipoprotein-TG as it is in strong linkage disequilibrium with the −482C/T variant. Both insulin response element variants were shown to be critical in conferring the response to insulin in vitro (Li et al., 1995). Hypothetically, in a postprandial situation, where insulin down regulates apo C-III to release its inhibitory effect on LPL, carriers of the −482T allele could maintain inappropriately high levels of apo C-III and, as a consequence elicit reduced TG hydrolysis by LPL and a delayed clearance of TRL and remnant lipoproteins. This is in agreement with a recent study where T-455C homozygotes influenced apo C-III levels (Olivieri et al., 2002). In another study, subjects homozygous for the G allele at the apo C-III T2854G polymorphism were associated with an increased postprandial TG response (Woo and Kang, 2003). The GG homozygotes had a 21% higher TG AUC than the T/T homozygotes and 22% higher TG area than the T/G heterozygotes.

Apolipoprotein E Apo E is a structural component of several lipoproteins and serves as a ligand for the LDL receptor and the LDL receptor-related protein (Beisiegel et al., 1989; Weisgraber et al., 1990). Therefore, apo E plays an important role in lipid metabolism by both promoting efficient uptake of TRL from the circulation and by taking part in the cellular cholesterol efflux and reverse cholesterol transport (Gylling et al., 2004). However, such functions are not uniformly effective because apo E is present in the population in three main isoforms (E2, E3, and E4), which determine apo E concentrations and differ in their affinity to bind to the specific receptors (Mahley, 1988; Mahley et al., 1995). In addition, apo E isoforms are important determinants of postprandial lipemia. It has been demonstrated that apo E2 homozygous subjects have the lowest affinity for TRL remnant receptor(s), and this genotype is associated with delayed postprandial clearance. Compared with apo E3 homozygous patients, apo E4 carriers tend to have enhanced clearance of remnants (Weintraub et al., 1987). However, several studies have found enhanced and/or prolonged postprandial lipid and apo responses in apo E4 carriers (Dart et al., 1997; Dallongeville et al., 1999). Patients with the metabolic syndrome who do not have the E3/3 genotype have a greater risk (OR 6,2 confidence interval, 1.41–16.08) of hyperuricemia and postprandial hypertriglyceridemia after a fat overload (Cardona et al., 2005). On the other hand, a polymorphism in the proximal promoter region of the *apo E* gene was recently described at position −219G/T (Mui et al., 1996; Artiga et al., 1998; Boisfer et al., 1999), which is associated with increased risk of myocardial infarction (MI) (Lambert et al., 2000) and CHD (Viitanen et al., 2001). The −219T allele was associated with decreased transcriptional activity (Artiga et al., 1998; Lambert et al., 2000), decreased plasma apo E concentration both in fasting and postprandial state (Lambert et al., 2000; Moreno et al., 2003) and prolonged and enhanced postprandial lipemic response (50% increase for homozygotes

T/T and 15% for heterozygotes T/G) (Moreno et al., 2003). It is probable that lower apo E plasma levels observed in subjects homozygous for the T allele are also associated with lower apo E TRL levels, thus reducing clearance by hepatic receptors. This phenomenon could be associated with the higher postprandial response observed, as well as the increased risk of MI and premature CHD associated with homozygote carriers of the T allele (Lambert et al., 2000).

17.4.4.2.2 Transport Proteins

Intestinal Fatty Acid-Binding Protein The intestinal fatty acid-binding protein (FABP-2) is located in the intestine and involved in long-chain fatty acid transport and metabolism (Matarese et al., 1989). A common alanine for threonine substitution at "FABP2" codon 54 (the A54T polymorphism), which occurs at a frequency of 0.28 in white populations, has been associated with hypertriglyceridemia, obesity, hyperinsulinemia, and insulin resistance (Baier et al., 1995; Hegele et al., 1996; Yamada et al., 1997). The "T54" allele is associated with a 41% increased postprandial lipemia in obese (Agren et al., 1998) and 80% increase in diabetic (Georgopoulos et al., 2000) subjects. However, not all studies have supported the associations with postprandial lipemia (Tahvanainen et al., 2000). It has been proposed that this association might depend on the type of fat ingested. Thus, in a recent study where subjects were given three oral fat tolerance tests (butter, safflower oil, and olive oil) the T54 group had increased chylomicron cholesterol only after the olive oil-containing test (Dworatzek et al., 2004). Another study in men from the Metabolic Intervention Cohort Kiel who underwent a standard glucose tolerance test and a standardized mixed meal test, demonstrated that the association of the FABP2 exon polymorphism A54T with postprandial triglycerides and postprandial insulin sensitivity depends on the combination with the homozygosity for FABP2 promoter B. Only the combination of FABP2 T54T with the homozygosity of promoter variant B caused higher postprandial plasma triglycerides and lower insulin sensitivity when compared with all other genotypic combinations (Helwig et al., 2007).

Fatty Acid Transport Proteins The fatty acid transport proteins (FATPs) have been implicated in facilitated cellular uptake of NEFA, thus having the potential to regulate local and systemic NEFA concentrations and metabolism. Hypothesizing that genetic variation within the FATP genes may affect postprandial metabolism, the G/A substitution at position 48 in intron 8 of the fatty acid transport-1 (FATP1) gene was studied. Although fasting plasma TG concentrations were not different, male A/A individuals had significantly higher postprandial TG concentrations and VLDL1 (Sf 60-400 apoB100)-to-VLDL2 (Sf 20-60 apoB100) ratio compared to male G/A and G/G individuals (Gertow et al., 2003).

17.4.4.2.3 Enzymes and Receptor Polymorphisms

Lipoprotein Lipase The LPL gene is an obvious candidate for studies of postprandial lipemia, inasmuch as it codes for the single-protein-hydrolyzing TG from chylomicrons and large VLDL. It also enhances the binding of apo E containing lipoproteins to the LDL receptor-related protein, thus affecting the catabolism of chylomicron remnants (Beisiegel et al., 1991). Talmud et al. (1998) have studied the

interaction between the functional variants involving the LPL-93T/G promoter polymorphism and the LPL D9N substitution that were identified with a combined population frequency of 3%–6%. Carriers of the haplotype constituting the rare LPL-93G variant (presumably higher transcriptional activity) and the common LPL9N variant (presumably secretion-defective LPL protein) exhibit higher plasma TG levels after meal intake than do carriers of other haplotypes (Talmud et al., 1998). The LPL A291S residue variant affects the specific activity of the enzyme and has a carrier frequency of 4%–6% (Fisher et al., 1997). Two studies show that carriers of this variant have significantly higher (41% higher AUC) postprandial triglyceridemia (Gerdes et al., 1997; Mero et al., 1999). In a recent study, the association of LPL HindIII (H1/H2) and Serine447-Stop (S447X) polymorphisms on postprandial lipemia was analyzed. Thus, carriers of the H1X447 genotypes presented a lower postprandial lipemic response (−42% lower AUC) than subjects with the H2S447 genotype (homozygote for the H2 allele of the LPL HindIII polymorphism and S447 allele), independently of baseline TG levels (Lopez-Miranda et al., 2004).

Hepatic Lipase HL has been implicated in the removal of remnant lipoproteins. The promoter of the HL gene contains several single nucleotide polymorphisms (van't Hooft et al., 2000). The rare variant of the −480C/T (also called −514C/T) polymorphism, present in 0.15%–0.21% of Caucasians, has been associated with lower HL activity. Jansen et al. observed that this polymorphism does not seem to affect total postprandial triglyceridemia but does affect the retention of a specific lipoprotein subspecies in the postprandial state like LpCIII:B particles, which are likely to reflect remnant lipoproteins (Jansen et al., 1999). However, in a recent study subjects homozygous for the T allele showed a lower postprandial response of TRL particles (−47% lower AUC) with a decrease in both total TG and in small and large TRL-TG postprandial response (Gomez et al., 2004).

Cholesterol Ester Transfer Protein CETP plays a major role in the remodeling of lipoprotein particles by mediating the transfer of cholesteryl ester from HDL to apo B-containing lipoproteins in exchange for TG. When the level of TRL is normal, CETP transfers from HDL cholesteryl esters are directed with preference towards LDL particles (Guerin et al., 2001). In contrast, when the level of TRL is increased, CETP transfers of HDL cholesteryl esters are directed towards larger VLDL particles, and there are high net transfer rates of TG to LDL and HDL (Barter et al., 2003). A common polymorphism detected using TaqI (TaqIB) has been shown to be a silent base change affecting the 277th nucleotide in the first intron of the CETP gene (Drayna and Lawn, 1987). The allele carrying the cutting site for the TaqI enzyme is called B1, whereas the one in which the cutting site is missing is known as B2. The more common B1 allele occurs at a frequency of 60% and is associated with higher CETP levels and reduced HDL-C levels compared with the less common B2 allele in most populations studied (Fumeron et al., 1995). This polymorphism has been found to account for 5.8% of the variance in HDL-C (Corella et al., 2000). Subjects with the B2 allele usually have lower levels of CETP, higher levels of HDL-C and a reduced risk of CHD in males compared to B1 subjects (Ordovas et al., 2000). A study carried out in patients with familial hypercholesterolemia, who

were previously subjected to an oral fat tolerance test, showed that B2 carriers had a lower postprandial triglyceride response compared to B1 carriers. There were no differences in TG levels between B1 and B2 carriers in patients with a negative oral fat tolerance test response. Therefore, at higher TG concentrations, the B2 allele may protect against an exaggerated postprandial TG increase and subsequent lowering of HDL-C concentrations (Kolovou et al., 2007).

Microsomal Triglyceride Transfer Protein Microsomal triglyceride transfer protein (MTP) plays a role in the formation of VLDL in the liver and that of chylomicrons in the intestine by transferring core lipids to the apo B molecule. Common polymorphisms have been described at position −493G/T, −400A/T, and −164T/C in the promoter region of the MTP. Homozygous carriers of the rare MTP-493T variant, which is associated with higher transcriptional activity of the gene in vitro (Karpe et al., 1998), showed markedly elevated accumulation of small apo B-48 containing lipoproteins in the postprandial state in healthy subjects and type 2 diabetics (Karpe et al., 1998; Lundahl et al., 2002). The −400A/T substitution gave very similar lipoprotein results, but there was significant linkage disequilibrium between the two polymorphisms.

Scavenger Receptor Scavenger receptor class B type I (SR-BI) is one of the intestinal proteins involved in the absorption of dietary cholesterol and triglycerides, this suggests that it may also play a role in postprandial responses (Hauser et al., 1998; Bietrix et al., 2006). Thus, the presence of the 2 allele at the SR-BI polymorphism in exon 1 was associated with a faster clearance of small-TRL and is probably related to a more rapid hepatic uptake (Perez-Martinez et al., 2004).

17.5 CONCLUSIONS

Postprandial lipoprotein metabolism is modulated by background dietary pattern, meal composition, lifestyle conditions (physical activity, smoking, and alcohol consumption), physiological factors (age, gender, and menopausal status), and pathological conditions (diabetes mellitus, insulin resistance, and obesity). Additionally, the postprandial lipid response has been shown to be modulated by polymorphisms within the genes for apo AI, apo E, apo B, apo C-I, apo C-III, apo A-IV, apo A-V LPL, HL, FABP-2, the FATPs, CETP, MTP, and SR-B1. Moreover, most of the past and current studies are being conducted using the simplest scenarios, i.e., one single dietary component, one single SNP, and one single risk factor. We have to evolve towards more realistic situations involving interactions of multiple genes, dietary components, and risk factors (Corella and Ordovas, 2005). This will require large genetic epidemiological studies and intervention studies involving groups of individuals selected for specific genotype combinations and phenotypic characteristics, who are then subjected to controlled dietary intervention protocols in order to establish specific gene–diet interactions. Nutrigenomics examines the effect of genetic variation on the interaction between diet and disease and presents it as several risk factors. This includes identifying and characterizing gene variants and factors associated or responsible for differential responses to nutrients or postprandial

responses. The goal of nutrigenomics is to generate recommendations regarding the risks and benefits of specific diets or dietary components for each individual. It has also been termed "personalized nutrition" or "individualized nutrition." Intervention and observational studies that attempt to examine gene–diet interactions need to include repeated sampling and measurement to provide an accurate measure of the phenotypes. To elucidate gene–environment interactions, and specifically gene–diet interactions, we need population sizes several orders of magnitude larger than those currently used for common multifactorial diseases. This will require the creation of international consortiums built along the models of the EPIC study or the Human Genome Project. Complex phenotype and genotype interactions require analysis of their combined effects. The information will need to be incorporated into predictive models that can be used clinically to improve disease assessment and prevention. This will probably happen within the umbrella of bioinformatics or computational biology.

ABBREVIATIONS

TG	triacylglycerol
C	cholesterol
LPL	lipoprotein lipase
VLDL	very low-density lipoprotein
HDL	high-density lipoprotein
TRL	triacylglycerol-rich lipoprotein
SFA	saturated fatty acid
PUFA	polyunsaturated fatty acid
MUFA	monounsaturated fatty acid
CHD	coronary heart disease
Apo	apolipoprotein
FABP-2	fatty acid-binding protein
MTP	microsomal triglyceride transfer protein
SR-BI	scavenger receptor class B type I
NEFA	nonesterified fatty acid
CETP	cholesteryl ester transfer protein

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Part VII

Lipids and Disease

18 Control of Fatty Acid Intake and the Role of Essential Fatty Acids in Cognitive Function and Neurological Disorders*

Kiran S. Panickar and Sam J. Bhathena

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18.1 INTRODUCTION

A fatty acid is a carboxylic acid often with a long unbranched aliphatic chain and is divided into two categories based on structural and chemical properties: (1) saturated and (2) unsaturated. Saturated fatty acids do not contain any double bonds or other functional groups along the chain. Unsaturated fatty acids contain at least one pair of carbon atoms linked by a double bond enabling the addition of other atoms to these carbons. Distinction between the two is simply that saturated fatty acids

* This chapter is dedicated to the memory of Dr. Sam Bhathena, an eminent scientist at the USDA, who passed away during the preparation of this manuscript.

are usually solid at room temperature whereas unsaturated fatty acids are liquid. Unsaturated fatty acids can be further divided into monounsaturated (which contains only one double bond) or polyunsaturated fatty acids (PUFAs), which contain more than one double bond. PUFAs are further grouped based either on the location of the double bonds and/or according to the chain length. An omega (ω) notation indicates the number of carbon atoms from the methyl end to the first double bond. Omega-3 (ω − 3 or n − 3) and Omega-6 (ω − 6 or n − 6) are two well-known fatty acids that are termed "essential fatty acids" (EFA). Such EFA are obtained from diet since they cannot be manufactured by cells (double bonds can be introduced into all positions of the fatty acid chain except the n − 3 and n − 6 positions). α-Linolenic acid (ALA) is an ω − 3 fatty acid that is converted to eicosapentaenoic acid (EPA) and subsequently from EPA to docosapentaenoic acid (DPA) and then to docosahexanoic acid (DHA). Linoleic acid (LA) is the parent fatty acid for ω − 6 class of fatty acids. LA is converted to γ-linoleic acid (GLA) which through subsequent conversions results in the formation of arachidonic acid (AA) and is a precursor for several classes of eicosanoids. In the nervous system, cell membranes contain relatively high concentration of PUFAs, such as docosahexaenoic acid (DHA) (Stillwell and Wassall, 2003).

In this review we provide a synthesis of evidence concerning the neural and hormonal control on food intake with a special emphasis on long-chain fatty acids. Fatty acids act on the central nervous system (CNS) as important physiological regulators of energy metabolism and overall energy homeostasis. In addition, we will examine evidence on key neural structures and systems influenced by fatty acids that are involved in feeding behavior. Lastly, we will review evidence on the role of EFA in preventing cognitive decline as well in neurological disorders.

18.2 NEURAL AND HORMONAL REGULATION OF FATTY ACIDS

Maintenance of energy homeostasis is critical to the well-being of an individual as excess energy balance can lead to increased adiposity. A complex physiological system regulates energy intake and expenditure composed of both afferent and efferent signals to and from the brain, respectively. These signals include glucose, lipids, peptides as well as steroids, all of which influence appetite. Links between peripheral signals and the brain to regulate food intake are complex and several hypotheses including the glucostatic (Mayer, 1953, 1955, 1996) and lipostatic (Kennedy, 1953; Mayer, 1955) hypotheses have been proposed to explain their interrelationship. Based on the observation that neurons primarily use glucose, the glucostatic theory posits that food intake is determined by the use of glucose by neurons and fluctuations in glucose availability or usage are linked to food intake. If glucose availability is low, neurons would be activated and hunger increased but when the rate of glucose utilization is high, the activity of the brain cells sensitive to glucose is diminished (see Levin et al., 2004). Thus energy intake would keep up with energy expenditure and thereby maintain energy balance. A major difference between peripheral tissues, such as muscle and the brain is that the neurons cannot store glucose, indicating that the metabolism of glucose may have a unique place in the regulation of appetite. Lipostatic theory is based on the observations that neurons monitor the levels of circulating lipids which is indicative of the nutritional status and that lipid storage

in the organism is connected to the neuronal or hormonal control of appetite. Thus, according to this theory, circulating levels of hormones including leptin or insulin, as well as substrates such as fatty acids, modulate hypothalamic function in determining appetite. While both glucostatic and lipostatic theories have studies supporting their hypothesis, energy regulation is far more complex and likely both mechanisms influence each other in the CNS to regulate overall energy homeostasis.

Investigations into the neural control of energy balance began with studies that showed lesions of specific nuclei in the hypothalamus could either produce increased or decreased food intake in animals depending on the location of the lesion (Anand and Brobeck, 1951; Stellar, 1954). In addition to the hypothalamus, other centers in the brain that contribute to appetite control include the brain stem (Carlisle and Reynolds, 1961), limbic structures, and the cerebral cortex (Grossman and Grossman, 1963; see Berthoud, 2002 for review). A neural network that includes these structures, with coordinated signals amongst them, likely influences energy regulation in the organism. Traditionally, adipose tissue, liver, and skeletal muscle have been the focus of such research, but the role of CNS in sensing the energy needs of the organism and conveying such information to the peripheral centers is vastly appreciated. Most research, however, centers on the role of the hypothalamus. The hypothalamus in the brain contains nuclei that control energy homeostasis as well as the regulation of food intake generally by altering the expression of neurotransmitters or neuromodulators (Kalra et al., 1999). The arcuate nucleus (ARC), which is located around the base of the third ventricle, responds to circulating levels of leptin, ghrelin, and insulin. The ARC, surprisingly, is not protected by the blood–brain barrier (BBB) (Brightman and Boradwell, 1976) but not all circulating hormones cross the BBB freely to influence the ARC (Banks et al., 1996; Banks, 2004) indicating a likely regulatory mechanism at the BBB. Two major nuclei in the ARC that play important roles in feeding are (1) the ventromedial nucleus of the ARC that contains the orexigenic (feeding promoting) center and expresses neuropeptides agouti-related protein (AgRP) and neuropeptide Y (NPY) (Broberger et al., 1998; Hahn et al., 1998) (2) ventrolateral nucleus of the ARC contains cells that are anorexigenic (feeding inhibitors) and expresses proopiomelanocortin (POMC)/alpha-melanocyte-stimulating hormone (α-MSH) and cocaine- and amphetamine-regulated transcript (CART; Elias et al., 1998; Kristensen et al., 1998). Melanocortin receptors, receptors for MSH, and adrenocorticotropic hormone (ACTH), are a subfamily of G protein-coupled receptors (Adan and Gispen, 1997) that consist of at least five subtypes. α-MSH secreted from POMC/CART neurons acts on melanocortin 3/4 receptors in the hypothalamus, whereas AgRP secreted from NPY/AgRP neurons acts on these receptors as an antagonist. Thus, melanocortin 3/4 receptors in the hypothalamic neurons play an integral role in the central control of appetite and energy expenditure.

Extensive reciprocal connections exist between the hypothalamus and the brainstem, particularly the nucleus of the solitary tract (NTS) (Ricardo and Koh, 1978). The NTS lies in close proximity to area postrema, which benefits from some lack of the BBB, and thus may be influenced by circulating levels of hormones or fatty acid metabolites. NTS has a high density of NPY-binding sites (Harfstrand et al., 1986) and NPY-expressing neurons (Sawchenko et al., 1985). In addition, melanocortin four receptors are also expressed in the brainstem (Mountjoy et al., 1994).

The corticolimbic structures play an important role in appetite regulation likely by modulating reward, cognitive, and emotional factors. Using functional magnetic resonance imaging (fMRI) to investigate activation of reward system in the brain, Stoeckel et al. (2008) reported that pictures of high-calorie foods produced significantly greater activation in the cortex, hippocampus, and striatum of obese subjects when compared to controls. Neurobiological responses in obese subjects to an implantable gastric stimulator (IGS), which induces stomach expansion via electrical stimulation of the vagus nerve, showed increased metabolism in the hippocampus as assessed by positron emission tomography and 2-deoxy-2[^{18}F]fluoro-D-glucose (Wang et al., 2006), indicating the importance of the hippocampus in modulating eating behaviors linked to emotional eating. A recent study indicated that in rodents, gastric electric stimulation may increase the expression of cholecystokinin (CCK), a hormone, in the hippocampus (Xu et al., 2008) and thus may be involved in inhibiting food intake. In addition, CART, POMC, NPY, and AgRP mRNAs are all expressed in the adult human hippocampus (Bai et al., 2005) as well as in rat hippocampus (Bai et al., 2005 and references therein), but their role in regulating appetite is not clear.

Numerous hormones play a key role in appetite regulation generally through their action on neural centers. Adipocytes, the gastrointestinal tract, and the pancreas are some of the major sites of hormone production in the periphery. One of the first hormones from the periphery implicated in the regulation of metabolism is CCK (see Beglinger, 2002, for review). CCK acts on the peripheral vagal afferent receptors to inhibit food intake, through its eventual actions on the brainstem. As mentioned above, CCK may also exert its effects on the hippocampus.

Leptin, an endocrine hormone and a product of *ob* gene, is secreted predominantly by adipocytes and acts on the hypothalamus. Leptin reduces adipocity and is another example of the endocrine-brain signaling pathway that regulates food intake. In the CNS, leptin acts to suppress appetite and increase energy metabolism (Halaas et al., 1995; Pelleymounter et al., 1995). Leptin administered peripherally (Pelleymounter et al., 1995; Hwa et al., 1997) or centrally (Hwa et al., 1996) to *ob/ob* mice reduces appetite, decreases fat mass, and increases metabolic rate. In the CNS, leptin receptors in the hypothalamus (Tartaglia et al., 1995) are hypothesized to mediate satiety by decreasing NPY levels (Campfield et al., 1995; Stephens et al., 1995) and/or by increasing metabolism through activation of the efferent sympathetic system (Campfield et al., 1996a,b). In the periphery, the mechanism of action of leptin, as studied in cell cultures and isolated tissue, is postulated to be through its effects on increasing metabolism primarily by intracellular lipolysis and fatty acid metabolism (Koyama et al., 1997; Shimabukuro et al., 1997).

Insulin is an important hormone that regulates food intake. Insulin is produced by the pancreas. Levels of insulin vary with adiposity (Bagdade et al., 1967). Central administration of insulin decreases food intake in primates (Woods et al., 1979) as well as in rats (Ikeda et al., 1986; Menendez and Atrens, 1991) and hypothalamus is implicated in insulin's action in reducing food intake (Strubbe and Mein, 1977; Menendez and Atrens, 1991).

Ghrelin is an orexigenic factor that is released from the stomach, duodenum, and ileum (Kojima et al., 1999; Date et al., 2000), and plasma ghrelin levels are inversely correlated with body mass index, a measure of obesity (Otto et al., 2001). Central

or peripheral administration of ghrelin increases food intake and body weight in rats (Tschop et al., 2000; Wren et al., 2001b). Intravenous administration of ghrelin to healthy human subjects also stimulates food intake (Wren et al., 2001a). Ghrelin is hypothesized to exert its action on the ARC in the rat hypothalamus (Tamura et al., 2002). Other hormones secreted by endocrine glands also affect energy balance including thyroid hormones, corticosterone, and growth hormones (see Kalra et al., 1999; Coll et al., 2007 for reviews). The exact mechanism by which such hormones act, whether alone or in concert, to regulate food intake is not clear.

Another class of molecules that also influence appetite is members of the fatty acid biosynthetic pathway including malonyl-CoA, an intermediate of fatty acid biosynthesis. Fatty acid oxidation plays a key role in the control of food intake. Evidence indicates an important role of fatty acids in the CNS where it plays a key role in physiological regulation of glucose metabolism and general energy homeostasis (Seeley and Woods, 2003; Lam et al., 2005a,b). Increased feeding by inhibition of fatty acid inhibition is hypothesized to send signals to the brain by vagal afferents. The brain plays an important role in the evaluation of energy status because the hypothalamus is the principal site that integrates signals from the periphery and other brain areas to regulate feeding behavior. The hypothalamus plays an important role in monitoring fatty acid metabolism as part of its energy-sensing function (Kim et al., 2002; Mobbs and Makimura, 2002; Obici et al., 2003). Fatty acids can be imported into the cell either from the blood circulation or synthesized *de novo* inside the cell. Within the cell, fatty acids either undergo oxidation in the mitochondria for energy production or may be directed towards glycerolipid synthesis (triglycerides or phospholipids) for later energy production or membrane function. In lipogenic tissues including liver and adipose, the fatty acid pathway is generally involved in the storage of excess energy in the form of triglycerides which can subsequently be oxidized to provide energy. Intracellularly, acetyl-CoA is the precursor for *de novo* synthesis of fatty acids. Acetyl-CoA carboxylase (ACC) catalyzes the conversion of acetyl-CoA to malonyl-CoA which is the basic unit for fatty acid synthesis by the enzyme fatty acid synthase (FAS) in the cytosol. To undergo mitochondrial oxidation, however, fatty acids must cross both the outer and inner mitochondrial membranes. This process is catalyzed by carnitine palmitoyltransferase-1 (CPT-1) which is bound to the outer mitochondrial membrane and subsequently by carnitine palmitoyltransferase-2 (CPT-2), an inner mitochondrial membrane enzyme. Two isoforms of ACC exist (ACC1 and ACC2) (Bianchi et al., 1990) with divergent functions for each and both isoforms are regulated by phosphorylation by 5′-adenosine monophosphate-activated kinase (AMPK), an energy-sensing enzyme. ACC1 (also known as ACC-α) catalyzes the carboxylation of acetyl-CoA to form malonyl-CoA. On the other hand, ACC2 (also known as ACC-β) colocalizes with CPT-1 and regulates mitochondrial fatty acid oxidation. Interestingly, malonyl-CoA is a potent inhibitor of CPT-1 (Ruderman et al., 2003) and has effects on both fatty acid oxidation in the mitochondria as well as the synthesis of various lipids. Disturbances in malonyl-CoA regulation may contribute to insulin resistance (Ruderman et al., 1999) and obesity (Ruderman et al., 1999; Loftus et al., 2000). ACC2-deficient mice accumulate less malonyl-CoA and show significant reduction in fat in adipose tissue, despite increased food intake, indicating that they were expending energy at an increased rate (Abu-Elheiga et al.,

2003). ACC has been detected in select neurons of the brain, notably in the ARC of the hypothalamus (McGarry and Brown, 1997), although which ACC isoforms are present in these neurons, however, is not clear. ACC has also been detected in other brain cell types including astrocytes in culture (Blazquez et al., 1998) and oligodendrocytes (Spencer et al., 1993), but the relative contribution of each cell type to food intake is currently not known. Hypothalamic inhibition of CPT-1 decreases food intake and suppresses endogenous glucose production (Ruderman et al., 1999).

One important enzyme that plays a key role in cellular energy homeostasis, as mentioned previously, is AMPK (Hardie and Hawley, 2001). AMPK is an energy-sensing kinase, which responds to changes in the energy levels of the cell and the whole body in order to maintain adequate adenosine triphosphate (ATP) levels in the cell. AMPK phosphorylates and inactivates ACC, thereby inhibiting fatty acid synthesis by decreasing malonyl-CoA availability (Frederich and Balschi, 2002). AMPK immunostaining in mouse brain sections reveals a predominantly neuronal distribution although it is also detected in activated astrocytes (Turnley et al., 1999). Significant interactions exist between fatty acids and the endocrine system (Bhathena, 2000, 2006). Hypothalamic malonyl-CoA responds to the level of circulating glucose and leptin, both of which affect energy homeostasis (Wolfgang et al., 2007). Leptin decreases AMPK activity in the hypothalamus (Minokoshi et al., 2004). Intracerebroventricular (ICV) injection of leptin, concomitant with inhibiting AMPK, activates ACC, the key regulatory enzyme in fatty acid biosynthesis, in the ARC and paraventricular nucleus (PVN) in the hypothalamus indicating that hypothalamic ACC activation makes an important contribution to leptin's anorectic effects (Gao et al., 2007). Orexigenic hormones such as ghrelin and cannabinoids stimulate hypothalamic AMPK leading to an increase in appetite while inhibiting AMPK activity in the liver and adipose tissue, thereby leading to lipogenic effects (van Thuijl et al., 2008).

Pharmacological modulation of fatty acid metabolism has been utilized to inhibit food intake (Loftus et al., 2000). C75 (*trans*-4-carboxy-5-octyl-3-methylenebutyrolactone), originally designed as a FAS inhibitor (Kuhajda et al., 2000), causes profound, reversible weight loss in lean mice, diet-induced obese (DIO) mice, leptin-deficient (*ob/ob*) mice (Loftus et al., 2000; Thupari et al., 2004), and normal lean rats (Loftus et al., 2000). FAS inhibitors were initially developed as inhibitors of tumor progression, but these inhibitors also produced weight loss in mice (Loftus et al., 2000). ICV administration of C75 also rapidly suppresses food intake (Clegg et al., 2002; Aja et al., 2008). When administered peripherally, C75 decreases NPY/AgRP levels (Loftus et al., 2000) and increases POMC/CART levels in the brain (Thupari et al., 2004; Tu et al., 2005). In addition, ICV administration of ghrelin in mice blocks the inhibitor effects of C75 and decreases NPY levels and increases POMC/CART levels (Hu et al., 2005) indicating the interrelationship that exists between FAS inhibition and these neuropeptides. However, multiple distinct events related to fatty acid metabolism in the CNS coordinate energy balance as glucose, insulin, amino acids, leptin, and other metabolites all regulate FAS directly or indirectly (Semenkovich et al., 1993; Dudek and Semenkovich, 1995; Fukuda et al., 1999). One example is the recent report by Chakravarthy et al. (2007) who used a FAS knockout mouse with a genetic inactivation of FAS in the hypothalamus and pancreatic β cell. While these mice had less adiposity and decreased food intake (similar to what is

observed with C75-treated animals), administration of a peroxisome proliferator-activated receptor-α (PPARα) agonist into the hypothalamus increased PPARα target genes and normalized food intake. This is novel since PPARα is a transcription factor that is believed to respond to starvation, but is regulated by FAS, an enzyme that is activated by feeding. Such complexity only further highlights increased modulation that exists intracellularly in the fatty acid metabolic pathway in the brain to maintain energy balance. Figure 18.1 shows the interaction of some hormonal and neural pathways in the control of food intake.

Cellular mechanisms by which fatty acids regulate neuronal activity are not clear, although their actions on ion channels in neurons as well as other cell types have been reported. Witt and Nielsen (2004) reported an increase in [^{3}H]Diazepam-binding in rat cortex *in vitro* upon treatment with fatty acids indicating an influence on the γ-aminobutyric acid (GABA)/benzodiazepine receptor/Cl^- ion channel. Their effect on G protein-gated K^+ channels by antagonizing the ATP-dependent gating

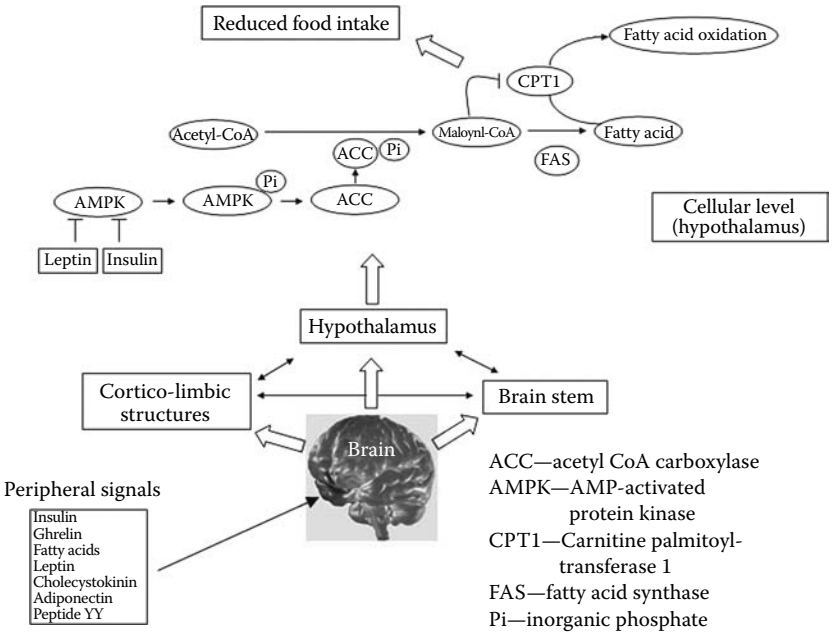

FIGURE 18.1 Schematic of the neural regulation of food intake. Hypothalamus, in concert with brain stem and corticolimbic structures receives and integrates hormonal and metabolic signals from the periphery to regulate energy homeostasis. At the cellular level, acetyl-CoA is carboxylated to malonyl-CoA by the enzyme acetyl-CoA carboxylase in the cytoplasm to eventually synthesize fatty acids. A decrease in AMPK activity results in reduced food intake. Anorectic hormones leptin and insulin inhibit AMPK activity. Malonyl-CoA inhibits oxidation of fatty acids by inhibiting CPT-1. Hypothalamic inhibition of CPT-1 reduces food intake. Inhibition of FAS also reduces food intake. Peripheral signals shown in the figure include both anorexigenic and orexigenic signals and, for the sake of simplicity, only effects of leptin and insulin are depicted at the cellular level.

in neurons and cardiac myocytes has also been reported (Kim and Pleumsamran, 2000). In gastric myocytes fatty acids markedly increase $I_{K(ca)}$, a calcium-dependent potassium current, and the enhancing potencies were related to the number of double bonds in the fatty acid chain (Zheng et al., 2005). Hyperpolarization of rodent hepatocytes by palmitate has also been reported (Rossi and Scharrer, 1995; Rossi et al., 1995). Whether these mechanisms occur in the brain in the hypothalamus or in other neural centers that are involved in the regulation of food intake is not known.

Sensitivity to EFA is observed in the periphery in the control of obesity. As a percentage of total fatty acids, $n-3$ EFA in liver and adipose tissue lipids were significantly lower in the obese mice than in the lean controls (Cunnane et al., 1985). Obese subjects have low EFA content in their circulating plasma lipids when compared to nonobese controls (Rössner et al., 1989). Serum and hepatic AA levels were elevated in obese compared to lean rats indicating abnormal arachidonate distribution in the obese Zucker rat (Phinney et al., 1993). A recent study reported that GLA reduced weight regain in humans following major weight loss (Schirmer and Phinney, 2007). While mechanisms underlying the effects of EFA in reducing obesity are not clear, LA significantly decreases adiponectin and leptin secretion, two adipokines known to influence weight gain and insulin sensitivity, in insulin-stimulated primary rat adipocytes (Pérez-Matute et al., 2007). Whether such a phenomenon occurs in the brain as well is not yet known.

18.3 ROLE OF FATTY ACIDS IN COGNITION

About 50%–60% of the dry weight of the brain consists of lipids and PUFAs constitute approximately 30% of the lipid content (Sastry, 1985). $\omega-3$ fatty acids including EPA and DHA play important roles in the development and maintenance of normal CNS structure and function. Chronic dietary intake of essential PUFAs may modulate learning and memory by being incorporated into neuronal plasma membranes. Representatives of two PUFA families, the $\omega-3$ and $\omega-6$ types become integrated into membrane phospholipids, where the actual $(\omega-6)/(\omega-3)$ ratio is hypothesized to determine membrane fluidity and thus the function of membrane-bound proteins. Animal studies suggest that a deficiency of $\omega-3$ fatty acids may lead to behavioral or cognitive deficits (Bourre et al., 1989; Yehuda et al., 1999; Hichami et al., 2007). In addition, supplementation of DHA enhances cognitive function in both adult and old mice (Shirai and Suzuki, 2004). This study also reported an enhancement when DHA was supplemented with catechin, a dietary polyphenol. Whether polyphenols and $\omega-3$ fatty acids act at multiple independent sites to improve cognition is not known and should be investigated further. While DHA has beneficial effects, an increase in lipid peroxides formation is also a possibility (Halliwell, 1992) which may result in oxidative stress that could be harmful to normal brain (Yavin et al., 2002). Dietary sources especially rich in $\omega-3$ fatty acids include fish such as tuna, trout, and salmon as well as some plant oils which are a rich source of ALA.

DHA is a key component of neural and retinal membranes, and rapidly accumulates in the brain during gestation and the postnatal period. Long-chain $\omega-3$ fatty acids are thought to be important for fetal neurodevelopment. Positive associations

have been shown between maternal intake of fish, seafood, and ω – 3 fatty acids during pregnancy and/or lactation and visual and cognitive development in human studies. Higher cord DHA concentration was associated with longer gestation, better visual acuity, and novelty preference on the Fagan Test at 6 months, and better Bayley Scale mental and psychomotor performance at 11 months. By contrast, DHA from breast-feeding was not related to any indicator of cognitive or motor development in this full-term sample (Jacobson et al., 2008). Despite encouraging results in human intervention studies (Oken et al., 2008; Olsen et al., 2008; Strain et al., 2008), several studies were unable to demonstrate a positive effect of prenatal ω – 3 supplementation (see Hadders-Algra, 2008 for review). Thus, limited evidence exists to support the notion that prenatal ω – 3 supplementation favors developmental outcome. Likewise, evidence for benefits of *n* – 3 long-chain PUFA on cognitive development in healthy children older than 2 years of age is too limited to allow a clear conclusion (Eilander et al., 2007). In adults, fatty fish and marine ω – 3 PUFA consumption was associated with a reduced risk of impaired cognitive function in a middle-aged population (Kalmijn et al., 2004). There was little evidence that the ω – 3 PUFAs were associated with cognitive change in an older population aged 65+ in the absence of any neurological disorder (Morris et al., 2005).

18.4 FATTY ACIDS IN NEUROLOGICAL DISORDERS

Deficiencies in ω – 3 fatty acids or an imbalance in the ratio of ω – 3 to ω – 6 fatty acids have been implicated in a variety of neurological disorders including Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), ischemic brain injury/stroke, and multiple sclerosis (MS) as well as psychiatric disorders. Neurons lack the enzymes for *de novo* synthesis of DHA and AA (Hardy and Higgins, 1992) and hence they are obtained from dietary sources or synthesized mainly in the liver from the dietary precursors ALA and LA. However, astrocytes produce DHA (Moore et al., 1991) some of which is likely exported to neurons although the mechanism by which fatty acids cross the plasma membrane to enter cells is not clear. A significant portion of studies have examined the role of DHA in neurological disorders.

18.4.1 Alzheimer's Disease

AD is an irreversible progressive neurodegenerative disorder that is the most common cause of dementia in the elderly. AD is characterized by cognitive and memory dysfunction. Neuropathologically, it is characterized by the deposition of extracellular amyloid beta (Aβ) protein and intracellular neurofibrillary tangles in the hippocampus and cerebral cortex (Khachaturian, 1985; Mirra et al., 1991). Associated synapse loss and neuronal death are key features of AD pathology. While the cause of sporadic AD is not known and is very likely multifactorial, AD is associated with several molecular and biochemical abnormalities including impaired energy metabolism/mitochondrial function and oxidative stress that may contribute to neuronal loss/dysfunction (Haass and Selkoe, 1993). More recently obesity has been recognized as an important risk factor for developing AD (Gustafson et al., 2003).

Reduced intake of ω − 3 or DHA deficiency has been reported as a risk factor for AD (Soderberg et al., 1991; Conquer et al., 2000; Tully et al., 2003; Pomponi and Pomponi, 2008). But no significant difference in the proportion of fatty acids, including those of the *n* − 6 and *n* − 3 series, in either the grey or the white matter, were observed in any of the regions studied in AD brain compared to controls (Skinner et al., 1993). Nevertheless, dietary DHA attenuates Aβ production, AD-like neuropathological changes as well as cognitive deficits in a transgenic mouse model of AD (Hashimoto et al., 2002, 2005) or in rats infused with Aβ (Hashimoto et al., 2006). In a similar transgenic mouse model of AD, safflower oil-induced ω − 3 deficiency induced apopototic-like cell death in the brain and this was partly protected by dietary supplementation with DHA (Calon et al., 2005). A protective role of DHA in AD transgenic mice has also been reported in other studies (Lim et al., 2005; Ma et al., 2007). However, Arendash et al. (2007) reported no improvement in AD-like neuropathology or cognition in transgenic mice when treated long term on diet rich in ω − 3. Whether the negative result was a consequence of assessing pathology as well as cognitive function at an advanced age as reported in this study is not known. If such is the case, however, then it is possible that the beneficial effects of DHA on cognition might be observed at an early stage of AD rather than when the symptoms of AD have advanced considerably. Gillette Guyonnet et al. (2007) reviewed 15 prospective cohort studies that focused on whether the risk of AD during aging is associated with low intake of total dietary fat, fish, and/or ω − 3 fatty acids from fish. While they concluded that there was no significant association between risk of AD and ω − 3 fatty acid or fish intake in some studies, a protective effect of higher fish and/or DHA intake on risk of AD was observed in others. A conclusive potential benefit of fish intake and/or DHA in reducing the risk of developing AD cannot be drawn at present.

18.4.2 Parkinson's Disease

PD is a progressive neurodegenerative disease that is caused by the loss of dopaminergic neurons in the basal ganglia. PD is characterized clinically by muscle rigidity, tremor, a slowing of physical movement (bradykinesia) and, in extreme cases, a loss of physical movement (akinesia). Cognitive dysfunction, subtle language problems, and depression are also observed.

In an animal model of Parkinsonism, mice fed on ω − 3 diet for 2–12 months showed a significant protection in preventing decline of dopamine and dopamine transporter mRNA levels when compared to controls, in response to 1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine (MPTP), a neurotoxin that produces symptoms resembling PD (Bousquet et al., 2008). A common treatment for PD patients is the administration of levodopa, which increases dopamine levels in the brain but can also cause unwanted dyskinesia with prolonged treatment. Pretreatment with DHA significantly reduced levodopa-induced dyskinesia without altering the antiparkinsonian effect of levodopa in a nonhuman primate model of PD (Samadi et al., 2006). In a prospective population-based cohort study of people aged ≥55, the association between intake of unsaturated fatty acids and the risk of incident PD was evaluated (de Lau et al., 2005). Intakes of total fat, monounsaturated fatty acids (MUFAs),

and PUFAs, were significantly associated with a lower risk of PD. A double-blind, placebo-controlled study showed that PD patients taking fish oil, with or without antidepressants, reduced depressive symptoms indicating that the intake of ω – 3 had an antidepressant effect or acted as adjuvant therapy with some other medication for PD (da Silva et al., 2008). However, more studies are needed to evaluate thoroughly the beneficial effects of ω – 3 in PD.

18.4.3 Huntington's Disease

HD also called Huntington's chorea is a progressive inherited neurodegenerative disease characterized by abnormal body movements and a lack of coordination (Harper, 1996). It is one of several polyglutamine-related diseases caused by a trinucleotide repeat expansion of Huntingtin gene. The disease is characterized by a profound neuronal degeneration in the striatum with some additional atrophy of the frontal and temporal cortex. The most common symptoms are jerky, random, and uncontrollable movements called chorea, although very slow movement and stiffness (bradykinesia, dystonia) sometimes appear instead or in later stages.

Early and sustained administration of EFA as a mixture of EPA, DHA, and LA to transgenic HD mice (R6/1 mice) protects against motor deficits when compared to placebo (Clifford et al., 2002). In a randomized, placebo-controlled, double-blind trial of highly unsaturated fatty acid therapy, HD patients showed a significant improvement in Dyskinesia Rating Scale (Vaddadi et al., 2002; see Das and Vaddadi, 2004 for review).

18.4.4 Ischemia/Stroke

Stroke is caused by an interruption of the blood flow to any part of the brain. It can be classified into two major categories: ischemic and hemorrhagic. Ischemia stroke is due to interruption of the blood supply due to blood clot, while hemorrhagic stroke is due to rupture of a blood vessel or an abnormal vascular structure. Stroke can lead to vascular leakage, inflammation, tissue injury, and necrosis. Long-term outcome of stroke depends on the region of the brain that is affected and the severity and generally paralysis, cognitive deficits, and speech and language problems may occur in survivors. Some major risk factors for stroke include age, obesity, diabetes, hypertension, high cholesterol, and poor diet.

Rats fed on ω – 3 diet for 6 weeks prior to middle cerebral artery occlusion show reduced ischemic damage compared to controls (Relton et al., 1993). Later studies have also shown protective effects of fish ω – 3 in animal models of ischemia (Bas et al., 2007; Ozen et al., 2008). ALA and DHA exert significant neuroprotective effects in animal models of focal or global ischemia (Lauritzen et al., 2000; Heurteaux et al., 2006). LA also prevents both necrosis and apoptosis of motor neurons in spinal cord ischemia in rats (Lang-Lazdunski et al., 2003). Chronic administration of DHA prior to inducing forebrain ischemia ameliorated spatial cognitive deficits in rats (Okada et al., 1996).

Administration of highly purified EPA reduced the risk of recurrent stroke in a Japanese population of hypercholesterolemic patients receiving low-dose statin

therapy (Tanaka et al., 2008). A prospective cohort study of women reported that higher consumption of fish and ω – 3 PUFAs reduced risk of total stroke and thrombotic infarction, but not the risk of hemorrhagic stroke (Iso et al., 2001). A population-based cohort from a community intervention program in Sweden, however, reported that increased levels of EPA and DHA did not decrease the risk for stroke (Wennberg et al., 2007). Balancing the risks and benefits of adequate fish consumption with the relative risk of toxic environmental contaminants in the fish should be considered (Domingo, 2007; Domingo et al., 2007a,b). Nevertheless, ω – 3 fatty acids appear to have a beneficial effect in preventing neural damage in ischemia/stroke.

18.4.5 Multiple Sclerosis

MS is an autoimmune condition in which the immune system attacks the CNS leading to demyelination of neurons. MS affects white matter in the brain and spinal cord. More specifically, MS destroys oligodendrocytes that form the myelin sheath around the axons of neurons, to help the neurons conduct electrical signals. MS results in a thinning or complete loss of myelin that results in the inability of neurons to conduct electrical signals effectively. Symptoms include muscle weakness, abnormal muscle spasms, difficulties with coordination and balance (ataxia), swallowing (dysphagia), acute or chronic pain syndrome, and bladder and bowel difficulties. Cognitive impairment has also been reported.

Swank et al. (1952) initially reported a lower incidence of MS in a coastal Norwegian community which had a high intake of fish when compared to a rural community. Decreased levels of ω – 3 in plasma as well as adipose tissue of MS patients have also been reported (Holman et al., 1989). Dietary supplementation of ω – 3 for 6 months to MS patients resulted in significant reduction in cytokines IL-1β, IL-2, IFNγ, and TNF-α as well as prostaglandin E2 (PGE2) and leukotriene B4 in peripheral blood mononuclear cells (Gallai et al., 1995; Calder, 1997). Reduced production of these proinflammatory eicosanoids and the decrease of some cytokines may underlie some of the beneficial actions of ω – 3 in MS.

Mechanisms underlying the neuroprotective effects of EFA are not clear. Antiapoptotic (Wu et al., 2007), neurotrophic (Rapoport et al., 2007), antioxidant (Packer et al., 1997), and anti-inflammatory effects (Das, 2007) have all been suggested as potential mechanisms based on *in vitro* or animal studies. In addition, regulation and normalization of intracellular Ca^{2+} by DHA in neural cultures has been reported (Sergeeva et al., 2005). Likely more than one pathway is involved, and possibly cross talk with other signaling pathways is also part of its protective action.

18.5 SUMMARY AND PERSPECTIVES

Obesity is a global health problem with nearly 1 billion people categorized as obese. It is estimated that in the United States ~60% of the adult population and nearly 13% of children are obese or overweight. Obesity is often associated with, or considered a major risk factor for diabetes, cardiovascular diseases, stroke, and cancer, but now it is also recognized as a risk factor for AD, a progressive neurodegenerative disease.

Obesity is also associated with declined cognitive function. Neural regulation of food intake is not only involved in behavior related to appetite but also in sensing the energy needs of the organism. The CNS including the hypothalamus, the brain stem, and the limbic system, with the help of autonomic nervous system, is sensitive to levels of metabolic and endocrine intermediates including glucose, fatty acids, insulin, and leptin that reflect peripheral energy status.

Both central and peripheral signals play important roles in regulating the complicated neuronal circuitry that regulates feeding and energy homeostasis. While much research into the role of fatty acids, based on *in vivo* and *in vitro* studies, have increased our knowledge, understanding their molecular mechanisms might provide new targets to prevent or attenuate obesity. Considering that multiple neural pathways function in concert to determine food intake, a more definitive role of how all these pathways relay information to one another and how various signaling molecules interact to eventually regulate energy homeostasis should permit a better understanding of appetite regulation. Furthermore, cellular mechanisms underlying the effects of fatty acids is not clear and should be investigated further in order to understand their effects on neural cells. While most studies have investigated the role of fatty acids on neurons, their effects on other cell types in the brain including astrocytes and oligodendrocytes are sparse. This would appear to be an important area of investigation since neuronal–astrocyte interactions are critical in determining the function of neurons especially since astrocytes provide energy substrates to neurons (see Panickar and Norenberg, 2005 for review). Further studies are also needed to examine the role of reward and cognition centers in the brain that modulate food intake. In addition, the function of orexigenic and anorexigenic peptides in the hippocampus to regulate appetite is not clear and needs further investigation. There is also evidence to indicate an important role for EFA in improving cognitive function and for neuroprotective effects of such are also appreciated, but more research and definitive data are required to reach conclusive answers.

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ABBREVIATIONS

ACC	acetyl-CoA carboxylase
AD	Alzheimer's disease
ALA	α-linolenic acid
AMPK	5′-adenosine monophosphate-activated protein kinase
AA	arachidonic acid
AgRP	agouti-related protein
ARC	arcuate nucleus
BBB	blood–brain barrier
CART	cocaine- and amphetamine-regulated transcript
CCK	cholecystokinin

CNS central nervous system
CPT carnitine palmitoyltransferase
DHA docosahexanoic acid
DPA docosapentaenoic acid
EFA essential fatty acids
EPA eicosapentaenoic acid
FA fatty acids
FAS fatty acid synthase
GLA γ-linoleic acid
HD Huntington's disease
LA linoleic acid
MS multiple sclerosis
MSH melanocyte-stimulating hormone
NPY neuropeptide Y
NTS nucleus of the solitary tract
PD Parkinson's disease
POMC proopiomelanocortin
PUFA polyunsaturated fatty acid

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19 What Is the Link between Docosahexaenoic Acid, Cognitive Impairment, and Alzheimer's Disease in the Elderly?

Michel E. Bégin, Mélanie Plourde, Fabien Pifferi, and Stephen C. Cunnane

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19.1 INTRODUCTION

Cognitive impairment in the elderly, particularly in the form of Alzheimer's disease (AD), has emerged in the past 20 years as a major challenge to the quality of life for the elderly and their caregivers, and to healthcare resources. AD is the most common form of dementia and the primary neurodegenerative disorder in the elderly. Once

it is clinically diagnosed, there is little prospect of improving the prognosis of AD. Cognitive deficits can progress gradually over many decades before reaching the clinical threshold for the diagnosis of AD (Petersen et al., 2001; Jorm et al., 2007). As the population ages, the prevalence of cognitive impairment leading to dementia and AD is expected to increase. Most of the subjects with mild cognitive impairment will progress to AD at a rate of 10%–15% per year compared with healthy control subjects who convert at a rate of 1%–2% per year (Petersen et al., 2001; Solfrizzi et al., 2006).

The cause of the progression of cognitive impairment to dementia and AD is not established. Genetic factors have been implicated and the apolipoprotein E ε4 allele is the genetic risk factor most associated with AD (Mahley et al., 2006). It is plausible that genetic factors, especially genes involved in lipid metabolism and transport, interact with environmental factors for lowering or increasing the risk of AD. Since aging is unavoidable and there is not yet a cure for AD, strategies to identify environmental factors lowering risk of AD are essential. Therefore, research on potentially modifiable risk factors for cognitive impairment, such as diet, is of great relevance.

Several studies showed that cognitive impairment in the elderly is associated with deficiencies of micronutrients and macronutrients (Rosenberg and Miller, 1992; Grant, 1999; Dye et al., 2000; Gonzalez-Gross et al., 2001; Gillette Guyonnet et al., 2007). Among macronutrients, there is increasing interest in the possible impact of dietary fatty acids on cognitive impairment and dementia. One class of dietary fatty acids closely associated with the function of the brain is the ω3 polyunsaturated fatty acids (PUFA), particularly docosahexaenoic acid (DHA), which is a major component of the membrane phospholipids in the brain. Fish and seafood (shellfish and crustacean) consumption is the main dietary source of preformed DHA.

Most epidemiological studies, but not all, suggest that fish and seafood consumption might protect the elderly from developing cognitive impairment or dementia including AD (reviewed in Gillette Guyonnet et al., 2007). Whether ω3 PUFA from fish and seafood, especially DHA, might be the principal contributors in preventing cognitive impairment and dementia in the elderly is presently debated. Previous reviews describing the relationship between ω3 PUFA and cognitive decline reported an inconclusive association (Maclean et al., 2005; Gillette Guyonnet et al., 2007; Plourde et al., 2007).

Therefore, this chapter examines the possible link between fish and seafood or DHA intakes and cognitive impairment and dementia including AD with emphasis on three types of human studies—evaluation of epidemiological studies on fish and seafood or DHA intake, analysis of DHA levels in blood or brain tissues, and clinical trials of supplementation with DHA-enriched oils in cognitively impaired nondemented (CIND) elderly and AD patients. In view of the literature as it stands presently, we sought to answer the following questions: (1) Does the intake of fish and seafood protect against cognitive impairment and its progression to dementia such as AD in the elderly?, (2) What is the biological evidence from tissue fatty acid analyses that DHA plays a significant role in the protective effect of fish and seafood consumption?, and (3) is DHA alone effective in the treatment of cognitive impairment and AD?

19.2 DEFINITIONS AND CLASSIFICATION

In this chapter, we will use the terms cognition, cognitive impairment, cognitive decline, dementia, and AD.

Cognition: Mental activities involved in the acquisition, storage, retrieval, and use of information are referred to here by the term "cognition" (Smith et al., 2002). Manifestations of cognitive behavior are achieved through the integration of a variety of processes and activities such as perception, imagery, memory, reasoning, problem solving, decision making, and language.

Cognitive impairment: Small deficits in cognitive performance may represent an early manifestation of the pathology of AD. These small deficits may progressively increase in severity to the levels reached in clinically diagnosed AD. Thus, the term cognitive impairment refers to a condition between normal aging and AD in which persons experience objective cognitive deficits to a greater extent than one would expect for age, yet, they do not meet currently accepted criteria for clinically probable dementia (Petersen et al., 2001; Panza et al., 2005). The expression mild cognition impairment has been described to represent preclinical AD or very mild AD, and clinical subtypes may be differentially related to risk of AD (Fabrigoule et al., 1998; Petersen et al., 2001; Solfrizzi et al., 2006; Dickerson et al., 2007). It differs from the term cognitive impairment in that it cannot be diagnosed by neuropsychological tests alone, and clinical judgement is always required.

CIND persons: Throughout this chapter, whether assessed by neuropsychological tests alone and/or clinically diagnosed, nondemented persons showing cognitive impairment or mild cognitive impairment, respectively, will be referred to as CIND persons. CIND persons have a higher risk of progression to dementia, but the evolution is heterogeneous, as some persons can improve over time or remain stable (Petersen et al., 2001; Solfrizzi et al., 2006). The term cognitive decline will specify the progression of cognitive impairment over time.

All-cause dementia: Dementia refers to a group of symptoms caused by damage or disease that affects the brain. It is characterized by a progressive decline in cognitive function beyond what might be expected from normal healthy aging. It constitutes a large category that includes AD, stroke or vascular dementia, and frontotemporal lobe dementia (Pick's disease). In most of the studies reported here, dementia was diagnosed according to the criteria of the *Diagnostic and Statistical Manual of Mental Disorders* after an initial screening of the participants with the minimental state examination (MMSE) or by neuropsychological tests and a clinical examination.

Alzheimer's disease: The most common form of dementia is AD. The neurodegeneration is characterized by progressive memory loss, decline in abstract reasoning, visuospatial perceptual changes, a reduced time of reaction, language difficulties, and eventually global cognitive impairment. The diagnosis of AD is based on the criteria defined by the National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association in 13 over 14 of the studies examined.

19.3 EPIDEMIOLOGICAL EVIDENCE ON THE ASSOCIATION OF FISH AND ω3 PUFA INTAKES WITH COGNITIVE IMPAIRMENT AND DEMENTIA

The epidemiological studies investigating the impact of fish and seafood consumption or ω3 PUFA on cognitive impairment and dementia including AD gave mixed findings. Two cross-sectional studies (shown in Table 19.1) reported that fish and marine ω3 PUFA intakes did not lower the risk of cognitive impairment in CIND old adults (Kalmijn et al., 1997a; van Gelder et al., 2007) whereas both fish and ω3 PUFA intakes lowered it in CIND middle-aged adults (Kalmijn et al., 2004). In prospective studies following CIND elderly during 3–5 years (Kalmijn et al., 1997a; Morris et al., 2005; van Gelder et al., 2007), an intake of more than 105 g of fish per week lowered from 10% to 75% the rate of cognitive decline (Table 19.2). However, the calculated ω3 PUFA or eicosapentaenoic acid (EPA) + DHA intakes corresponding to the amount of fish consumed gave conflicting results in that they may (van Gelder et al., 2007; Beydoun et al., 2008) or may not (Kalmijn et al., 1997a; Morris et al., 2005; Solfrizzi et al., 2006) be associated with reduced risk of cognitive decline. Hence, the effects of EPA + DHA or long-chain ω3 PUFA intake do not appear to always reflect that of fish intake. However, it cannot be ruled out that the possible difference in ω3 PUFA composition among different species of fish con-

TABLE 19.1
Cross-Sectional Studies Showing Fish or ω3 PUFA Intake Lowers (A) or Does Not Lower (B) the Risk of Cognitive Impairment in Nondemented Middle-Aged and Old Adults

	Participants			
Reference	***N***	**Age (Years)**	**Exposure[a]**	**Risk Value**
(A) *Lower risk*				
Kalmijn et al. (2004)	1613	45–70	3 g fatty fish/day	0.77[b]
			0.17 g EPA + DHA/day	0.81[c]
(B) *No risk reduction*				
Kalmijn et al. (1997a)	476	69–89	>20 g fish/day	NS
			0–2 g EPA + DHA/day	NS
van Gelder et al. (2007)	210	70–89	0 to >20 g fish/day	NS
			0.02–0.4 g EPA + DHA/day	NS

[a] The fish and ω3 PUFA intakes were derived from food frequency questionnaires or dietary histories.

[b] Odds ratio with $p = 0.05$.

[c] Odds ratio with $p = 0.03$.

DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; PUFA, polyunsaturated fatty acids; NS, not significant.

TABLE 19.2
Prospective Studies Showing Fish or ω3 PUFA Intake Lowers (A) or Does Not Lower (B) the Risk of Cognitive Decline in Cognitively Impaired Nondemented Adults

Reference	Participants		Follow-up (Years)	Exposure[a]	% Rate Reduction or Risk Value
	N	Age (Years)			
(A) *Lower risk*					
Morris et al. (2005)	3718	≥65	6	≥1 fish serving/week	**10**[‡]
Beydoun et al. (2008)	2251	50–65	6	Increase of 1 SD in EPA + DPA + DHA (% energy intake)	**0.79**[b,†] (verbal fluency only)
Van Gelder et al. (2007)	210	70–89	5	>140 g fish/wk 0.38 g EPA + DHA/day	**75**[§] **73**[§]
(B) *No risk reduction*					
Kalmijn et al. (1997a)	342	69–89	3	105 g fish/week	NS
Kalmijn et al. (1997a)	342	69–89	3	0.0–2 g EPA + DHA/day	NS
Morris et al. (2005)	3718	≥65	6	1.6 g ALA + EPA + DPA + DHA/day	NS
Solfrizzi et al. (2006)	278	65–84	2.6	≥9 g PUFA[c]/day	NS

[a] The fish and ω3 PUFA intakes were derived from food frequency questionnaires or dietary histories.
[b] Odds ratio.
[c] Amounts and types of ω6 or ω3 PUFA not reported.
Significant values are marked in bold. [‡]$p = 0.03$, [†]$p < 0.05$, [§]$p = 0.01$.
ALA, alpha-linolenic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; NS, not significant; PUFA, polyunsaturated fatty acids; SD, standard deviation.

sumed may partly account for the variation in the amounts of the calculated ω3 PUFA intakes between the studies.

Most support for a protective role of fish and ω3 PUFA intakes against the risk of dementia or AD comes from prospective studies. As shown in Table 19.3, four studies revealed that one to two servings of fish per week lowered the risk of all-cause dementia significantly (Kalmijn et al., 1997b; Barberger-Gateau et al., 2002; Huang et al., 2005; Barberger-Gateau et al., 2007). Similar findings were obtained for AD (Kalmijn et al., 1997b; Barberger-Gateau et al., 2002; Huang et al., 2005; Barberger-Gateau et al., 2007). The study by Morris et al. (2003) also showed an inverse association between fish consumption and the risk of AD but did not reach statistical significance of $p \leq 0.05$. However, the calculated ω3 PUFA or ω6 + ω3

TABLE 19.3
Prospective Studies Showing Fish or ω3 PUFA Intake Lowers (A) or Does Not Lower (B) the Risk of Dementia (D) or AD

Reference	Participants *N*	Participants Age (Years)	Follow-Up (Years)	Amount Fish/Week PUFA g/Day (Mean or Range)	Risk Value D	Risk Value AD
Fish intake[a]						
(A) *Lower risk*						
Kalmijn et al. (1997b)	5386	68	2.1	>130 g serving	**0.4**[b,†]	**0.3**[b,¶]
Barberger-Gateau et al. (2002)	1416	≥68	7	≥1 serving	**0.66**[c,§]	**0.69**[c,§]
Morris et al. (2003)	815	65–94	3.9	≥1 serving		0.4[b]
Huang et al. (2005)	1570	≥65	5.4	>2 servings	**0.72**[c,d,†]	**0.59**[c,d,†]
Barberger-Gateau et al. (2007)	5944	≥65	4	2–3 servings	**0.53**[c,d,†]	**0.65**[c,‡]
(B) *No risk reduction*						
Huang et al. (2005)	474	≥65	5.4	>2 servings	NS[e]	NS[e]
Barberger-Gateau et al. (2007)	1479	≥65	4	2–3 servings	NS[e]	
ω3 PUFA intake[a]						
(A) *Lower risk*						
Morris et al. (2003)	815	65–94	3.9	1.8 g ALA + EPA + DHA		**0.3**[b,§]
				0.06 g DHA		**0.4**[b,†]
Laitinen et al. (2006)	1449	65–80	21	0.5–0.8 g ω6 + ω3	**0.4**[e,f,‡]	NS
				0.9–2.9 g ω6 + ω3	**0.5**[e,f,‡]	**0.36**[e,f,‡]
Barberger-Gateau et al. (2007)	413	≥65	4	ω3[g]	**60**[h,‡]	NS
(B) *No Risk Reduction*						
Engelhart et al. (2002)	5395	68	6	16.6 g ω6; 1.3 g ω3	NS	NS

[a] Derived from food frequency questionnaires or dietary histories.
[b] Relative risk.
[c] Hazard ratio.
[d] For apolipoprotein E ε4 noncarriers only.
[e] For apolipoprotein E ε4 carriers only.
[f] Odds ratio.
[g] From ω3-rich oils, amount and type of ω3 PUFA not reported.
[h] % rate reduction.
Significant values are marked in bold. [†]$p < 0.05$, [¶]$p < 0.005$, [§]$p \leq 0.01$,[‡]$p \leq 0.05$.
ALA, alpha-linolenic acid; D, all-cause dementia; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; NS, not significant; PUFA, polyunsaturated fatty acids.

PUFA intake was associated with lower risk of dementia in two of three studies (Laitinen et al., 2006; Barberger-Gateau et al., 2007) and with no risk reduction of AD in three of four studies (Engelhart et al., 2002; Morris et al., 2003; Laitinen et al., 2006; Barberger-Gateau et al., 2007). Thus, again, the effects of calculated EPA + DHA or long-chain ω3 PUFA intake do not consistently reflect that of fish intake. The lack of consistency between fish intake and ω3 PUFA intake in lowering the risk of dementia or AD may be related partly to the varying length of the follow-ups, the variability of ω3 PUFA composition among different species of fish consumed, and other nutrients in fish such as proteins, iodine, and/or selenium, which may interact with ω3 PUFA and assist in lowering the risk for dementia and AD (Barberger-Gateau et al., 2007; Gillette Guyonnet et al., 2007).

Recently, it was suggested that the lower cognitive decline following the intake of fish and seafood or ω3 PUFA may be important in specific cognitive domains. Five studies have investigated the association of fish and seafood or ω3 PUFA intakes with specific cognitive domains such as memory, language, speed, and visuospatial skills. It was reported that moderate intakes of fish and seafood products or ω3 PUFA could lower the risk of decline in verbal fluency (Beydoun et al., 2007) and in speed domains (Kalmijn et al., 2004; Dullemeijer et al., 2007). Conversely, other authors found no improvement in language (Dullemeijer et al., 2007) or speed domains (Whalley et al., 2004; Beydoun et al., 2007). In a population of 98% fish and seafood consumers (70–74 years) who reported eating a lot of marine products (about 85 g/day), episodic memory, perceptual speed, visuospatial skills, and verbal fluency were significantly improved (Nurk et al., 2007). Significant improvement in memory was found only in those consuming a lot of marine products. Whalley et al. (2004) found that fish oil users improved only in visuospatial skills but not in the other specific cognitive domains. Collectively, most of the studies support the hypothesis that moderate consumption of fish and seafood products is protective against cognitive decline in speed, language, and visuospatial domains. Since the authors used a variety of different neuropsychological tests, the inconsistencies of the findings in some specific cognitive domains might be due to the choice of the neuropsychological tests used.

We also noticed that in two prospective studies, carriers of the apolipoprotein E ε4 allele were not protected by higher fish intake (Huang et al., 2005; Barberger-Gateau et al., 2007). Others reported that moderate PUFA intake in midlife was protective against both dementia and AD, especially among apolipoprotein E ε4 carriers (Laitinen et al., 2006). However, their PUFA data were limited to fat coming from spreads and they did not distinguish between ω3 and ω6 PUFA. Recently, we have shown that, compared to normal controls, apolipoprotein E ε4 carriers have 59% higher plasma ω3 PUFA levels and are less amenable to increasing ω3 PUFA incorporation in the plasma following ω3 PUFA supplementation (Plourde et al., 2008).

Overall, we suggest that, with few exceptions, the available epidemiological literature provides good arguments for the potential of fish and seafood consumption to protect against cognitive impairment and dementia including AD. However, the effects of EPA + DHA or long-chain ω3 PUFA intakes do not consistently reproduce those obtained by fish and seafood consumption.

19.4 BIOLOGICAL DATA: THE BLOOD DHA CONNECTION TO COGNITIVE DECLINE

Measurements of ω3 PUFA in plasma or red blood cells are generally considered better estimates of ω3 PUFA status than those obtained from dietary assessments since they can be regarded as an integrated measure of short- to medium-term dietary fatty acids intake, absorption, and individual biologic response (Stanford et al., 1991; Bonaa et al., 1992; Ma et al., 1995a,b; Nikkari et al., 1995; Katan et al., 1997; Arab, 2003; Baylin et al., 2005). Because of the importance of DHA in brain functioning (Horrocks and Farooqui, 2004), DHA measurements in plasma or red blood cells are valuable in providing insight into its connection with protection against cognitive impairment. In plasma total lipids, there are four major lipid classes: phospholipids, triglycerides, cholesteryl esters (CE), and free fatty acids. Among phospholipids, the two major classes are phosphatidylcholine (PC) and phosphatidylethanolamine (PE). There are a limited number of studies reporting DHA in plasma total lipids, total phospholipids, CE, or red blood cells in CIND subjects (Table 19.4). Among all the studies, only two showed a significant lower DHA in CIND subjects compared to healthy controls, one in plasma total phospholipids (Conquer et al., 2000) and the other in red blood cells total phospholipids (Heude et al., 2003).

DHA levels in plasma total lipids, total phospholipids, PC and CE, or in red blood cells of AD patients are shown in Table 19.5. Among all the studies, only three showed a significant lower DHA in AD compared to healthy controls, two in plasma total phospholipids (Corrigan et al., 1991; Conquer et al., 2000), and one in CE (Tully et al., 2003). Unexpectedly, one study reported significantly higher DHA in AD compared to healthy controls in plasma CE and in red blood cells total phospholipids (Corrigan et al., 1991). Inconsistent findings between some studies were reported within each of the plasma total phospholipid and CE fractions.

The differences in blood DHA data between studies could be related to differences between the age-matched healthy controls as much as to those in CIND and demented subjects. We have reported that healthy elderly (70–79 years) have a greater capacity to incorporate DHA into plasma than young adults (18–30 years) after fish oil supplementation (Vandal et al., 2008). However, the studies reviewed here involved healthy controls that were matched to CIND and demented patients of the same age group, that is 60–80 years old. Hence, assuming that the metabolism of DHA and its incorporation into blood lipids are comparable among 60–80 years olds (Rodriguez-Palmero et al., 1997; Plourde et al., 2007), the inconsistencies of the findings might be better explained by differences in food intake patterns of different populations and different geographical areas. Another possibility might be the difference in the number of demented patients. However, the difference in the number of demented patients across the studies cannot explain the inconsistencies observed because although the majority ($N = 190$) of demented subjects showed lower blood DHA levels, close to the same number ($N = 158$) of demented individuals showed no difference in their blood DHA levels whereas a minority ($N = 36$) of demented patients had higher blood DHA levels.

TABLE 19.4
Blood DHA in CIND Elderly

Reference	Cognitive Status	*N*	Age (Years) Mean	Follow-Up (Years)	DHA % of Total Fatty Acids	DHA in CIND (% Control)
Plasma TL						
Cherubini et al. (2007)	Control	725	74		2.3 ± 0.1	
	CIND	153	81		2.2 ± 0.1	96
Plasma TPL						
Conquer et al. (2000)	Control	19	77		4.6 ± 0.4	
	CIND	27	83		3.7 ± 0.2	**80**†
Laurin et al. (2003)	Control	79	77		2.1 ± 0.8	
	CIND	43	79		2.1 ± 0.8	100
Manzato et al. (2003)	Control	98	≥65		3.3 ± 1.1	
	CIND	93	≥65		3.1 ± 1.1	94
Laurin et al. (2003)	Control	52	82	5	2.0 ± 0.7	
	CIND	11	84	5	2.1 ± 0.8	105
Beydoun et al. (2007)	Control	2111	56	6	2.9 ± 0.9	
	CIND	140	57	6	3.0 ± 0.9	103
Plasma CE						
Beydoun et al. (2007)	Control	2111	56	6	0.5 ± 0.2	
	CIND	140	57	6	0.5 ± 0.2	100
Red blood cells TPL						
Heude et al. (2003)	Control	219	69	4	6.3 ± 1.1	
	CIND	27	69	4	5.9 ± 1.0	**94**†

Significant values are marked in bold. †$p < 0.05$.

ALA, alpha-linolenic acid; CE, cholesteryl esters; CIND, cognitively impaired nondemented; LC-PUFA, long-chain polyunsaturated fatty acids (EPA + DPA + DHA); TFA, total fatty acids; TPL, total phospholipids.

Therefore, the commonly discussed link between lower blood DHA and AD does not appear to be consistently supported by the present data. Because of the inconsistencies of the findings within the same lipid fraction, the limited number of studies in some lipid fractions (plasma total lipids and PC or red blood cells total phospholipids), and the possible bias related to healthy controls, no lipid fraction in particular appears as the most representative or ideal to use for the evaluation of blood DHA in AD. On the other hand, the possibility of substantial variability in blood DHA levels between AD patients or other types of dementia and healthy elderly cannot be excluded.

TABLE 19.5
Blood DHA in Dementia (D) and AD

Reference	Cognitive Status	*N*	Age (Years) Mean	DHA % of Total Fatty Acids	DHA in D, AD (% Control)
Plasma TL					
Cherubini et al.	Control	725	74	2.3 ± 0.1	
(2007)	D	57	85	2.0 ± 0.2	87
Plasma TPL					
Corrigan et al.	Control	49	74	5.6 ± 0.6	
(1991)	MID	6	81	5.5 ± 0.2	98
	AD	36	81	4.4 ± 0.9	**79**[‡]
Conquer et al.	Control	19	77	4.6 ± 0.4	
(2000)	AD	13	83	3.1 ± 0.2	**67**[‡]
Laurin et al.	Control	79	77	2.1 ± 0.8	
(2003)[a]	AD	52	79	2.3 ± 0.8	110
Laurin et al.	Control	52	82	2.0 ± 0.7	
(2003)[b]	AD	11	84	2.7 ± 1.2	135
Plasma PC					
Schaefer et al.	Control	800	76	>4.2%	**0.53**[c,†] 0.61[d]
(2006)	D & AD	99	76	<4.2%	
Plasma CE					
Corrigan et al.	Control	49	74	0.4 ± 0.4	
(1991)	MID	6	81	2.0 ± 1.0	**500**[¶]
	AD	36	81	0.6 ± 0.5	**150**[¶]
Tully et al.	Control	45	69	1.2 ± 0.9	
(2003)	AD[e]	42	75	0.6 ± 0.5	**50**[‡]
Red blood cells TL					
Boston et al.	Control	10	N/A	41.2 ± 20.9[f]	
(2004)	AD	22	81	35.3 ± 21.5[f]	86
Wang et al.	AD[g]	13	77	5.4 ± 1.6[h]	
(2008)	AD[i]	10	76	4.2 ± 1.2[h,j]	
Red blood cells TPL					
Corrigan et al.	Control	49	74	3.2 ± 2.5	
(1991)	AD	36	81	4.4 ± 2.2	**138**[§]

[a] Cross-sectional study.
[b] Prospective study.
[c] Relative risk for D.
[d] Relative risk for AD.
[e] Quartile with lowest MMSE (score = 12.9 ± 3.9).
[f] μg/g.
[g] Mild AD (MMSE score = 24–27).
[h] mmol/L.
[i] Moderately severe AD (MMSE score = 16–19).
[j] Values between moderately severe AD and mild AD are not significantly different.

Significant values are marked in bold. [‡]$p \leq 0.001$, [†]$p < 0.05$, [¶]$p = 0.01$, [§]$p = 0.02$.

AD, Alzheimer's disease; CE, cholesteryl esters; D, all-cause dementia; MID, multi-infarct dementia; MMSE, minimental state examination; N/A, not available; PC, phosphatidylcholine; TL, total lipids; TPL, total phospholipids.

19.5 BIOLOGICAL DATA: THE BRAIN DHA CONNECTION TO ALZHEIMER'S DISEASE

Since cognitive dysfunction is the central clinical feature of AD and since DHA is a major component of membrane phospholipids in the brain, comparison of DHA in brain phospholipids of AD subjects and age-matched healthy controls might provide relevant information on the association between DHA status and cognitive impairment in AD. Since healthy aging has been associated with a decrease of about 43% in brain total phospholipid concentrations (Svennerholm et al., 1997), changes in brain PE and PC concentrations, the two major phospholipid classes, have been evaluated in AD patients relative to healthy elderly. In AD, brain PE and PC concentrations were 20%–30% less in the frontal cortex, but about 14% less in the hippocampus compared to those of healthy elderly, respectively (Soderberg et al., 1991; Guan et al., 1999, data not shown). Thus, the decrease in brain PE and PC concentrations is more pronounced in AD than in the healthy elderly.

Results of analyses of the proportions of DHA (% DHA) in phospholipids in three brain regions normally affected by AD are shown in Table 19.6. The studies revealed that only DHA in PE of the hippocampus is significantly lower in AD than in healthy controls (Soderberg et al., 1991; Prasad et al., 1998). No significant change in DHA in PE or PC between AD and healthy controls was reported in the parahippocampus or the frontal cortex, except the study of Soderberg et al. (1991) showing lower DHA in both PE and PC in the frontal cortex. Bias in the selection of healthy controls may partly explain the apparent inconsistency in the findings for the frontal cortex.

In AD, the relative decrease in brain DHA (absolute data not shown) does not appear to be specific for DHA: either arachidonic acid (ARA) and/or adrenic acid were also decreased (Soderberg et al., 1991; Corrigan et al., 1998; Han et al., 2001), or the lower DHA was a function of a decrease mostly in plasmalogen PE (Guan et al., 1999). Conversely, increases (up to 50%) for DHA (Skinner et al., 1993; Prasad et al., 1998; Guan et al., 1999) and fourfold for adrenic acid (Skinner et al., 1993) have also been reported for some regions of AD brain. Hence, the commonly discussed link between lower brain DHA and AD does not appear to be consistently supported by the data or to be specific to AD. As a whole, the available literature shows that, besides lower DHA in the hippocampus, the elderly with AD may have very similar DHA proportions to those found in the healthy elderly and suggests that the changes in brain phospholipid pool size during AD may be equally or more important than the possible changes in fatty acid composition of these lipid classes (Cunnane et al., 2007; Plourde et al., 2007).

Taken together, in view of the discrepancies of the data between studies, our assessment of the literature is that there is not yet a consistent association between AD and low brain DHA.

19.6 EFFICACY OF DHA TREATMENT OF ALZHEIMER'S DISEASE

The central issue in linking low DHA to risk of AD is whether higher DHA intake can be clinically beneficial in preventing or reducing cognitive impairment, especially in AD. As shown in Table 19.7, very few studies have investigated whether

TABLE 19.6
DHA in Brain Phospholipids in Healthy Elderly (Controls) Compared to Elderly with AD

Reference	Cognitive Status	*N*	Age (Years)	DHA[a]			DHA in AD (% Control)		
				TL	PE	PC	TL	PE	PC
Hippocampus									
Soderberg et al.	Control	8–10	N/A		16.9	0.9			
(1991)	AD	8–10	N/A		7.9	0.6		**47**†	67
Prasad et al.	Control	9	78		1017[b]	121			
(1998)	AD	9	78		557	84		**55**†	69
Parahippocampus									
Corrigan et al.	Control	6	73		20.7	1.6			
(1998)	AD	8	77		20.5	1.4		99	88
Skinner et al.	Control	10	68	16.9					
(1993)[c]	AD	12	79	16.6			98		
Skinner et al.	Control	8	68	5.1					
(1993)[d]	AD	7	79	5.0			98		
Frontal cortex									
Soderberg et al.	Control	8–10	n/a		23.5	1.4			
(1991)	AD	8–10	n/a		12.6	0.8		**54**†	**57**†
Guan et al.	Control	13	72		4.7[e]	1.1			
(1999)	AD	15	80		5.7	0.8		121	73
Skinner et al.	Control	10	68	18.6					
(1993)[c]	AD	9	79	17.3			93		
Skinner et al.	Control	8	68	3.6					
(1993)[d]	AD	7	79	4.6			130		

[a] Unless otherwise noted, the DHA values are % of total fatty acids.
[b] nmol/g wet tissue.
[c] Total lipids of gray matter.
[d] Total lipids of white matter.
[e] µmol/mg DNA.
Significant values are marked in bold. †$p < 0.05$.
N/A, not available; PC, phosphatidylcholine; PE, phosphatidylethanolamine; TL, total lipids.

increase in dietary DHA intake reduces the occurrence and/or the symptoms of AD and other dementias.

Only one intervention study examined the impact of DHA supplementation in CIND elderly (Kotani et al., 2006). They reported that ingestion of 200 mg of DHA + ARA for 3 months in 12 CIND subjects (67 years) improved their scores in attention and memory tests by 8%–15% compared to age-matched placebo controls.

The direct effect of DHA on cognitive impairment in demented patients was assessed in four intervention studies. Two studies reported that DHA supplementation improved cognitive functioning modestly. Suzuki et al. (2001) reported an

TABLE 19.7
Impact of DHA Supplementation in Elderly with Cognitive Dysfunction

Reference	Cognitive Status	*N*	Age (Years)	Supplement	Dose (g/Day)	Duration (Month)	Measures
Kotani et al. (2006)	CIND	9	70	P	0.2	3	No change
	CIND	21	67	DHA + ARA	0.2	3	8–15% higher scores[a]
Suzuki et al. (2001)	Control	8	78	DHA + EPA	0.6 + 0.5	6	75% of subjects improved[b]
	AD	22	78	DHA + EPA	0.6 + 0.5	6	55% of subjects improved[b]
Terano et al. (1999)	vD	10	83	None		12	No change
	vD	10	83	DHA	4.3	12	17% higher scores[c]
Freund-Levi et al. (2006)	AD	85	73	P/DHA + EPA	4.0/1.7 + 0.6	6/6	No change[d]
	AD	89	73	DHA + EPA	1.7 + 0.6	12	No change[d]
Kotani et al. (2006)	AD	8	67	DHA + ARA	0.2	3	No change[e]

[a] Attention ($p < 0.05$) and memory ($p < 0.01$) tests.
[b] % of subjects with improved intelligence score.
[c] $p < 0.05$ at 3 and 6 months, but not at 12 months after supplementation.
[d] Except in subjects with scores >27 on the MMSE.
[e] Relative to placebo.

AD, Alzheimer's disease; ARA, arachidonic acid; Vd, thrombotic cerebrovascular dementia; D, dementia; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; CIND, cognitively impaired nondemented; P, placebo.

improved cognitive performance in 55% of elderly with AD and in 75% of healthy controls but could not be matched to their respective blood DHA levels. In a pilot study, Terano et al. (1999) reported that subjects with mild to moderate vascular dementia improved the dementia scores by 17%; this improvement was accompanied by the increase in the serum DHA content by 179%. No significant change was observed in those without supplementation.

In contrast, two studies found that DHA supplementation in the presence of EPA or ARA (at combined doses ranging from 0.2 to 2.3 g/day) did not improve cognitive performance of AD patients (Freund-Levi et al., 2006; Kotani et al., 2006). In the study by Freund-Levi et al. (2006), patients with AD were randomized into a double-blind placebo-controlled clinical trial and received a supplement of EPA + DHA or placebo during the first 6 months of the study, after which all patients received the EPA + DHA supplement for a further 6 months. After the first 6 months, the rate of cognitive decline in patients with mild to moderate AD was not delayed. However, for a small group of elderly with very mild AD, the cognitive decline was significantly slower in the

EPA + DHA-treated group (Freund-Levi et al., 2006). However, since the latter 6 months of the study was not placebo controlled, the possibility of better cognitive performance in the subjects with very mild AD could have been due to practice effects rather than to the DHA + EPA supplements (Freund-Levi et al., 2006). In that study, serum DHA levels were measured and, after 1 year of supplementation, were shown to be similar in placebo-treated subjects and in subjects supplemented with fish oils.

Thus, these clinical trials reported a modest clinical benefit of DHA intervention in demented and nondemented individuals. Three factors affect the interpretation of these studies. First, they were small and of short duration. Second, since they have used DHA in combination with either EPA (Terano et al., 1999; Suzuki et al., 2001; Freund-Levi et al., 2006) or ARA (Kotani et al., 2006), whether DHA (or other PUFA including EPA or ARA) has a beneficial clinical effect on its own remains to be established. It is possible that more than one PUFA besides DHA is necessary to produce a beneficial clinical effect on cognitive impairment in AD. Third, the doses of DHA used covered a 36-fold range—120 mg/day to 4.3 g/day (Cunnane et al., 2007; Plourde et al., 2007). Besides the problem posed by the large range of the effective dose, the pertinent question as to why the necessary dose exceeds manyfold the brain turnover of DHA needs to be answered (Rapoport et al., 2007).

19.7 WHY ARE BLOOD AND BRAIN DHA DATA DISCONNECTED FROM FISH INTAKE DATA?

Overall, an inverse association is emerging between higher fish and seafood consumption and risk of AD but the DHA data derived from plasma, red blood cells, and brain do not consistently explain the protective effect of fish and seafood consumption against cognitive impairment in AD and other types of dementia. Compared with the fish and seafood intake data, blood and brain DHA data in AD have too much variability to indicate any significant association between low blood/tissue DHA and increased risk of AD.

If there really is no link between low DHA and risk of AD but there is a link with low fish and seafood intake, other nutrients in fish and seafood products that are currently drawing less attention than DHA or EPA may actually be the nutrients protective against AD, i.e., selenium, iodine, zinc, manganese, antioxidants, niacin, etc. (Morris et al., 2004; Barberger-Gateau et al., 2007; Gillette Guyonnet et al., 2007; Bégin et al., 2008). Indeed, a nutrient may be the marker of the dietary intake of another one to which it is closely linked in fish and seafood products or in other foods that are part of the diet of regular fish and seafood consumers.

Another possibility is that, owing to methodological differences between studies or biological changes in DHA metabolism due to AD itself, there is a link that is not revealed in the literature as it stands. In that case, further research is needed to find the reasons why the blood and brain DHA data do not support the fish and seafood intake data. Since DHA synthesis from alpha-linolenic acid and β-oxidation are both very low in humans (Plourde and Cunnane, 2007), preformed DHA intake plays an important role in human whole body DHA homeostasis. Brain DHA levels may depend on factors linked to DHA degradation such as peroxidation or its loss due to cell death in the brain as well as on the input variables (mostly dietary DHA intake

and its transport into the brain). The link between DHA intake and brain DHA may be more complex than anticipated and there is no *a priori* reason to believe that there should necessarily be a direct or simple link between DHA intake, blood DHA, and brain DHA in the healthy elderly, let alone in AD.

Finally, isolated biological measures of DHA may have little meaning given the opposite effects of ω3 and ω6 PUFA on cognitive impairment (Kalmijn et al., 1997a), cognitive decline (Heude et al., 2003), dementia (Barberger-Gateau et al., 2007), and neuroinflammation which is involved in neurodegenerative pathology (Lovell et al., 1995; Gillette Guyonnet et al., 2007). For example, DHA composition in relation to other ω3 PUFA, ω6 PUFA, overall fatty acids, or cholesterol might be more informative than DHA alone. Indeed, a higher percentage of a specific fatty acid or group of fatty acids will automatically reflect a lower percentage of another. Hence, there is a problem of interdependence which makes it difficult to interpret the effect of a single constituent or group of constituents.

19.8 LIMITATIONS OF STUDIES

Several studies have investigated the associations between fish and seafood consumption, ω3 PUFA or DHA intakes, and cognitive impairment in demented and nondemented individuals with inconsistent findings. The inconsistencies of these findings may be due to various factors: differences in sample size, heterogeneity of study populations, type of population analyses (cross-sectional vs. prospective), different methods of estimating ω3 PUFA intakes, choice of tissues and lipid fractions examined, adjustments for different confounders, and the wide variety of the applied cognitive tests.

Lack of uniform quantification of fish and seafood or ω3 PUFA and DHA intakes makes comparison between different studies difficult. DHA intake may vary following cooking methods as well as species of fish (Candela et al., 1998; Mozaffarian et al., 2003; Huang et al., 2005). Since many food products such as eggs and milk are now enriched in long-chain ω3 PUFA, it seems necessary that future trials should take into account the intake of these products in their food frequency questionnaire (Meyer et al., 2003; van Gelder et al., 2007). Differences of food intake patterns in different populations and cultural backgrounds are another confounder that complicates the comparison of findings from populations in different geographical areas.

Some of the lack of agreement between studies of fish and seafood intake vs. DHA levels and lower risk of AD is potentially attributable to artifacts caused by differences in methodology, including different definitions of cognitive function, different analytical methods, possible different definitions of "healthy" age-matched controls, and differences between normal age-related cognitive decline and pathological cognitive decline. Therefore, standardization of definitions, clinical classifications of severity of pathological cognitive decline and of analytical procedures is urgently needed in order to draw valid general conclusions.

Some differences in the methodology across the studies are worth noting. For example, although the methods used for the diagnosis of all-cause dementia and AD were comparable in most of the studies examined here, the neuropsychological measures found to be predictors of dementia or AD differed across studies. In general, most of

the studies examined defined a global measure of overall cognitive performance using a wide variety of neuropsychological tests. Some of the studies reported performance in specific cognitive domains separately, such as memory function, speed of cognitive processes, executive functions, cognitive flexibility, and language. Participants in most studies were screened for cognitive impairment or dementia with the MMSE (Folstein et al., 1975) or a composite of specific neuropsychological tests selected by the authors. For example, different MMSE scores were used between studies as the cutoff point indicative of cognitive impairment. MMSE was sometimes used as a screening test by some or as a diagnostic test by others. The number and types of neuropsychological tests selected differed also between studies. These differences in the evaluation of global cognition and specific cognitive domains may be important in interpreting some differences in the findings of the studies.

Regular fish consumers may have particular dietary habits (intake of fruits, vegetables, and alcohol) and lifestyles, socioeconomic characteristics (education, income), and medical conditions (hypertension, past stroke, and depressive symptoms) which could confound the relationship between fish intake and risk of cognitive impairment, dementia, and AD. It was suggested that they should be systematically considered when studying dietary risk factors of dementia (Barberger-Gateau et al., 2005). Most studies reviewed here, but not all, adjusted the association between fish, DHA, or ω3 PUFA intake and cognitive impairment, dementia, or AD for certain confounders, but the particular confounders examined varied between studies.

Cross-sectional studies comparing CIND elderly or demented patients to normal age-matched controls are of limited value since they cannot ascertain whether the diet was the cause or the consequence of the cognitive impairment. Indeed, CIND subjects or demented patients may have altered their diet as a consequence of the disease, or the diet of demented patients may have been modified by their caregivers and therefore may not reflect their past dietary habits. Prospective studies linking fish and seafood consumption and blood DHA content to dementia with at least 6 year follow-ups are needed but they are still very scarce. This follow-up period is suggested because it was shown to be less prone to bias due to subclinical dementia (Engelhart et al., 2002) and was the time needed for significant cognitive decline in specific cognitive domains to become more apparent in susceptible individuals compared to a 3 year follow-up period (Wahlin et al., 2005).

19.9 FUTURE RESEARCH DIRECTIONS

It remains to be determined whether and/or how DHA status plays an etiological role in the pathogenesis and/or progression of dementia, especially AD or may simply be a secondary marker of the disease process. It is crucial that the true nature of the impact of DHA on AD be investigated. The study of DHA transporters (lipoproteins and other circulating DHA-binding proteins) or free DHA for example, in relationship to DHA in the brain rather than other plasma lipid fractions may help in clarifying the controversy about the link between plasma and brain DHA status and progression to, or severity of, dementia and AD.

Aside from this new direction of biological analysis, it seems plausible that cognitive impairment in dementia and AD changes DHA metabolism in such a way as

to raise plasma DHA, thereby counterbalancing the normal relation between low DHA intake and low plasma DHA. This is purely speculative but could explain the discrepancy between the epidemiological studies and those in which DHA measurements have been made. It may also be that the amount of fish or DHA needed to protect against AD does not reproducibly change plasma DHA. Proper design studies directed at these specific issues should be carried out.

19.10 CONCLUSION

Prospective studies on fish and seafood consumption are most supportive for a protective role of fish and seafood products against pathological cognitive decline in the elderly, but DHA intake, blood and brain DHA data are not, due mainly to the inconsistencies of the findings and to the large dispersion of the data. The inconsistencies between dietary DHA, plasma/tissue DHA, and possible DHA efficacy in AD could be more methodological than biological. At present, there is no clear explanation for this apparent lack of a consistent association between low DHA (whether examined in the diet, the plasma, the red blood cells or the brain) and cognitive impairment during aging. Three years of research following the initial report by Maclean et al. (2005) have failed to provide new data susceptible to clarify whether and, if so, how DHA is linked to AD.

Developing effective strategies to reduce the risk of AD and possibly improve its treatment is a pressing matter considering its medical and socioeconomic importance. If DHA is to play a role in its development, clinical studies reporting DHA intake and plasma levels of lipid fractions including DHA transporting proteins while also undertaking a DHA intervention in AD would presumably help resolve some of the issues. Establishing whether the discrepancies between the epidemiological and tissue DHA analysis in AD is methodological or biological also needs to be resolved. Accordingly, appropriate strategies could be developed both to maximize the protective impact of fish and other seafood products and determine what ingredients in fish and other seafood products are protective against cognitive impairment in AD or other types of dementia.

In line with the growing interest in elucidating the role of dietary lifestyles in AD, accumulating evidence suggests that unhealthy dietary lifestyles can lead to an increasing incidence of the metabolic syndrome and of obesity which has been associated with dementia including AD. Conversely, the Mediterranean diet, an example of a healthy dietary lifestyle, has been linked with reduced dementia.

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ABBREVIATIONS

AD	Alzheimer's disease
ARA	arachidonic acid
CE	cholesteryl ester
CIND	cognitively impaired nondemented
DHA	docosahexaenoic acid
EPA	eicosapentaenoic acid
MMSE	minimental state examination
PC	phosphatidylcholine
PE	phosphatidylethanolamine
PUFA	polyunsaturated fatty acids
ω3	omega-3
ω6	omega-6

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20 Hypothalamic Fatty Acid Sensing in the Normal and Disease States

Madhu Chari, Carol K.L. Lam, and Tony K.T. Lam

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20.1 INTRODUCTION

Obesity, which encompasses the accumulation of excess fat in peripheral tissues and its associated health risks, is increasingly prevalent worldwide and has reached epidemic proportions. With over 1.1 billion overweight [body mass index (BMI) exceeding 25] and 312 million obese (BMI exceeding 30) adults worldwide, the rates of obesity have effectively tripled in the developing world, thanks to the increasingly popular Western lifestyle which generally involves the abundant consumption of energy-dense food and decreased physical activity (Hossain et al., 2007). The latent health risks associated with obesity are potentially devastating—in addition to metabolic disease, being obese increases the risk of type 2 diabetes (T2D), cardiovascular disease (CVD), and even various cancers (Calle and Kaaks, 2004).

Despite the undeniable influence of genetic and environmental factors, obesity is ultimately a resultant of, and perpetuated by, a disruption in energy homeostasis, whereby energy (food) intake exceeds energy expenditure. Normally, there is a

series of concerted physiological and biochemical checks and balances initiated to handle acute, day-to-day fluctuations in energy balance: for example, an elevation in adiposity resulting from increased energy intake leads to counter-regulation via an increase in adipose-derived hormones, such as leptin (Friedman, 2003), and an increase in energy expenditure (López et al., 2007). Conversely, the fasting state shifts the energy balance such that energy stores are maintained while an increased propensity for food intake results (Lelliott and Vidal-Puig, 2004). As a result of these feedback signals that relay changes in energy status, the caloric storage/body weight is generally stable for most humans over long periods of time despite the wide variations in day-to-day food intake that occur. These above homeostatic responses are poised to handle subthreshold fluctuations in food intake, but clearly, chronic hypercaloric excess combined with reduced energy mobilization (i.e., exercise) limits their effectiveness (López et al., 2007), and leads to increased adiposity. In addition, this physiological cross-regulation also provides a reason for the inherent difficulty in losing large amounts of weight and sustaining that weight loss, as massive weight loss is capable of triggering rebound hunger (Friedman, 2003). Further impacting the effectiveness of the energy balance mechanism is the influence of the inherent sensory circuitry that mediates the pleasure and reward on feeding (Flier, 2004). Thus, identifying the nature of the satiety and hunger signals generated in the body that are involved in the regulation of feeding behavior has historically been a necessary preoccupation in obesity research.

By the mid-twentieth century, the glucostatic (Mayer, 1953) and lipostatic (Kennedy, 1953) hypotheses, which proposed that circulating nutrients (glucose and lipids, respectively) generated in amounts proportional to peripheral storage depots serve as signals to the brain in order to mediate alterations in energy intake and expenditure, were in place. As a result, research then shifted to focus on the primary energy storage sites in the periphery, including the adipose tissue, skeletal muscle, and the liver, and their potential ability to sense energy and mediate the control of energy intake. The liver, given that it is exposed to the postabsorption nutrient flow (Langhans, 1996) and that hepatocytes are essentially able to metabolize all fuels (Seifter and Englard, 1988), was a natural target that in particular was quite convincingly demonstrated as a possible mediator of the hunger/satiety signal (Langhans, 1996).

However, the hypothalamus in particular has long been championed as a key mediator of whole body energy homeostasis. Presently, it is generally accepted that it is involved in the day-to-day regulation of a number of factors including body temperature, blood pressure, thirst, and hunger, and is a vital structure for the integration of the nervous and endocrine systems. The first demonstrations of the hypothalamus serving as a satiety centre were conducted several decades ago, wherein hyperphagia and obesity resulted after the ventromedial nucleus of the hypothalamus was subjected to bilateral lesions (Hetherington and Ranson, 1942). Furthermore, the observed hyperphagia following the administration of the classical 2-deoxyglucose (2-DG) antimetabolite into the third ventricle of the brain (Miselis and Epstein, 1975) demonstrated a central fuel-sensing component to the regulation of energy homeostasis.

Numerous landmark studies—the vast majority of which were conducted in the past decade—have demonstrated that the latter possibility holds much promise. The central nervous system (CNS) has been shown to sense hormones and nutrients

in order to regulate not only food intake (Cota et al., 2006; Lam et al., 2008; Luheshi et al., 1999; Morton et al., 2006; Turton et al., 1996; Wolfgang and Lane, 2006) but also glucose homeostasis (Bence et al., 2006; Coppari et al., 2005; Gelling et al., 2006; Inoue et al., 2006; Kievit et al., 2006; Lam et al., 2005a,b,c; Obici et al., 2002a,b 2003). Of particular interest and relevance, changes in hypothalamic fatty acid levels and metabolism have been shown to regulate both food intake (Loftus et al., 2000) and glucose homeostasis (Obici et al., 2002a, 2003). As obesity and diabetes are characterized by hyperphagia and hyperglycemia, the characterization of defects in the hormone- and nutrient-sensing pathways in the hypothalamus that regulate energy and glucose homeostasis will shed light on the central component that initiates and perpetuates these metabolic diseases.

In order to gain a full appreciation of the physiologic control of nutrient input and output that is mediated by the brain, a closer look at the key molecular and enzymatic targets of the hypothalamus and their regulation is an absolute must.

20.2 HYPOTHALAMUS AND HOMEOSTASIS

Within the CNS, the hypothalamus appears to be the primary processor of peripheral signals—theoretically mechanical, neural, hormonal, or metabolic in nature—which indicate nutrient availability (Horvath et al., 2004). In terms of its composition, the hypothalamus comprises a set of anatomically distinct nuclei that are interconnected via axonal projections (López et al., 2007). These nuclei are equipped to respond to acute alterations in energy status by governing the expression of specific, homeostasis-relevant neuropeptides and neurotransmitters. The ventromedial hypothalamic nucleus (VMH) in particular has long been perceived as a "satiety centre," due to the aforementioned finding that bilateral lesions in the VMH result in pronounced hyperphagia and obesity (Hetherington and Ranson, 1942). However, the molecular mechanisms underlying the feeding control by the VMH have, to date, yet to be satisfactorily tackled.

Based on the rather remarkable experimental findings of the recent past, the arcuate nucleus (ARC) has appeared to emerge as the "master" nuclei for the regulation of energy and glucose homeostasis. From an anatomical perspective, it can be seen why, situated around the base of the brain's third ventricle, the ARC lies immediately above the median eminence where the capillary endothelium lacks tight junctions (Williams et al., 2001), effectively forming an incomplete blood–brain barrier (BBB); thus it is more conducive to allowing larger proteins and hormones to readily access the ARC neurons from circulation.

There are at least two distinct neuronal subtypes in the ARC that are able to process peripheral metabolic and feeding signals (Figure 20.1). The first are neurons that express the anorexigenic products of the peptide proopiomelanocortin (POMC). POMC is posttranslationally cleaved to a series of smaller peptides, the most notable of which are adrenocorticotropic hormone (ACTH) and α-melanocyte stimulating hormone (α-MSH), the latter of which exerts a net catabolic action that is important in the stimulation of feeding (Seeley and Woods, 2003). Once released, α-MSH carries out its theorized anorexigenic action by binding the MC4 receptor (MC4R), one of five known melanocortin receptors (MCRs) (Seeley and Woods, 2003), in

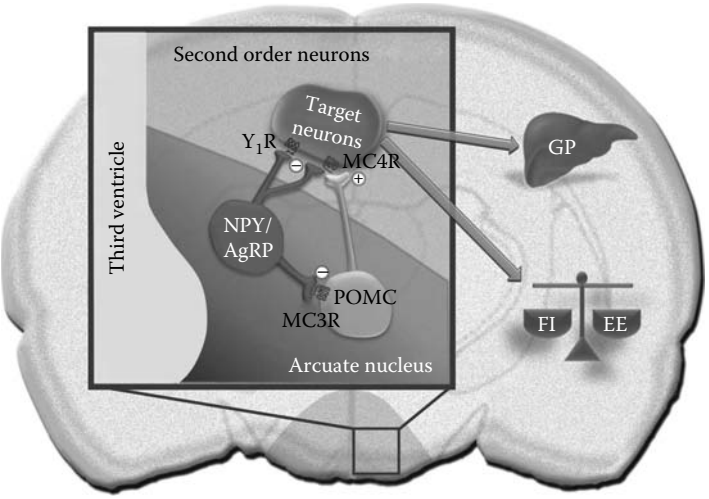

FIGURE 20.1 (See color insert following page 166.) The hypothalamic melanocortin system and the regulation of energy and glucose homeostasis. The hypothalamic ARC is a critical mediator of energy and glucose homeostasis. Two groups of ARC neurons ("first-order" neurons), the NPY and AgRP coexpressing neurons and the POMC expressing neurons, are proposed to play a major part in mediating these regulatory effects. Projections from the ARC neurons reach adjacent hypothalamic nuclei ("second-order" neurons) including the PVN. The melanocortin signaling progressing along these nuclei and to downstream brain areas is mediated by the α-MSH, a cleavage product of POMC, which binds the melanocortin receptor MC4R. Inhibition of this "melanocortin tone" is mediated by (1) synaptic inhibition of POMC neurons by NPY/AgRP neurons (via MC3R) and of target second-order neurons by neuropeptide Y1 receptor (Y_1R) and (2) AgRP acting as an endogenous antagonist of MC4R. Insulin and leptin carry out their anorectic effects via inhibiting NPY/AgRP neurons and stimulating POMC neurons, while ghrelin activates NPY/AgRP neurons to promote feeding behavior. Nutrients such as LCFAs have been shown to downregulate the expression of AgRP to mediate the hypothalamic anorectic signal. GP, glucose production; FI, food intake; EE, energy expenditure.

downstream effector neurons. Belonging to the second subtype are neurons that coexpress the orexigenic peptides neuropeptide Y (NPY) and agouti-related peptide (AgRP). The activation of these orexigenic neurons leads to the inhibition of anorexigenic signaling in two ways: (1) AgRP/NPY neurons synapse directly with POMC neurons, providing an inhibitory tone and (2) AgRP itself is an inverse agonist at MC4Rs, effectively antagonizing the effects of α-MSH. Both the AgRP/NPY and POMC neurons ("first-order" neurons) have axonal projections to other hypothalamic nuclei (housing downstream "second-order" neurons) as well: the AgRP/NPY neurons project primarily to the paraventricular nucleus (PVN), while POMC neurons project more broadly, reaching other nuclei such as the lateral hypothalamus (LH) in addition to the PVN (López et al., 2007). Collectively, the interplay between these orexigenic and anorexigenic neuronal subsets and their downstream effector signaling form the melanocortin signaling system (Figure 20.1); it is the activation

of this so-called hypothalamic melanocortin tone (Cone, 2005) that is thought to be instrumental in the regulation of homeostasis.

Numerous studies have shown the role of these neurons' activity in the regulation of energy balance and glucose homeostasis. Central NPY injections, which have been previously shown to potently stimulate feeding within minutes (Clark et al., 1985), were recently shown to markedly diminish the ability of a hyperinsulinemic clamp to lower glucose production (GP) (van den Hoek et al., 2004). Interestingly, rates of peripheral glucose disposal and lipolysis were unaffected in the NPY-treated group (van den Hoek et al., 2004). Overall, the data suggests that NPY release must be downregulated in order for insulin to suppress hepatic GP, and that an increase in NPY secretion can lead to insulin resistance. In the case of AgRP, ubiquitous expression of human AgRP in transgenic mice caused obesity, while in a similar vein, AgRP was found to have an eightfold increase in expression in the leptin-deficient ob/ob mice (Ollmann et al., 1997). As for the importance of melanocortin signaling, van Dijk and colleagues demonstrated that relative to vehicle-treated controls, the third intracerebroventricular (i.c.v.) administration of the MCR antagonist SHU9119 to rats doubled not only water but also food intake, resulting in increased body weight (Adage et al., 2001). When other metabolic parameters were assessed, there was increased fat and glycogen content, and elevated plasma cholesterol, leptin, insulin in i.c.v. SHU9119-treated animals (Adage et al., 2001).

The hypothalamic melanocortin signaling system is also heavily regulated by circulating hormones. AgRP/NPY neurons are inhibited by both leptin and insulin (Schwartz et al., 2000) while conversely, POMC neurons are stimulated by input from leptin (Cowley et al., 2001) and insulin (Plum et al., 2006). The administration of i.c.v. insulin increases the expression of POMC mRNA, and reduces food intake; however, the latter anorexic effects were prevented with a subthreshold dose of SHU9119 (Benoit et al., 2002). Furthermore, while it had been shown that hypothalamic insulin signaling is required for the suppression of hepatic GP (Obici et al., 2002b), Jens Brüning and colleagues recently revealed with the use of AgRP neuron-specific insulin receptor (IR) knockout mice that insulin action in AgRP-expressing neurons is required for this GP suppression (Konner et al., 2007).

Overall, it is apparent that key neurons in the hypothalamus, most likely in the ARC, are poised to integrate a variety of hormonal and metabolic signals in order to interpret the state of energy balance and in turn, mediate the necessary metabolic and behavioral responses in order to compensate for deviations from homeostasis. But in particular, this review will focus on the recently determined ability of hypothalamic fatty acid metabolism to regulate homeostatic mechanisms.

20.3 HYPOTHALAMIC FATTY ACID METABOLISM AND HOMEOSTASIS

The brain is heavily reliant on the oxidation of glucose to meet its significant energy demands. And while the brain does not, to our knowledge, use fatty acids as a primary source of energy, it has been demonstrated recently that select enzymes and intermediates of fatty acid metabolism contribute to the hypothalamus' ability to serve as a monitor of energy status. In 2002, Rossetti and colleagues were the first to

demonstrate that the central administration of long-chain fatty acids (LCFAs) triggered a hypothalamic response to regulate energy as well as glucose homeostasis.

Specifically, the administration of oleic acid (a type of LCFA) centrally into the third cerebral ventricle (i.c.v.) of rodents was shown to produce a modest yet significant decrease in plasma insulin and glucose levels within 4 h of the infusion (Obici et al., 2002a,b). This indicates that i.c.v. oleic acid may have enhanced insulin sensitivity, and to better assess this, the effects of central oleic acid infusion were coupled to a pancreatic-euglycemic clamp protocol. The infusion of i.c.v. oleic acids for 6 h did indeed result in a reduction in hepatic GP under clamped, basal insulin conditions, indicating an enhancement in insulin sensitivity; interestingly, the infusion of the medium-chain fatty acid octanoic acid did not yield the same results, revealing specificity in the nature of the hypothalamic signal (Obici et al., 2002a,b). The coadministration of a K_{ATP} channel blocker, the sulfonylurea glybenclamide, with the i.c.v. oleic acid was able to nullify the insulin-sensitizing effect of i.c.v. oleic acid alone (Obici et al., 2002a,b). Thus, as seen with insulin and leptin (Spanswick et al., 1997, 2000), LCFAs appear to activate hypothalamic neurons via a K_{ATP} channel-dependent mechanism. Furthermore, this was in line with a later finding demonstrating that alterations in hypothalamic K_{ATP} channel activity *per se* can regulate GP (Pocai et al., 2005).

The same study looked at the effects of an acute increase in central LCFAs with respect to the regulation of energy balance, namely food intake. Following a bolus administration 1 h prior to the dark cycle, i.c.v oleic acid-treated rats had decreased food intake—the effects of which, remarkably, lasted for 2 days; this is likely mediated by the fact that i.c.v. oleic acid decreased the hypothalamic NPY mRNA levels versus vehicle-injected control rodents (Obici et al., 2002a,b).

Thus, the LCFA oleic acid serves as a central signal of surfeit/nutrient abundance, which in turn triggers the series of neuronal signaling cascades necessary to regulate nutrient intake and production. But how effective is this signal in models of obesity and/or diabetes? In a follow-up study, the same group evaluated whether short-term changes in nutrient availability can affect the ability of central oleic acid to regulate energy and glucose homeostasis. In rats fed a highly palatable, high-fat diet for 3 days (~140 kcal/day), the bolus administration of i.c.v. oleic acid was unable to recapitulate the significant decrease in food intake observed over 2 days in rodents fed a standard chow (Morgan et al., 2004). As for a possible underlying mechanism, the loss in the anorexigenic signal initiated by i.c.v. oleic acid in hyperphagic rats may be due to an incomplete inhibition of the orexigenic NPY expression: i.c.v oleic acid was only able to inhibit hypothalamic NPY expression in hyperphagic rats to 50% of the levels that were seen in standard chow-fed rodents (Morgan et al., 2004). In a similar vein, i.c.v oleic acid was able to inhibit the hypothalamic expression of the orexigenic AgRP by 75%, but this inhibition was lost in the high-fat diet fed, hyperphagic rodents (Morgan et al., 2004). With regard to the effect of central oleic acid on insulin action, the ability of i.c.v. oleic acid to suppress GP under conditions of a pancreatic-euglycemic clamp was nullified in rodents that had received the high-fat diet for 3 days; interestingly, by limiting the high-fat diet caloric intake to ~80 and ~55 kcal/day (versus ~140 kcal/day seen in hyperphagia), the ability of i.c.v. oleic acid to suppress hepatic GP was progressively restored (Morgan et al., 2004). This

provides compelling evidence that the hypothalamic responses triggered by an acute increase in central LCFAs are nutritionally regulated, and presents a startling reality in terms of how rapidly intrinsic homeostatic mechanisms can fail.

Circulating fatty acids can readily access the brain, where they equilibrate with neuronal long-chain fatty acyl-CoAs (LCFA-CoAs) (Miller et al., 1987). Illustrating that circulating plasma fatty acids can access the brain and recapitulate the effect of directly administered i.c.v. oleic acid on glucose homeostasis would undoubtedly further the physiological relevance of this finding. Rossetti and colleagues designed a series of elegant experiments to look at this in greater detail. When lipids were infused intravenously for 4 h to induce hyperlipidemia, glucose uptake and GP were unaffected during a pancreatic-euglycemic clamp; however, when an i.c.v. infusion of the K_{ATP} channel blocker glybenclamide was administered during the lipid infusion, there was a significant elevation (50%) in GP, which was attributed to an increase in glycogenolysis (Lam et al., 2005b). As i.c.v. glybenclamide alone had no effect on GP in the absence of the intravenous lipid infusion (Lam et al., 2005b), this demonstrates that circulating lipids lower hepatic GP via a hypothalamic K_{ATP} channel-dependent mechanism, which is in line with the requirements of the i.c.v.-administered oleic acid to regulate GP (Obici et al., 2002a,b). The results of these pharmacological findings were confirmed with a genetic approach, in which the ability to restrain GP in the presence of increased lipid availability was lost in mice deficient in the K_{ATP} channel subunit Sur1 (Lam et al., 2005b). Once LCFAs gain access to the brain, they are esterified to LCFA-CoAs by the enzyme long-chain acyl-CoA synthetase (ACS), the activity of which can be pharmacologically inhibited by triacsin C. The bilateral infusion of triacsin C into the mediobasal hypothalamus was able to disrupt the hepatic autoregulation of GP in response to lipid infusion (Lam et al., 2005b), much like what was seen with the use of glybenclamide. This demonstrates that the generation of LCFA-CoAs from LCFAs selectively in the mediobasal hypothalamus—triacsin C infusions into the PVN failed to reproduce the effect (Lam et al., 2005b)—is necessary in the generation of the central signal to regulate glucose homeostasis. In the final part of the study, the authors investigated the descending pathway responsible for carrying out the hypothalamic effect of circulating LCFAs on GP. In rats that underwent the hepatic branch vagotomy, a procedure in which the hepatic branch of the vagus nerve is surgically transected, lipid infusions resulted in an increase in GP; the glucose kinetic parameters were unaffected in the sham-operated control group (Lam et al., 2005b). Taken together, the study illustrates that circulating LCFAs can regulate GP via a hypothalamically triggered mechanism that is dependent on (1) the esterification of LCFAs to LCFA-CoAs, (2) functional K_{ATP} channels, and (3) neural transmission via the vagus nerve.

At this point, the studies convincingly demonstrated the importance of the hypothalamic LCFA signal in regulating energy and glucose homeostasis, but the importance of fatty acid oxidation in this signal remained unclear. Carnitine palmitoyltransferase-1 (CPT1) regulates the transportation of LCFAs into the mitochondria, where they undergo β-oxidation (Figure 20.2), and its activity is likely a key determinant in the level of cytosolic pool of LCFAs. Based on the observation that i.c.v. oleic acid but not octanoic acid, a medium-chain fatty acid which does not require CPT1 for mitochondrial entry (Obici et al., 2002a,b), has suppressive effects on food intake

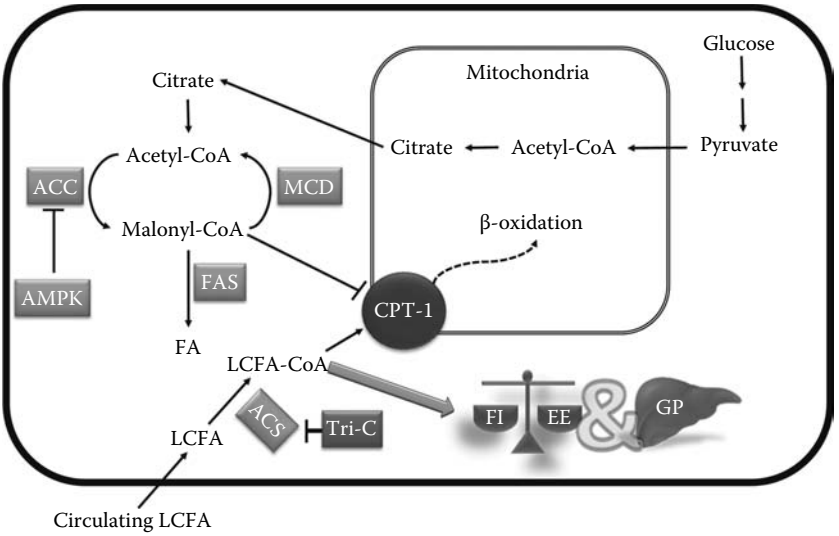

FIGURE 20.2 Generation of the hypothalamic LCFA-CoA surfeit signal. LCFA-CoAs gain access to the mitochondria to undergo β-oxidation via the acyltransferase CPT1, which is located on the outer mitochondrial membrane. Cellular fat oxidation is regulated by the availability of malonyl-CoA, which binds CPT1 and potently inhibits its activity. Malonyl-CoA, in turn, is mainly derived from acetyl-CoA—a glycolytic end-product—via the enzyme ACC; thus, the pathway is in a prime position to accurately monitor cellular energy status. Finally, ACC activity is allosterically inhibited by AMPK-mediated phosphorylation. LCFA-CoAs are also generated from LCFAs transported from the circulation, via ACSs, the activity of which can be inhibited by triacsin C (Tri-C). FAS, fatty acid synthase; GP, glucose production; FI, food intake; EE, energy expenditure.

and GP, Rossetti and colleagues then tested if CPT1 activity-mediated changes in cytosolic LCFAs can recapitulate the observed effects with i.c.v.-administered LCFAs. Specifically, the study made use of molecular and pharmacological approaches to inhibit CPT1 with the use of a ribozyme designed to cleave the "CPT1A" (predominant hypothalamic isoform) mRNA and CPT1-specific inhibitors, respectively (Obici et al., 2003). Before assessing the physiological effect of CPT1 inhibition, molecular analyses were conducted, and it was determined that i.c.v. delivery of the ribozyme did indeed substantially decrease "CPT1A" mRNA, and that i.c.v. ribozyme as well as the pharmacological inhibitors resulted in a marked decrease in CPT1 activity, both resulting in an increase in the concentration of LCFA-CoAs (Obici et al., 2003). Feeding behavior was then assessed as per previous studies (Obici et al., 2002a,b), and it was determined that i.c.v. injection of the CPT1 ribozyme as well as the pharmacological inhibitors were all able to produce a marked reduction in food intake for 48 h versus the i.c.v. infusions of the control ribozyme or the inactive stereoisomer of the CPT1 inhibitor (Obici et al., 2003). Once again, this was the result of a downregulation in mRNA of both the orexigenic neuropeptides AgRP and NPY (Obici et al., 2003). In terms of the effects of central CPT1 inhibition on whole body insulin action, it was found that the i.c.v. administration of the CPT1 ribozyme (3 days before

pancreatic-euglycemic clamp) or CPT1 inhibitor (concurrent infusion) resulted in a substantial and significant decrease in GP (Obici et al., 2003). Based on the results, it can be concluded that the inhibition of hypothalamic CPT1 activity, and the resultant increase in hypothalamic LCFA-CoA levels, was sufficient to produce a surfeit signal to suppress food intake as well as GP (Obici et al., 2003).

In a parallel finding, M. Daniel Lane's group showed that mice that were deficient in CPT1c, a recently discovered CNS-specific isoform of CPT1 that is expressed in high amounts in the hypothalamus in addition to CPT1a (Price et al., 2002), weighed significantly less than their wildtype littermates at the end of a 15-week period and consumed less food after a fast (Wolfgang et al., 2006). However, despite the homology to the established CPT1a and CPT1b isoforms, CPT1c was found *in vitro* to lack the ability to catalyze the acyl transfer from LCFAs to carnitine, which CPT1a and CPT1 are capable of (Wolfgang et al., 2006). Thus, it is likely that CPT1c's role in energy balance is independent of the altered β-oxidation mechanism that has been set forth by Rossetti's group. Indeed, in a follow-up study, it was determined that CPT1c knockout mice exhibited similar levels of hypothalamic LCFA-CoAs as their wildtype littermates, either in the fasted or fed state (Wolfgang et al., 2008). Thus, further work needs to be completed in order to identify the nature of the metabolite or intermediate that is necessary in generating the CPT1c-mediated hypothalamic surfeit signal.

Now that the hypothalamic lipid-sensing mechanism is in place, it is time to make a return to the effectiveness of this surfeit signal in the obese state. Recall that short-term (3 day) overfeeding on a highly palatable, high-fat diet was able to negate the ability of central oleic acids to regulate food intake and GP (Morgan et al., 2004). Rossetti's group asked if defective lipid-sensing mechanisms in the hypothalamus are partly responsible for this negation, and in particular, they focused on the role of CPT1 activity. As the rise in LCFA-CoAs is a critical initiator of this signal, the authors specifically postulated that, in the overfed model, the increase in lipid availability fails to translate into this increase in the intracellular pool of LCFA-CoA (Pocai et al., 2006). This was indeed the case: when rats fed a standard chow were administered a systemic lipid emulsion designed to double plasma LCFAs, this resulted in a doubling in hypothalamic LCFA-CoAs as previously seen (Lam et al., 2005a–c); however, in the overfed rats, this increase in circulating lipids failed to increase hypothalamic LCFA-CoAs (Pocai et al., 2006). The lack of hypothalamic LCFA-CoA increase in overfed rats was also seen when oleic acid was directly infused into the hypothalamus (Pocai et al., 2006), thus confirming that in overfed rats central oleic acids lack the metabolic and anorectic effects, and that the failure to increase hypothalamic LCFA-CoAs was not likely due to impeded BBB LCFA transport. A possible explanation for the observed effects is that there might be an enhanced rate of LCFA-CoA metabolism in the hypothalamus; indeed, hypothalamic CPT1 activity was significantly increased in the overfed rats (Pocai et al., 2006). Remarkably, by hypothalamically administering a CPT1a inhibitor or ribozyme to suppress CPT1a activity or expression, respectively, the authors were able to suppress food intake as well as GP in overfed rodents (Pocai et al., 2006). Thus, inhibiting hypothalamic lipid oxidation via the inhibition of the CPT1 activity is sufficient to restore energy balance as well as glucose homeostasis in overfed rodents.

Taken together, these noteworthy studies highlight the importance of central fatty acid metabolism in initiating the hypothalamic behavioral and metabolic responses necessary to regulate energy and glucose homeostasis. Thus, it is important to acquire an understanding of the upstream biochemical processes that are involved in the formation of LCFA-CoAs, as they are all ultimately responsible for the generation of this hypothalamic surfeit signal.

20.4 FATTY ACID BIOSYNTHESIS—GENERATING THE LCFA-COA SURFEIT SIGNAL

The access of circulating LCFAs to the CNS is proportional to the plasma fatty acid concentration, and once in neuronal cells, they are rapidly esterified by ACSs to form LCFA-CoAs (Lam et al., 2005c). Yet, this is not the sole way that LCFA-CoAs can be generated in the brain (Figure 20.2). In fact, LCFA-CoAs can also be made *de novo* with the aid of upstream biosynthetic enzymes and metabolite intermediates (Wakil et al., 1983).

As described earlier, LCFA-CoAs gain access to the mitochondria to undergo β-oxidation via the acyltransferase CPT1, which is located on the outer mitochondrial membrane. The two most well-characterized isoforms of CPT1 are CPT1a (the liver isoform) and CPT1b (the muscle isoform), and the former is most predominant in the brain. Cellular fat oxidation is regulated by the availability of malonyl-CoA, which binds CPT1 and potently inhibits its activity. Malonyl-CoA, in turn, is mainly derived from acetyl-CoA—an end-product of glycolysis—via the enzyme acetyl-CoA carboxylase (ACC); thus, the pathway is in a prime position to accurately monitor cellular energy status (Lam et al., 2005c). Finally, ACC activity is allosterically inhibited by the adenosine monophosphate-activated protein kinase (AMPK)-mediated phosphorylation.

Collectively, these regulatory enzymes and metabolite intermediates of the fatty acid biosynthesis pathway are poised to be key players in triggering the hypothalamic response to regulate energy as well as glucose homeostasis, and there is ample evidence suggesting that this may be the case.

20.4.1 AMP-Activated Protein Kinase

AMPK is an evolutionarily conserved energy sensor that in essence acts as a fuel gauge of mammalian cells (Hardie and Carling, 1997). It is expressed in most tissues, including the hypothalamus (Stapleton et al., 1996), and has a vast array of functions systemically and, more recently identified to function, centrally. AMPK operates by phosphorylation of various targets and by responding to an increasing cellular AMP:ATP ratio.

AMPK in the periphery responds to a diverse range of hormonal, physiological, and pathological stimuli (Ramamurthy and Ronnett, 2006). Its activity is responsive to a large number of hormonal cues: leptin has been found to stimulate glucose uptake (Kamohara et al., 1997) and fatty acid oxidation (Minokoshi et al., 2002) in skeletal muscle by activation of AMPK while adiponectin stimulates glucose utilization and fatty-acid oxidation in muscle and liver also by activating AMPK (Yamauchi et al.,

2002). In general, activation of AMPK by ATP-depleting cellular stresses switches on ATP-generating catabolic pathways while ATP-consuming processes, including parameters such as glucose homeostasis, lipid metabolism, and mitochondrial biogenesis (Kahn et al., 2005) are switched off.

The hypothalamus has been suggested as a central mediator of energy homeostasis. Recently, AMPK in the hypothalamus has been investigated for its role in this regulation. Under normal physiological conditions, AMPK activity increases during fasting and decreases upon refeeding (Minokoshi et al., 2004). Conversely, modulating AMPK activity *per se* changes feeding behavior. It has been shown that central administration of 5-amino-4-imidaszole carboxamide riboside (AICAR), a pharmacological activator of AMPK, can raise food intake in rodents (Andersson et al., 2004; Hu et al., 2005; Kim et al., 2004). Moreover, dominant negative AMPK expression in the hypothalamus decreases feeding and body weight whereas constitutively active hypothalamic AMPK expression promotes feeding and increases body weight (Minokoshi et al., 2004). These changes in feeding patterns are accompanied by changes in neuropeptide expressions. Particularly, under *ad libitum* fed conditions, hypothalamic dominant negative AMPK decreased NPY and agouti-related protein (AgRP) mRNA levels whereas constitutively active AMPK increased both under fasted conditions. The AMPK-induced NPY fluctuation is suggested to be mediated by cAMP response element-binding protein (CREB) phosphorylation (Kim et al., 2004). *In vitro* studies with neuronal cell lines expressing AgRP are in support of these findings as high cellular ATP concentration decreased phosphorylation of AMPK and AgRP expression while low cellular ATP concentrations increased both (Lee et al., 2005).

As discussed earlier, energy regulation is largely based upon neuropeptide release from specific neuronal populations, namely the POMC and AgRP/NPY neurons in the ARC of the hypothalamus. The above findings would suggest that energy regulation signaling through AMPK selectively impacted AgRP/NPY neurons given that only the expression of orexigenic peptides AgRP and NPY, but not the anorexigenic peptide (POMC), was altered. However, recent genetic knockout models shed light on the possible involvement of both neuronal types. In particular, selective knockout of AMPK in either AgRP/NPY or POMC neurons in mice disrupted energy homeostasis, where the former exhibited an age-dependent lean phenotype and the latter developed obesity due to dysregulation in food intake and energy expenditure (Claret et al., 2007). These studies, along with the recent finding that an obesity-resistant hypophagic rat strain (Lou/C) exhibited impaired AMPK responses to starvation (Taleux et al., 2007), collectively established that AMPK signaling is necessary for proper energy balance.

Associated findings suggest that circulating nutrients, which are known to control energy balance, also change hypothalamic AMPK activity. Central administration of glucose significantly decreased AMPKα2 activity (Minokoshi et al., 2004). In support of this, central 2-deoxyglucose administration, which inhibited glucose utilization, activated hypothalamic AMPK activity (Kim et al., 2004). In addition, α-lipoic acid, a short-chain fatty acid that is a cofactor of mitochondrial enzymes with anorectic properties, also inhibited hypothalamic AMPK activity and was associated with decreased food intake and body weight (Kim et al., 2004). Further, as in the periphery, various

hormonal signals involved in appetite control are able to modulate hypothalamic AMPK activity. Initial studies of leptin, an anorectic peptic hormone, administration peripherally found it to reduce hypothalamic AMPK activity (Andersson et al., 2004). Consistent with this, hypothalamic administration of either leptin or insulin also decreased hypothalamic AMPK activity (Andersson et al., 2004; Minokoshi et al., 2004). Interestingly, leptin's alteration of AMPK activity was absent in a mouse model with melanocortin-4 (MC4) receptor knockout, indicating that the MCR signaling mediates leptin's effect on AMPK activity (Minokoshi et al., 2004). Further, constitutively active AMPK expression in the hypothalamus blocked leptin's anorectic effect, suggesting that leptin's appetite regulation requires modulation of AMPK activity (Minokoshi et al., 2004). More recently, the acute effects of the important anorectic gut peptide, glucagon-like peptide 1 (GLP-1) has been found to be mediated, at least in part, by hypothalamic AMPK as the food intake lowering effect of i.c.v. GLP-1 is accompanied by reduction of hypothalamic AMPKα2 mRNA levels (Seo et al., 2008).

Contrary to anorectic peptides, orexigenic peptides such as ghrelin (Andersson et al., 2004; Kola et al., 2005), AgRP (Minokoshi et al., 2004), and orexigenic cannabinoids (Kola et al., 2005) stimulated hypothalamic AMPK activity. More recently, it was shown that the adipocyte-secreted hormone adiponectin also enhanced AMPK activity in the ARC to stimulate food intake and reduce energy expenditure (Kubota et al., 2007). To strengthen the point, it was previously identified *in vitro* that both insulin's anorexigenic and ghrelin's orexigenic alteration of NPY were only evident in the presence of glucocorticoids (Goto et al., 2006; Sato et al., 2005). More interestingly, it was recently identified that this glucocorticoid-induced increase in NPY was via AMPK signaling (Shimizu et al., 2008). Taken together, the discussed findings show that AMPK is a downstream enzyme that converges and coordinates various nutrient and hormonal signals, anorexigenic and orexigenic, in the hypothalamus to regulate energy homeostasis.

It is perhaps interesting to note that the pattern of neuropeptide regulation elicited by the manipulations of hypothalamic AMPK activity, i.e., changes in NPY/AgRP but not POMC levels (Minokoshi et al., 2004), is similar to that seen with central fatty acids administration (Obici et al., 2002a,b) or manipulation of hypothalamic lipid metabolism by inhibition of CPT1 (Obici et al., 2003). This hints at a possible parallel or converging mechanism at work bridging AMPK to fatty acid sensing in the brain. In fact, one of the best characterized targets of AMPK is the phosphorylation of ACC. AMPK negatively regulates ACC activity through phosphorylation (Munday et al., 1988; Sim and Hardie, 1988). This inhibition hinders the conversion of acetyl-CoA to malonyl-CoA, the latter of which is known to inhibit CPT1 action (McGarry et al., 1978). It is therefore reasonable to postulate that the effects of energy regulation elicited by AMPK are mediated by the downstream fatty acid sensing mechanism via the "malonyl-CoA $\rightarrow$ CPT1 inhibition $\rightarrow$ LCFA-CoA accumulation" hypothesis of appetite regulation. To compliment this hypothesis, it was found that the suppression of food intake by C75, a fatty acid synthase (FAS) inhibitor, is accompanied by decreased AMPK activity (Kim et al., 2004).

While the role of AMPK in facilitating nutrient and hormonal regulation of energy homeostasis is clear—with strong evidence pointing to its role in altering

hypothalamic neuropeptide gene expression in specific neuronal populations, possibly involving the fatty acid metabolism pathway—the precise upstream mechanisms by which AMPK is phosphorylated and activated to exert its energy regulation is open to speculation. AMPK catalytic activity is triggered upon two conditions, namely the conformational change of the γ subunit of AMPK under sufficient AMP concentration and the phosphorylation of its α-subunit by an upstream AMPK kinase (AMPKK) that leads to consequent activation of AMPK. LKB1, the Peutz-Jeghers syndrome tumor suppressor gene is a known AMPKK (Hawley et al., 2003; Woods et al., 2003). However, while it is widely expressed in various tissues, LKB1's role in brain AMPK activation remains largely unknown (Rowan et al., 2000; Ramamurthy and Ronnett, 2006). More recently, the calcium/calmodulin-dependent protein kinase β (CaMKK2) has also been identified as an AMPKK (Hawley et al., 2005; Hurley et al., 2005; Woods et al., 2005). In fact, CaMKK2 has now been shown to mediate hypothalamic AMPK activity to regulate production of NPY, and mice with CaMKK2 inhibition had decreased NPY and AgRP mRNA accompanying weight loss and inhibited appetite (Anderson et al., 2008).

20.4.2 Acetyl-CoA Carboxylase

Fatty acids that are present in the cells are either imported from the circulation or generated *de novo*. The committed step of *de novo* fatty acid biosynthesis is the initial conversion of acetyl-CoA to malonyl-CoA, which is mediated by the enzyme ACC (Wakil et al., 1983). There exists two isoforms of ACC, ACC1, and ACC2, both of which are present in the brain (Gao and Lane, 2003; Kim et al., 2002). It remains unclear, however, whether one or both isoforms in the hypothalamus are responsible for monitoring energy homeostasis. Using a mouse knockout model, it was first identified in mice lacking ACC2 that although these animals had 20%–30% higher food consumption in comparison to wildtype, their body weights were significantly lower than that of wildtype animals. Further, these ACC2 knockout mice were leaner, with less fat in their adipose tissues (Abu-Elheiga et al., 2001). The unmatched food intake and body weight might in part be accounted for by increased energy expenditure in ACC2 knockout mice (Choi et al., 2007). Unfortunately, as the aforementioned mice are whole body ACC2 knockouts with changes occurring in the periphery, one is unable to pinpoint from these two studies the precise contribution of hypothalamic ACC in the observed energy regulation changes. The advantages of knockout models in analyzing hypothalamic ACC comes to a halt at this stage given that global knockout of the ACC1 gene is embryonically lethal (Abu-Elheiga et al., 2005) and hypothalamic-specific ACC knockouts have not yet been reported (Wolfgang et al., 2006).

With this said, however, a few recent studies have highlighted the importance of hypothalamic ACC in energy balance through other pharmacological and molecular approaches. Of note, it was identified that ACC makes critical contributions to leptin's inhibition of food intake (Gao et al., 2007). It was found in this study that i.c.v. leptin, which inhibits AMPK activity, activates ACC. The fact that constitutively active AMPK prevented ACC activation in response to i.c.v. leptin strengthens the claim that AMPK lies upstream of ACC's energy regulatory effects. This is perhaps

no surprise given that AMPK is a well-established inhibitor of ACC, as discussed in previous sections. Also of interest is the finding that blockage of ACC activity eliminated the anorectic effect of leptin and prevented the drop in NPY mRNA usually observed with i.c.v. leptin. In fact, such ACC blockade also abolished the iv leptin-induced rise in malonyl-CoA level, highlighting that the energy regulation of ACC is due to a rise in its product, malonyl-CoA (Gao et al., 2007). This, again, is in concordance with the LCFA-CoA energy homeostasis hypothesis, i.e., malonyl-CoA inhibits CPT1, increasing cytosolic LCFA-CoA levels. While most studies thus far have focused on energy homeostasis, a recent study pointed at ACC's possible involvement in glucose homeostasis (Cesquini et al., 2008). Citrate, an intermediate metabolite produced in the mitochondria in the citric acid cycle, had previously been shown to promote satiety when administered into the hypothalamus (Roman et al., 2005). Given that citrate is an allosteric effector of ACC activity, the authors, upon confirming that ACC activity is significantly reduced by i.c.v. citrate, found that such administration not only decreased food intake and body weight, but also resulted in lower blood glucose levels during glucose tolerance test and increased glucose uptake during hyperglycemic–euglycemic clamp settings (Cesquini et al., 2008). Of note is the striking finding that the anorectic phenotype induced by ACC activation and subsequent malonyl-CoA formation can indeed be reversed by promoting the reverse reaction, namely, the conversion of malonyl-CoA back to acetyl-CoA by enzyme malonyl-CoA decarboxylase (MCD). It was found in a set of elegant molecular manipulation experiments that overexpressing MCD using adeno-associated viruses injected into the mediobasal hypothalamus of animals led to a rapid increase in food intake in such animals accompanied by a gradual development of obesity (He et al., 2006). Further, these MCD-overexpressing animals exhibited impaired suppression of GP during insulin-clamped settings (He et al., 2006). In accordance with the fatty acid sensing hypothesis, these MCD-overexpressed rats not only had a significant reduction in malonyl-CoA levels, but also a decrease in LCFA-CoA abundance. Notably, the LCFA-CoA levels in these animals are highly comparable to those seen in animals with pharmacological inhibition of LCFA esterification with triacsin-C in the mediobasal hypothalamus, which also exhibited impaired glucose homeostasis (Lam et al., 2005a–c).

20.4.3 Fatty Acid Synthase and the Malonyl-CoA Hypothesis

FAS catalyzes the synthesis of LCFA-CoAs from malonyl-CoA in a downstream reaction of the reductive synthesis of LCFAs. The potential role for fatty acid intermediates in the regulation of energy balance originally stemmed from findings revealing that the systemic as well as central treatment of mice with the FAS inhibitor cerulenin—a fungus-derived compound originally developed as an anticancer agent (López and Vidal-Puig, 2008)—led to the marked inhibition of feeding and subsequently, weight loss (Loftus et al., 2000). However, the authors believed that this agent may have toxic properties because of its epoxide structure, and synthesized a closely related compound termed C75 (Loftus et al., 2000), a FAS inhibitor that has since acquired immense popularity in studies investigating the physiological effects of the *in vivo* inhibition of fatty acid synthesis.

In the seminal paper by Kuhajda and colleagues, the physiological effect of inhibiting fatty acid synthesis on global lipid metabolism was initially assessed with a single intraperitoneal (i.p.) injection of C75, and the result in the treated mice was profound weight loss which was primarily due to a remarkable 90% reduction in food intake within the first day (Loftus et al., 2000). The feeding behavior as well as the body weight returned to normal as the drug effect wore off, indicating that the observed effect was not due to a toxicity-induced state of permanent wasting (Loftus et al., 2000). As far as the neuropeptide control of feeding is concerned, hypothalamic NPY mRNA levels in C75-treated mice were lower than in fed control mice, indicating that the C75-induced mechanism of anorexia may work via the inhibition of NPY-induced feeding (Loftus et al., 2000). This mechanism likely lies upstream of NPY activation, as i.c.v. NPY treatment in C75 pretreated mice led to pronounced feeding behavior (Loftus et al., 2000). Leptin had long been championed as a primary anorexigenic factor, however, the ability of C75 treatment to work in leptin-deficient "ob/ob" mice (Loftus et al., 2000) illustrates that the anorexigenic behavior initiated by FAS-inhibition is actually independent of leptin action. The potential of a central impact of C75 was demonstrated in two ways: (1) studies with a radiolabeled variant of the drug illustrated that C75 indeed enters the brain and (2) direct, i.c.v. administration of C75, much like i.c.v. cerulenin, inhibited feeding by greater than 80%. Overall, the data is supportive of the aforementioned hypothesis, according to which malonyl-CoA signals a fuel status to trigger satiety signals in the hypothalamus.

In the attempt to link the role of malonyl-CoA with FAS in the regulation of feeding behavior, it was demonstrated in a subsequent study that i.c.v. administration of C75 in fasted animals increased hypothalamic malonyl-CoA concentrations by fourfold (Hu et al., 2003). By administering an ACC inhibitor centrally to prevent the rise in malonyl-CoA accumulation, it was found that the C75-induced anorexia and neuropeptide mRNA changes were abolished (Hu et al., 2003).

It is clear from such findings that hypothalamic regulation of energy homeostasis is a complex series of processes involving many metabolites and enzymes. Particularly, the anorexic effects triggered by ACC activation combined with the opposing orexigenic effects of AMPK activation would strongly imply that malonyl-CoA, a downstream product of ACC, must hold a critical role in such sensing pathway. Indeed, the concentration of malonyl-CoA in the hypothalamus falls during fasting and rises after refeeding (Hu et al., 2003). Further, malonyl-CoA is also involved in nutrient and hormonal sensing in the hypothalamus. Specifically, central glucose and leptin have recently been confirmed to regulate hypothalamic malonyl-CoA concentrations (Wolfgang et al., 2007). It was found that malonyl-CoA level in the hypothalamus rises upon peripheral glucose administration, but is blocked when central glucose metabolism is pharmacologically inhibited. Further, a single dose of i.c.v. leptin induces a sustained increase in hypothalamic malonyl-CoA level, which flawlessly compliments previous findings that central glucose and leptin lowered hypothalamic AMPK activity (Andersson et al., 2004; Minokoshi et al., 2004) while the latter was also found to augment hypothalamic ACC activity (Gao et al., 2007). These kinetic changes in AMPK, ACC, FAS, and malonyl-CoA are perhaps not merely coincidental and strongly support the existence of an underlying link between these individual factors.

The above finding suggests a potential integration and codependence between the fatty acid biosynthesis and glucose metabolism pathways in the hypothalamus when it comes to the regulation of energy balance. Noting that neuronal metabolism is essentially solely dependent on glucose supply for energy, Wortman and colleagues tested the idea that the anorexic effects of C75 administration requires increased glucose utilization as opposed to decreased lipid utilization (Wortman et al., 2003). In this study, rats maintained on a very low-carbohydrate diet to induce ketosis did not exhibit the decreased short-term food intake or body weight in response to i.c.v. C75; interestingly, i.c.v. C75's anorectic ability was restored when ketogenic animals were provided with a 10% sucrose drink (Wortman et al., 2003). To further assess the need for increased central glucose utilization due to C75's anorectic effects, the authors coadministered glutamine or lactate—energy sources provided to neurons by glial metabolism—with i.c.v. C75. In the presence of glutamate or lactate, there is a reduced need for neurons to metabolize glucose, so according to the proposed theory, C75's effect should be negated in this environment; indeed, they found that administering glutamate or lactate was sufficient to negate the anorexia induced by i.c.v. C75 (Wortman et al., 2003). Thus, it is quite likely that the regulation of energy balance mediated by inhibition of FAS is reliant on central glucose use.

The studies thus far have focused on FAS inhibition and its role in the suppression of food intake, however, it has also been shown that C75-induced weight loss is also due to an increase in energy expenditure (Kumar et al., 2002). A follow-up study by M.D. Lane's group investigated the possibility that an increased rate of fatty acid oxidation accounted for this observed increase in energy expenditure. The central administration (i.c.v.) of C75 to fasted mice caused a significant increase in (1) whole-body fatty acid oxidation, as determined *in vivo* and (2) skeletal muscle fatty acid oxidation, as determined *in vitro*; in response to this, there was also a concomitant increase in the expression of PPARα, which activates the expression of genes encoding enzymes of fatty acid oxidation (Cha et al., 2005). Interestingly, when phentolamine, an agent that blocks α-adrenergic (sympathetic nervous system) transmission, was used, the effects of i.c.v. C75 on whole body and skeletal muscle fatty acid oxidation were negated (Cha et al., 2005). Thus, the results indicate that the hypothalamic signal mediated by FAS-inhibition to increase energy expenditure occurs via nervous transmission to the skeletal muscle to increase fatty acid oxidation.

Though the evidence for the role of FAS in hypothalamic energy regulation thus far appears indisputable, a few legitimate concerns persist. First, while the pharmacological inhibition of FAS with C75 provided the basis of much of the evidence on FAS, it is made clear in recent years that C75 is, in fact, nonspecific, having complex effects including activation of the sympathetic nervous system (Cha et al., 2005; Chakravarthy et al., 2007), as mentioned previously. Further, while i.c.v. inhibition of FAS is known to increase the expression of PPARα in the periphery, the effects of central PPARα remain unknown. To tackle these questions, brain-beta cell-specific FAS knockout mice were generated (Charkravarthy et al., 2007).

Using a rat insulin promoter, FAS was elegantly and specifically knocked out in the beta cells and brain. It was first established in the study that FAS knockout had no effect on beta cell function or visible changes in islet morphology, indicating

that FAS was not required for beta-cell function in adult mice (Charkravarthy et al., 2007). With that established, it was reasonable to attribute the observed changes to the absence of central FAS. In line with the findings previously described, central FAS knockout had disrupted energy balance, with altered feeding behaviors and energy expenditure. These mice exhibited a lean, hypophagic phenotype with increased physical activity compared to control animals (Charkravarthy et al., 2007). *In situ* hybridization revealed a suppression of mRNAs for orexigenic neuropeptides, i.e., NPY and AgRP, and induction of anorexigenic neuropeptides, i.e., POMC and cocaine-amphetamine-related transcript (CART) in the ARC of the hypothalamus (Charkravarthy et al., 2007). Furthermore, it was seen that central FAS, besides activating peripheral PPARα (Cha et al., 2005), also activates brain PPARα to coordinate feeding behaviors (Charkravarthy et al., 2007). The authors reported a 50% reduction of mRNA levels of acyl-CoA oxidase (ACO), CPT1, and MCD, all of which are known targets of PPARα (Charkravarthy et al., 2007). However, in order to confirm that PPARα is indeed a critical downstream effector of FAS' energy regulation, the authors administered a potent PPARα agonist Wy14643 centrally in an attempt to rescue the FAS knockout phenotype. Strikingly, such agonist administration was able to rescue the food intake and body weight decrease in the knockout animals under fasted conditions and restored the mRNA levels of PPARα target genes, i.e., ACO, CPT1, and MCD. Further, Wy14643 injected in the ventral hypothalamus in these knockout animals actually resulted in increased food intake under *ad libitum* settings compared to wild-type controls (Charkravarthy et al., 2007).

20.5 HYPOTHALAMIC SENSING MECHANISMS—IMPLICATIONS FOR DISEASE

The recent past has yielded novel and exciting experimental data that have furthered the concept that the hypothalamus is a key regulator of energy balance and glucose homeostasis, revealing new metabolic and hormonal signaling factors along the way. However, a necessary step is to flesh out these findings in models of metabolic disease (obesity, insulin resistance and/or diabetes) in order to gain an appreciation of what went wrong—and possibly what is still going strong—when it comes to the centrally-mediated homeostatic mechanisms in pathology.

As mentioned earlier, an increase in central fatty acid levels is able to trigger a hypothalamic mechanism to curb food intake and GP (Obici et al., 2002a,b), the latter of which is K_{ATP} channel-dependent; circulating fatty acids also were demonstrated to initiate a similar regulatory response in the suppression of GP (Lam et al., 2005a–c). Remarkably, providing rats with a high-fat diet for a mere 3 days was sufficient to nullify these homeostatic effects (Lam et al., 2005a–c; Morgan et al., 2004). It appears as if overfeeding elevated the basal activity of the enzyme CPT1, resulting in an absence of the LCFA-CoA signal, as the inhibition of this enzyme with multiple approaches was able to restore the ability of hypothalamic lipids to suppress food intake and GP (Pocai et al., 2006). These methodical studies by the Rossetti group clearly demonstrated the role of fatty acid oxidation shunting in the hypothalamus in generating the local LCFA-CoA trigger to serve as a behavioral and metabolic surfeit signal.

Long known to serve as a cellular energy sensor, even in the most simplest of organisms, AMPK has recently been shown to have a regulatory role in the hypothalamus-mediated control of energy balance (Minokoshi et al., 2004). With the fatty acid biosynthetic pathway in mind, the accumulation of intracellular malonyl-CoA and LCFA-CoA would be antagonized by AMPK hyperactivity, and a number of studies have examined this potential dysregulation of AMPK activity in obese or diabetic models. Streptozotocin (STZ)-induced diabetic rats are eventually characterized by marked hyperphagia, and compared with control rodents, it was found that hypothalamic AMPK activity was higher in diabetic rats; this activity (and the resultant food intake) was normalized by the i.c.v. administration of an AMPK inhibitor, as well as insulin and leptin (Namkoong et al., 2005), confirming the previously established central anorectic effects of those hormones. Additionally, in a 12-week model of diet-induced obesity, leptin's ability to inhibit AMPK activity in various hypothalamic nuclei, including the ARC, was lost (Martin et al., 2006). Conversely, it was recently found out that mice that were absent in CaMKK2, a regulator of AMPK activity, were protected from diet-induced obesity, insulin resistance, and glucose intolerance (Anderson et al., 2008).

Obesity and diabetes are typically characterized by hyperphagia, hyperinsulinemia, and hyperleptinemia, and as a result, profound insulin and leptin resistance can occur. Studies have shown that central pathways play a part in bestowing these resistant phenotypes. In a landmark study nearly 30 years ago, Woods and colleagues demonstrated that the chronic central infusion of insulin reduces food intake and body weight (Woods et al., 1979); however, in animals receiving a high-fat diet for 14 days, a chronic, one-week infusion of central insulin failed to reproduce these anorectic effects (Arase et al., 1988). Furthermore, the i.c.v. infusion of a phosphatidylinositol 3-kinase (PI3K) inhibitor blunted the ability of peripheral insulin injection to normalize glucose levels in STZ-diabetic rodents, convincingly demonstrating the importance of central insulin signaling in the brain during insulin treatment (Gelling et al., 2006). The adipokine leptin, once hailed as the next possible "miracle cure," faces a particularly interesting problem as far as the brain is concerned. Peripherally administered leptin normally acts through a hypothalamic STAT3-signaling pathway to exert its anorectic effects, however, a prolonged high-fat diet was found to eradicate this ability in rodents (El-Haschimi et al., 2000). Interestingly, these diet-induced obese rodents were still responsive to the effects of centrally administered insulin (El-Haschimi et al., 2000). Thus in the obese state, it is not necessarily the action of leptin that is compromised; rather, there is likely defective leptin transport from circulation into the CNS.

A noteworthy study recently implicated apolipoprotein E (ApoE) as yet another mediator of the central control of energy homeostasis. Predominantly produced in the brain and the liver, and shown in the brain to have effects on oxidative stress protection and regulating local immune responses, i.c.v. ApoE was demonstrated to have markedly decreased food intake via a POMC-stimulating mechanism in lean mice (Shen et al., 2008). Dysregulation of central ApoE may be a critical event in hyperphagia and resultant obesity, as in both diet-induced obese and leptin-deficient "ob/ob" rodents had significantly reduced hypothalamic "apoE" mRNA levels (Shen et al., 2008).

While each of these observed defects hint at varying alterations in metabolic signals and/or enzymatic activities *per se* as a potential root cause in the defects mediating the loss in hypothalamic signal effectiveness to regulate homeostasis, a very recent publication hints that there may indeed be a common defect underlying them all. Bouret and colleagues observed that rats that were selectively bred to develop diet-induced obesity develop defective ARC neuronal projections (Bouret et al., 2008). Leptin, which is essential for the normal development of ARC projections, was found to be ineffective in activating ARC neuron signaling in diet-induced obese neonates (Bouret et al., 2008). Thus, it is quite likely that the genetically governed structural defects persist into adulthood and play a key role in initiation and progression of ineffective hypothalamic surfeit signaling.

But interestingly, not all central sensing mechanisms are disrupted in models of obesity and/or diabetes. For example, an acute increase in central or hypothalamic lactate has been shown to regulate glucose homeostasis by suppressing GP in normal rodents (Lam et al., 2005a–c). Interestingly, we have also observed that administration of central lactate—at the same dose used in normal rodents is able to lower GP in an early-onset model of STZ-Diabetes (Chari et al., 2008). Furthermore, central lactate suppressed GP in normal rodents with experimentally induced hypoinsulinemia, and more significantly, in diet-induced insulin resistance resulting from a 3 day high-fat diet (Chari et al., 2008). It is intriguing that in a similar model of acute diet-induced insulin resistance, central oleic acid was ineffective in suppressing food intake and GP (Morgan et al., 2004). Clearly, further investigation is necessary to elucidate the mechanism underlying this selective loss in central nutrient sensing.

20.6 CONCLUDING REMARKS

Although the glucostatic and lipostatic theories were presented well over half a century ago, the nature of the input signal required for the hypothalamic regulation of nutrient and energy homeostasis and the related biochemical mechanism had remained unclear until the recent past. Numerous landmark studies have made significant contributions to the advancement of the field, and have collectively proven that certain hypothalamic nuclei respond to shifts in energy and nutrient status relayed by circulating factors, including metabolites as well as hormones, to initiate behavioral and metabolic changes to restore energy and glucose homeostasis (Sandoval et al., 2008). Thus, there is an undeniable importance of the CNS in the development of what seems like primarily peripheral metabolic diseases of obesity and diabetes.

In specific, this review highlights the importance of generating the LCFA-CoA surfeit signal as a result of hypothalamic fatty acid biosynthesis and utilization in mediating these homeostatic responses. Keen observers will be quick to notice that this idea is at complete odds with the effect of circulating LCFAs—namely, the induction of insulin resistance and an increase in GP (Lam et al., 2003). This might seem paradoxical; however, we propose that these two seemingly contradictory processes, as far as diabetogenic processes and metabolic dysfunction is concerned, can work harmoniously in the monitoring of energy and nutrient status. In fact, we propose that a balance in central and hepatic lipid sensing exists (Caspi et al., 2007), whereby the opposing effects of lipid sensing in these two organs achieve an equilibrium in

GP regulation. In the event that this balance is disrupted, such as in the obese state, a dysregulation in GP will result, facilitating the progression of diabetes.

But the picture is far from complete. While the transmission from the first-order neurons in the ARC to second-order neurons in other hypothalamic nuclei is generally accepted, it can still be argued in the present that the efferent mechanism by which hypothalamic signaling is linked to metabolism in peripheral tissues remains largely unknown (Horvath et al., 2004; Schwartz and Porte, 2005). One possible mechanism may use vagal outflow to the liver, as hepatic vagotomy has been shown to nullify the ability of central inhibition of fat oxidation to suppress GP (Pocai et al., 2005). And while the bulk of the research has focused on the ARC, other brain nuclei (hypothalamic or otherwise) could likely be involved in the processing of peripheral hormone and nutrient signals (Schwartz and Porte, 2005).

Thus, continued efforts are necessary in order to fully characterize the nutrient, nervous, or hormone-mediated responses in the brain and the extent to which they may be altered in the diseased metabolic state. This will undoubtedly lead to a prioritization of possible central targets when it comes to developing novel therapeutics to combat the rapidly increasing obesity and diabetes epidemic.

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21 Dietary Fat and Carbohydrate Composition: Metabolic Disease

Marc A. Brown, Len H. Storlien, Xu-Feng Huang, Linda C. Tapsell, Paul L. Else, Janine A. Higgins, and Ian L. Brown

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21.1 INTRODUCTION

The term "metabolic syndrome" is used to describe a cluster of disease states including blood lipid disorders, hypertension, propensity for thrombus formation, low-grade chronic inflammation, abdominal obesity, and type-2 diabetes. Insulin resistance is the key to the metabolic syndrome, particularly the relative failure of insulin to exert its multiple biological effects on carbohydrate and lipid metabolism.

The current epidemic of obesity and type-2 diabetes in developed and developing countries has focused major attention on the metabolic derangements critical to their etiology. The Banting lecture of 20 years ago by Reaven sparked great interest in the clustering of diseases, which he termed "Syndrome X" and subsequently is more commonly referred to as the metabolic syndrome (Reaven, 1988). The basic symptomatology included dyslipidemias (high cholesterol and triglycerides), insulin resistance, obesity, and hypertension. With continuing research, subsequent iterations have added central (and in particular visceral) adiposity and chronic low-grade inflammation as key elements. Diet plays a powerful role in modulating expression of the metabolic syndrome and it is increasingly clear that both amount and type of both fats and carbohydrates, and the interaction between them, are important variables.

21.2 DIETARY FAT AS A PROPORTION OF CALORIES

Fat is the most calorically dense of macronutrients. A recent review by Mendoza, Drewnowski, and Christakis (Mendoza et al., 2007) has very nicely covered the energy density literature and the conclusion is clear: "Dietary energy density is an independent predictor of obesity, elevated fasting insulin levels, and the metabolic syndrome in U.S. adults." Fat, while certainly not the only factor, is a strong contributor. It then makes intrinsic sense that increased proportion of calories from fat would be associated with increased obesity and related disorders. Since obesity is the result of a very long-term excess of intake over expenditure and may consist of many life phases of both subtle caloric imbalances and more dramatic episodes, it is difficult to assign a role to the proportion of calories as fat in the development of obesity. Indeed, some studies suggest a reduction in fat calories during the 1990s, a period of explosive growth in obesity (Prentice and Jebb, 1995; Arnett et al., 2000). This overall obesity rise will reflect the interplay with reduced energy expenditure attendant on a society with much less demand for hard physical work both in the home and workplace, but even these data are not consistent with a message that lowering the proportion of fat in the diet will automatically lower body weight.

More useful data have come from intervention studies for weight loss. Studies from 2000 to 2002 were somewhat conflicting. Astrup and colleagues suggested a significant effect of reducing the proportion of fat calories on top of the weight loss of caloric restriction without proportional fat reduction (see Astrup et al., 2000).

The effect was very modest but nevertheless represented, over a year, an excess loss of 4–500 g per percentage of fat reduction. In contrast, a Cochrane review (Pirozzo et al., 2002) concluded "that fat-restricted diets are no better than calorie-restricted diets in achieving long-term weight loss in overweight and obese people." More recently this conclusion has been reinforced by work from the excellent Nugenob Consortium from Europe (Petersen et al., 2006) which showed no difference in weight loss, nor any difference in measures of dyslipidemia, insulin, or glucose, between a 10 week low- or high-fat hypocaloric dietary intervention.

In the United States, the focus has been on comparing the sometimes outrageous claims of the various "wonder" diets (Foster et al., 2003; Gardner et al., 2007). The media hysteria over fad diets has encouraged all sorts of dietary interventions for weight loss ranging from high-fat to very low-fat diets. The studies done scientifically to test these claims are interesting for a few reasons. First, they speak to the issue of percentage of fat and weight loss. Here, we recommend reading the Gardner et al. (2007) paper where weight loss was compared across the so-called Atkins, Zone, Ornish, and LEARN (Lifestyle, Exercise, Attitudes, Relationships, and Nutrition) diets. The latter three were quite similar in weight loss (very little at 1–2 kg in a year or less than 1 BMI point) while the effect of the low carbohydrate, high-protein, and high-fat diet (Atkins) did better at 4.7 kg (still less than 2 BMI points). This can be compared with the study of Foster et al. (2003) who compared the Atkins to a more "conventional" low-fat diet in obese individuals. As later confirmed by the Gardner study, the Atkins diet resulted in greater weight loss by 3 and 6 months. However, if one looks at the weight curves, then the trajectory of weight change is upwards for the low-fat diet such that the differences between diets are no longer significant at 12 months. Indeed, the pattern is very similar to the Gardner study (compare Figure 2 in Gardner with Figure 1 in Foster) but in this case statistical significance is still there at 12 months. One wonders if the significance would still be present at 2 years?

Of course, dietary interventions are aimed at more than just weight loss. Glycemic control, dyslipidemias, and inflammation are also therapeutic targets. These will be covered more in the sections under dietary fat subtypes. However, there is one particular issue of importance in the discussions about Atkins style diets. As noted, chronic low-grade inflammation is now seen as a critical component of the metabolic syndrome. Recent work (Rankin and Turpyn, 2007) has shown a potential danger of the high-fat approach. While weight loss was again somewhat larger with the high- vs low-fat diet, and blood glucose was similarly improved, markers of inflammation increased on the high-fat diet. This was indexed, for example, by a 20% increase in C-reactive protein (CRP) compared to a reduction of 43% in the low fat. These results provide a cautionary note about ensuring that the full metabolic picture is viewed with any dietary intervention.

What is clear in almost all "diet" studies is that weight regain is pervasive after a few months of weight loss. This is despite continued reporting of caloric deficit compared to baseline. A commentary on the Gardner paper by Heymsfield and Blackburn (2007) points out the difficulty this creates for the laws of thermodynamics (or, more properly, the other way around) as noted many years ago by Grande (1968). The only way this could occur would be for metabolic rate to drop substantially in the dieting individuals when they have reached their nadir of weight. Certainly this is possible

as early work from Leibel et al. (1995) showed quite substantial drops in energy expenditure on a per kilo of lean mass (i.e., the metabolically active component of body weight) basis with 10%–20% weight loss maintained. Equally, an outstanding study of a 9 month diet and exercise weight loss intervention in adolescents from Lazzer et al. (2004) showed a quite substantial (~10%) decrease in all types of energy expenditure (resting, sleeping, exercise, etc.) beyond that which would have been predicted on the basis of lean tissue loss which, in this case, was minimized by a focus on greatly increased physical activity.

Two-year follow-up data are available from a 1 year intervention study, also in adolescents (Rolland-Cachera et al., 2004), and similar to the Lazzer work. In the initial phase over 30 kg were lost but the same, odious pattern of weight regain was seen as in most work on adults despite reported intake levels substantially below baseline. Is this truth or misreporting? Certainly the reduction in energy expenditure beyond that expected by lean tissue loss would seem to be correct from both the Leibel and Lazzer data. Whether or not misreporting is also a substantial component explaining the effect is unknown but very likely. Recent data have shown that no matter the macronutrient mix in a weight loss diet, compliance is initially quite good and then deteriorates (see in particular Dansinger, 2005). The point with most diets is that there is some success, particularly over 3–6 months, but in the few diet studies that go out beyond 1 year, regain is universal. This is a critical issue in the pursuit of antiobesity therapy. It seems clear that regardless of dietary strategy, calorie intake is the main determinant of success.

21.2.1 Dietary Fat Subtypes

Lipids play multiple roles in metabolism. First, they are a source of energy-dense calories and, given the body's very limited ability to modulate carbohydrate and protein stores, fat balance over time effectively determines development of, or resistance to, obesity. However, the roles of lipids as major structural components of membranes and as potent metabolic intermediates in cellular signaling are equally important in expression of the metabolic syndrome disease cluster.

21.2.1.1 *n* − 6/*n* − 3 Ratio

The ratio of *n* − 3/*n* − 6 polyunsaturated fatty acids (PUFAs) is an important composite variable that has received a good deal of attention. Unfortunately, there are few good long-term studies of the effect of increasing *n* − 3 intake on either insulin action or obesity, and the data from those that exist generally show no effect (see, for example, Vessby et al., 2001). As noted above, the issue that always occurs with altering fatty acid composition is the length of the intervention, and we may not have yet had studies of appropriate duration. In addition, it is still not known if the beneficial effects are due to the *n* − 6/*n* − 3 ratio or to the absolute amounts of either, but it seems to be dependent on the pathway(s) of interest.

One aspect of the metabolic syndrome that is receiving increased attention is that of chronic, low-grade inflammation. Here the story around the *n* − 6/*n* − 3 ratio is much clearer. We highly recommend a very nice review by Robinson et al. (2007),

which comprehensively analyses the available literature in the area of $n-3$ fatty acids and inflammation as well as touching on the obesity issue. One can summarize by saying that there is clear evidence for the anti-inflammatory effects of $n-3$ fatty acids and certainly the interrelationship between $n-3$ and $n-6$ polyunsaturated fatty acid (PUFA) metabolism would suggest an increased $n-6/n-3$ would be deleterious. However, again the authors must conclude that the critical studies are lacking. Fortunately, since their review one area has started to be explored. They comment that "… the relationship between low-grade inflammation and acute postprandial response remains largely unknown." The recent work by Erridge et al. (2007) has shown that, even in healthy men, a high saturated fat meal generated an endotoxemia measurable by increased plasma lipopolysaccharides (LPS). How this postprandial inflammation is modulated, by changing the fatty acid profile is currently unknown, but seems to be a fruitful line of investigation to pursue.

21.2.1.2 *Trans* Fatty Acids

The role of *trans* fatty acids in cardiovascular disease is now quite clear from epidemiological studies and has been recently reviewed by Willett and colleagues (Mozaffarian et al., 2006; Mozaffarian and Willett, 2007). Equally the startling data in Figure 21.1 suggests *trans* fatty acids to be a potent driver of diabetes. To summarize the conclusions of the review articles, there is evidence that, in humans, intake of trans fatty acids results in dyslipidemia (increased triglyceride and LDL cholesterol), inflammation (circulating CRP, IL-6, and TNFa activity), and insulin resistance (at least in overweight, if not healthy, individuals) leading to increased cardiovascular disease. Of course, as noted above in nonhuman primates, we can

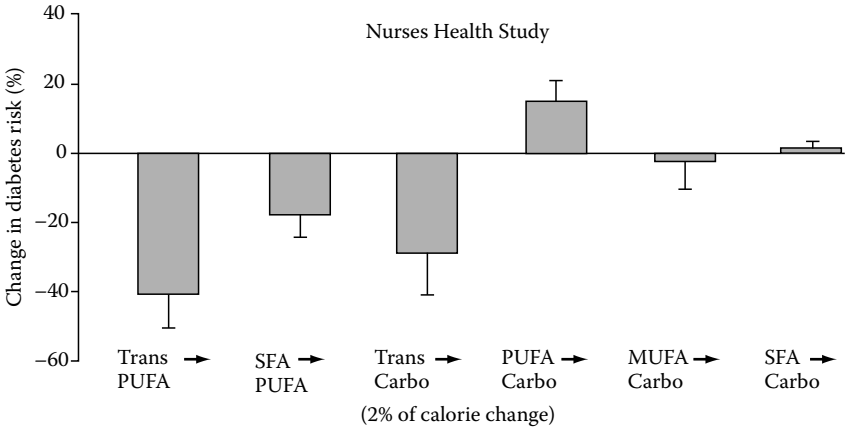

FIGURE 21.1 Data showing the change in diabetes risk which would theoretically be achieved by only a 2% substitution of one type of fatty acid with another, or by carbohydrate. These are quite remarkable changes in major disease risk for such a small dietary change. (Redrawn from Salmerón et al., *Am. J. Clin. Nutr.*, 73, 1019, 2001.)

add increased visceral adiposity. The good thing with *trans* fatty acids is that the level of their intake over time can be indexed by membrane *trans* fatty acid levels and correlations with the above markers of dysmetabolism adds good strength to the other cross-sectional data. Pleasingly, this weight of evidence has led to successful public health campaigns to bring legislation to severely limiting *trans* fatty acids in the food supply. It is a shocking result that a 2004–2005 survey in European countries showed that fried chips from a certain fast food chain had 28 times the amount of *trans* fats in Hungary than in Denmark where the legislation has led the world.

21.2.1.3 Conjugated Linoleic Acids

Conjugated linoleic acids (CLAs) are another subgroup of dietary fats largely, but not solely, of ruminant metabolism origin. They have a controversial history in terms of human health, which has been very nicely reviewed by Tricon and Yaqoob (2006) and that relatively brief review is recommended. CLAs were touted as a body fat loss supplement but the vast majority of studies in humans have shown no effect. As noted in that review, very careful studies by Vessby's group showed reduced insulin sensitivity as well as an increase in markers of inflammation (Smedman et al., 2005), albeit in both cases with differences between isomers. The opposing effects of isomers on cholesterol metabolism is illustrated in the Tricon and Yaqoob review from their own work (Figure 1 from Tricon and Yaqoob, 2006). Overall, the conclusion would seem to be that there are inconsistent effects on metabolic parameters and that specific isomers may have different, and opposing, effects. This is a confusing area in which the power input has created considerable heat but less illumination.

In 1996 some of us wrote a review on dietary fat subtypes and insulin action (Storlien et al., 1996). At that time there was a stunning lack of data, from both animal and human studies, on the effects of fats and fat subtypes on this key variable related to development of the prevalent diseases of the metabolic syndrome. However, since then, there has been an explosion of interest and there are a number of excellent recent reviews, covering both the experimental animal and human literature. Rather than reiterate the content of these we will reference them in the context of their particular focus; summarize the main messages; and then concentrate on potential underlying mechanisms.

21.2.2 Obesity, Dyslipidemia, and Insulin Resistance—Animal Studies

Experimental animals are particularly useful in this area because precise control of both amount and fatty acid profile of the diet can be achieved. The picture from many studies in rats and mice is fairly consistent and has been comprehensively reviewed last year by Buettner et al. (2007). This is really an excellent, succinct review nicely covering the obesity angle and effects in the key tissues. We highly recommend it, and their comprehensive investigation report from a year earlier (Buettner et al., 2006), and will not try to reprise it here. Overall, $n-3$ fatty acids are generally good. This has been shown repeatedly in a range of studies now going back almost 20 years and applies to countering the obesogenic, dyslipidemic, and insulin resistance provoking qualities of other types of dietary fat. Saturated fat, in comparison, is

deleterious, but not hugely more than either monounsaturated fat or polyunsaturated fat of the $n-6$ class, all of which impinge negatively on insulin action, at least in the context of diets with a high proportion of calories as fat. It is striking that $n-3$ fats seem to be even protective against high-fat diet induced weight gain (see Pan and Storlien, 1993 and Figure 1 from the Buettner 2007 review). $n-3$ fats also tend to reduce blood triglyceride levels. Olive oil is generally seen to be beneficial, particularly in the context of a Mediterranean diet. However, most rodent studies using olive oil as the source of oleic monounsaturated fat show both excess weight gain and insulin resistance, albeit not as severe as with primarily saturated fat diets (Storlien et al., 1991). Most work in rodents show that diets high in dietary $n-6$ fatty acids induce both obesity and insulin resistance despite their being very unsaturated.

One further area of dietary lipid subtypes not well covered by rodent studies is that of *trans* fatty acids. However, here we have an excellent study in primates (Kavanagh et al., 2007). The authors fed *cis* or *trans* fatty acids representing 8% of the total calories ingested over a 6 year period as part of a diet intended to maintain stable weight levels. The results were clear and disturbing. *Trans* fatty acid-fed monkeys gained weight and deposited more central fat even without increased food consumption. They also displayed impaired glucose tolerance as predicted during severe insulin resistance as well as impaired insulin signal transduction at the cellular level. Altogether, the results demonstrated a very negative profile.

Given the excellent reviews of Buettner and colleagues covering this aspect in rodents, we will focus on human studies drawing parallels with rodent work in the context of cellular mechanisms. However, we will also focus on the effects of dietary lipid subtypes on neural circuits controlling energy balance as this aspect has not been comprehensively reviewed yet.

21.2.3 Fats and Effects on Brain Mechanisms of Energy Balance—Fat Subtypes and Satiety Hormones

The classic monoamine (norepinephrine, serotonin, and dopamine) systems of energy balance were described many years ago although their roles are constantly being refined. Since the discovery of leptin over 10 years ago, there has been an explosion of knowledge about novel neuropeptides acting in concert to regulate energy balance. By quick oversimplification, one can note some key elements of two opposing systems, one orexigenic or appetite stimulating and one anorexigenic or appetite suppressing. Of particular importance is the fact that neuropeptide Y (NPY) and agouti-related protein (AgRP) are orexigenic, such that increased activity of either system will augment food intake and support obesity. On the other hand, melanocortin (α-MSH) an anorexigenic nonopioid peptide encoded by the pro-opiomelanocortin (POMC) gene, is distributed throughout the hypothalamus and acts via the melanocortin receptor 4 (MC4-R) and possibly melanocortin receptor 3 (MC3-R) to inhibit food intake. These two systems interact through AgRP a potent and selective antagonist of MC3-R and MC4-R. Since NPY-producing neurons in the arcuate nucleus of the hypothalamus (Arc) project to the paraventricular nucleus where they release NPY thereby stimulating feeding, the co-release of AgRP may act as a modulator of the balance between NPY and α-MSH.

There is comparatively little research on the effects of dietary fat subtypes on the neural systems controlling energy balance. However, in the following section we briefly outline some of this work, concentrating on NPY, AgRP, leptin, and serotonin.

21.2.3.1 Fats and Neuropeptide Y

As noted above, diets particularly high in saturated fat induce obesity in mice, whereas diets emphasizing PUFAs do not. The reduction in Arc NPY mRNA with obesogenic high-fat feeding has been reported from a number of laboratories including our own (Giraudo et al., 1994; Guan et al., 1998; Stricker-Krongrad et al., 1998; Wang et al., 1998; Piggott et al., 2002). Arc NPY protein levels follow mRNA levels down with longer feeding periods. As well, NPY protein in the paraventricular hypothalamic nucleus falls with long-term feeding and development of obesity (Stricker-Krongrad et al., 1998). The argument has then been made around the carbohydrate/fat ratio as a controller of hypothalamic NPY. Compared to high $n-3$ fat diet, a high-fat diet with a higher proportion of saturated fat perturbs the NPY (and AgRP) system. This suggests that it is not the level of dietary fat per se which influences NPY expression, but that it is some obesogenic property or properties of saturated fats, which are detected either peripherally or centrally. The NPY/AGRP systems then could be seen to be reacting in a homeostatic manner. In the case of the PUFA diets, there is no evidence for increased body fatness and the lack of change in Arc NPY mRNA is appropriate. The issue is clearly not dietary fat vs carbohydrate.

The NPY and AgRP systems (remember they are orexigenic) are reacting to some signal indexing positive energy balance (driven by the high saturated fat diet) and are reacting appropriately. What is equally apparent is that the reduced Arc NPY and AgRP are insufficient to maintain energy homeostasis. High-fat diets emphasizing PUFAs do not act to impair energy balance and the Arc NPY or AgRP systems are, appropriately, not responding. This strongly argues for a dysregulation, not in the NPY/AGRP system, but in other parts of the energy balance organization induced specifically by saturated fat or at least by the fatty acid profile of that diet.

As a potent regulator of the NPY system, leptin is one such candidate. However, we showed that at week 1 of a high saturated fat diet, Arc NPY mRNA levels were reduced by half, but no increase in circulating leptin and no change in leptin receptor mRNA expression could be detected. That leptin is not responsible for the decline in NPY with fat feeding supports earlier suggestions (Stricker-Krongrad et al., 1998). Other regulatory mechanisms must therefore exist to modulate Arc NPY and AgRP mRNA expression in the mice fed a high saturated fat diet.

A good deal can be learned about the control of energy balance by attempting to ameliorate the obesity of prolonged high saturated fat diets. This dietary "reversal" stage can be achieved in mice by both change in dietary fat profile (diet emphasizing $n-3$ fatty acids) and alteration in fat/carbohydrate balance (i.e., low-fat diet). However, it is striking that the effects are very modest with the low-fat diet compared to a major effect to reverse obesity with change only in dietary fat profile (Wang et al., 2002). This is equally reflected in the leptin levels. In contrast, both dietary reversal interventions resulted in "normalization" (to the chronic low-fat control) of NPY and AgRP mRNA expression.

In summary, changing the fatty acid profile of the diet alone can profoundly alter the expression of major hypothalamic neuropeptides of energy balance. The effects would appear to relate to whether a particular dietary fatty acid profile is obesogenic or not, but dysregulation appears independent of leptin, and of the NPY/AgRP system, as mediators.

21.2.3.2 PUFAs and Serotonin Receptor

Serotonin (5-HT) is a monoamine of primary interest for energy balance because of its long history of investigation and many decades of marketed drugs like fenfluramine which is thought to act via the 5-HT receptors. The hypophagic effect of serotonin is believed to be mediated mostly via postsynaptic 5-HT_{2C} receptors. Knockout mice lacking 5-HT_{2C} receptors are hyperphagic and become obese.

Our work has shown that different fat types can increase or decrease serotonin receptor binding, depending on the area being examined in rat brain in chronic feeding conditions (du Bois et al., 2006). For example 5-HT_{2A} receptor binding was increased in the caudate putamen but reduced in the mamillary nucleus by long-term feeding of diets high in saturated fat or $n-6$ PUFA fatty acids, but not $n-3$ PUFA diets. 5-HT_{2C} receptor binding was similarly reduced in the mamillary nucleus of saturated fat group and $n-6$ PUFA, and here also in the $n-3$ PUFA group.

$n-6$ PUFAs may be the most influential on serotonin receptor binding as this diet has previously been shown to decrease density of rat opioid (Oktem and Apaydin, 1998) and adenosine A1 (Cunha et al., 2001) receptors in several brain regions. Since only major changes in membrane viscosity can modify adenosine 1A receptor density (Casado et al., 1992), the results from Cunha et al. (2001) would suggest that $n-6$ PUFA interact directly with adenosine receptors to alter density levels. Consistent with this, we previously found that $n-6$ PUFA was the most influential fatty acid on muscarinic receptor binding, which is likely due to direct interactions between arachidonic acid, acetylcholine, and muscarinic receptor downregulation (du Bois et al., 2005).

No effect on 5-HT_{2A} receptor density was observed following $n-3$ PUFA diet treatment in most areas of the brain, consistent with Chalon et al. (1998). However, the dentate gyrus, an area not examined by Chalon et al. (1998), was a specific area where 5-HT_{2A} receptor density was solely affected by the $n-3$ PUFA treatment. Conversely, an $n-3$ PUFA-deficient diet had a number of effects on monoaminergic receptors including an increase in 5-HT_{2A} receptor binding (Delion et al., 1994) and decrease in D2 receptor binding in the frontal cortex (Delion et al., 1996). Interestingly, suicide victims have been reported to show a similar increase in 5-HT_{2A} receptor density in the cortex as $n-3$ PUFA-deficient rats (Stanley and Mann, 1983). The links between depression and obesity are well established.

In summary, while changes in binding density as a result of high-fat diets can be attributable to numerous global factors, including altered membrane fluidity, gene expression of receptors, receptor affinity, or to dynamic interactions between essential fatty acids, our results support that they are direct receptor-mediated interactions as receptors were affected differentially in a regional-specific manner. This area of research may be particularly fruitful in the pursuit of antiobesity agents.

21.2.4 Obesity, Dyslipidemia, and Insulin Resistance—Human Studies

The diets of free range humans are notoriously hard to control or even to monitor. In addressing the issues of amount and type of fats in the diet and effects on obesity and insulin resistance, one relies on cross-sectional studies and the far fewer longer term (3 months or greater) diet intervention studies. In both, one has to make allowance for the high degree of uncertainty around compliance. Equally, when we are discussing insulin resistance and obesity, the whole-body pool of fatty acids is very high. The length of time needed to achieve a change in metabolic outcome may be several months or even years.

One marvelous human longitudinal study is the well-known Nurses Health Study. One graph of an analysis of this study from a publication some years ago provides a remarkable testament to the likelihood that even relatively small changes both in the fatty acid profile of the fat component, and the balance between fat and carbohydrate calories (which we will explore later) will have a major impact on diseases related to insulin secretion and action. These data, shown above, suggest that exchanging as little as 2% of, for example, *trans* fatty acids with PUFA (not even specifying which class of PUFA) can have the effect to reduce diabetes by 40% (Salmerón et al., 2001). One limitation of this study however, was that the analysis was based on a hypothetical model of shifting patterns of dietary intakes, not on an actual intervention. These results appear consistent with knowledge from both cellular and animal research models, but more translational research is needed at this level to confirm effects with actual food patterns.

There are very few human intervention studies of any duration which compare diets of similar percentage of fat calories but where the fatty acid profile has been manipulated. Cross-sectional work is informative but the measurement uncertainty noted above limits the useful to only those few with very large subject numbers. Two recent papers have reviewed this limited literature from the perspective of insulin sensitivity (Galgani et al., 2008; Riserus, 2008 and see Vessby, 2003). The reader is referred to these for the detail, but in summary, they all conclude that the evidence is tantalizing and support the deleterious effects of saturated fats while more unsaturated fats are better, consistent with the cross-sectional and longitudinal studies. All point out the paucity of data and the need for more studies of appropriate length, rigor, and power.

Data also comes from a particular instance of type-2 diabetes, that occurring during pregnancy (gestational diabetes mellitus, GDM). Occurrence of glucose intolerance and GDM in relation to diet has been studied in a Chinese urban population (Wang et al., 2000). Dietary fat as a percentage of total energy intake was lower in GDM women compared to those with normal pregnancies. This was consistent with earlier work (Major et al., 1998), but not with others (Moses et al., 1997). In addition, glucose intolerance and GDM were associated with low polyunsaturated/saturated (P/S) ratio. In other words, a higher intake of PUFAs appeared to protect against glucose intolerance and GDM. This conclusion was consistent with the Moses et al. work, and supported by Bo et al. (2001) who also found in an Italian cohort that saturated fat was associated with increased incidence of glucose intolerance and diabetes during pregnancy, and PUFA with a decreased incidence.

One final key point that is worth noting here comes from the initial KANWU Study publication (Vessby et al., 2001). When the authors analyzed the effect of dietary fat subtype in relation to total fat intake, a significant improvement in insulin sensitivity was seen in the monounsaturated diet group compared with the more saturated fat group in the half of subjects with the lowest total proportion of calories as fat (in this study those consuming <37% fat calories). This beneficial effect of monounsaturates was lost when the total percentages of fat calories was high, suggesting the differential effect was swamped by total fat overload. This is an important point to remember when evaluating this literature, and in guiding further studies.

In relation to obesity, the evidence for a major effect of changes in dietary fatty acid profile is very limited and inconsistent (Moussavi et al., 2008). However, there are some positive studies where increasing $n-3$s in the fat component of a diet did lead to increased fat oxidation (Couet et al., 1997) and increased weight loss (Kunesová et al., 2006) during a VLCD diet. As Moussavi et al. (2008) conclude "there is an urgent need for conducting more studies—particularly case-control studies and analyses of large data sets (cross-sectional or longitudinal)."

21.2.5 Mechanisms for Fatty Acid Effects at the Cellular Level

There are multiple ways in which membrane and membrane-derived lipids can influence cellular metabolism. These range from the simple mechanical to provision of intracellular second messenger and potent signaling molecules.

The fatty acid composition of structural membrane lipids is influenced both by genetic predisposition and by environment, particularly dietary fatty acid profile. Phospholipid is the major membrane structural lipid, and evidence linking obesity and insulin resistance to the fatty acid composition of phospholipids has existed for some time. Most of these studies have been carried out in skeletal muscle, the major tissue for insulin-stimulated glucose disposal (see, for example, Pan et al., 1995). This work shows that an increased proportion of saturated fatty acids in skeletal muscle phospholipid relate directly and positively to impaired insulin action and to various measures of regional and total increased adiposity. Conversely, PUFAs in phospholipid, and particularly the highly unsaturated $n-3$ PUFA class, convey protection against both insulin resistance and development of obesity and type-2 diabetes.

Two mechanisms have been proposed to underpin such observations, which have been proposed and which are in the "mechanical" class. First, increased saturation of membrane lipids will be associated with higher density of membrane lipid packing because of the lack of double bonds to provide "shape" to phospholipids. This should decrease membrane fluidity and leakiness to ions and protons through interactions between the lipids and proteins within the membrane. Since proton/ion leak and the necessary pumping to maintain ionic homeostasis contributes heavily to the basal metabolism of the cell, increased saturation of membranes will decrease metabolic rate (Hulbert et al., 2005). This, in turn, will predispose increased accumulation of body fat stores for any given level of nutrient ingestion (positive fat balance). Of course such positive fat balance will result in whole body fat accretion as well as accumulation in the liver and skeletal muscle, both critical for insulin action. There is

now a considerable body of convincing literature showing increased intramyocellular and hepatic lipid accumulation in close association with muscle insulin resistance is now large and convincing (van Herpen and Schrauwen-Hinderling, 2008). Conversely, there are data in support of the notions that more unsaturated fats will increase membrane proton/ion leakiness, increase metabolic rate, and impact favorably on fat balance (Hulbert et al., 2005). Interestingly, increased membrane unsaturation provides an environment conducive for the improved intrinsic activity of ion transporters thus providing the conditions, in concert, to allow maintenance of ion homeostasis.

A second mechanical process which would allow for membrane fatty acid composition to influence insulin action by a "mechanical" means is via the alteration of membrane proteins, both in terms of affinities of receptors and in translocation to membrane and intrinsic activity of membrane nutrient transporters. Such changes in affinity of the insulin receptor were demonstrated many years ago. Early in vitro studies showed impaired insulin binding with saturated fatty acids added to the medium (Grunfeld et al., 1981; Field et al., 1988). Conversely there is also some evidence that highly unsaturated $n-3$ fatty acids were beneficial (Sohal et al., 1992; Clandinin et al., 1993). It is interesting in this context that insulin sensitizers such as bezafibrate (Matsui et al., 1997) also unsaturate membrane lipids and this effect on the insulin receptor may be a part of their beneficial action.

While one can clearly see the direct implications of changes in insulin receptor binding, other data has shown that beta adrenergic receptor affinity is decreased with dietary treatment emphasizing saturated fat intake (Matsuo et al., 1995), an observation which is also consistent with decreased metabolic rate. That is also true in brain (Matsuo and Suzuki, 1997). Taken together with the results of diets of differing fatty acid profiles on brain neuropeptides/neurotransmitters of energy balance, one could hypothesize that these changes could come about via modulation of membrane receptor binding.

In addition to the more mechanical processes that relate membrane structural lipid composition to key metabolic derangements of the metabolic syndrome, there are a number of ways in which membrane lipid components can influence intracellular lipid and carbohydrate metabolism, either directly by contributing metabolic products which themselves are involved or by generating second messenger molecules which modulate activities of key enzymes. Following is a figure of some elements of intracellular lipid and carbohydrate metabolism to orient the reader during the following discussion of some of these mechanisms.

One prominent mechanism involves diacylglycerols (DAGs), which are lipid second messengers that are produced during signal transduction by hydrolysis of membrane phospholipids. Protein kinase Cs are a family of DAG responsive enzymes that are recruited to cellular membranes as a consequence of DAG production where they phosphorylate specific target proteins responsible for regulating many cellular processes. In the current context, PKC inhibition of glucose transporter translocation will impair insulin-stimulated glucose uptake by making unavailable the specific proteins responsible for its transport. From the perspective of a functional validation of this hypothesis, it has been shown that increased availability of DAG, specifically from palmitate, impairs insulin-stimulated glucose uptake in primary myocyte cultures. In terms of specificity of the fatty acids on the DAGs for PKC translocation,

the work of Montell et al. (2001) clearly shows the effect of palmitate on translocation of PKC (indexed by PKC activity) to the membrane where it can have its deleterious effects on glucose transport. An equal exposure of cells to the monounsautrated fatty acid, oleate, had no such effect.

A further mechanism involves the complex interaction of the membrane lipid class, sphingomyelins as a sink, and source, for ceramides. Ceramides, as can be seen diagrammatically in Figure 21.2, are deleterious for glucose transport and storage via, *inter alia*, inhibition of PKB/Akt activation. This has been shown to impair insulin-stimulated glucose uptake in primary myocyte cultures, and to inhibit insulin stimulation of glycogen synthase kinase-3, thus impairing cellular capacity for glycogen synthesis (Schmitz-Peiffer et al., 1999). Ceramides are on the synthetic pathway for sphingomyelin, and are also formed from its breakdown.

Sphingomyelin concentrations in adipose tissue and plasma have shown to be positively related to obesity and to insulin resistance (Zeghari et al., 2000). While there are excellent mechanistic links between membrane sphingomyelins, ceramides, and deleterious effects on glucose uptake and storage, there are also ceramide effects on cell apoptosis, and on lipid uptake as well, that should not be forgotten in the context of the metabolic syndrome. A review some time ago from Unger and Orci (2001) showed a pathway by which elevated ceramide levels might induce apoptosis. Apoptosis now appears to be the major reason for reduced β-cell mass, and hence impaired insulin secreting capacity—the final stage going from insulin resistance

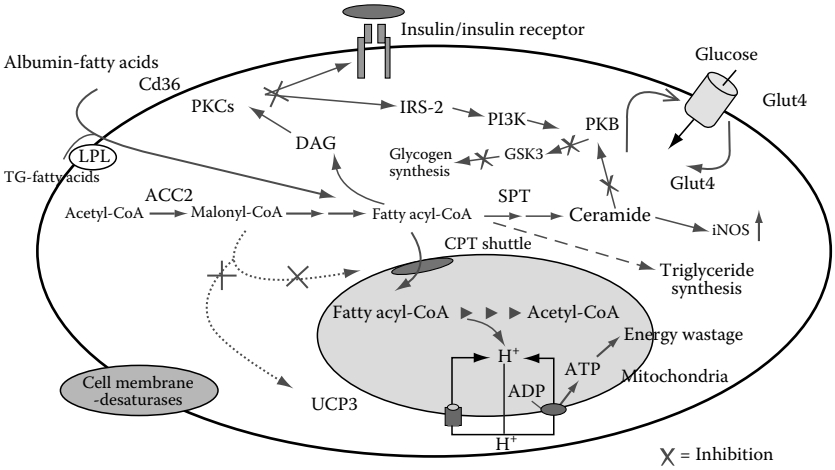

FIGURE 21.2 **(See color insert following page 166.)** Some intracellular pathways of fatty acid handling which impinge strongly on insulin-stimulated glucose handling. Please see the text for further detail. ACC2, acetyl CoA carboxylase; ADP, adenosine diphosphate; ATP, adenosine triphosphate; CPT, carnitine palmitoyl tranferase; DAG, diacylglycerol; Glut4, glucose transporter 4; GSK3, glycogen synthase kinase 3; iNOS, inducible nitric oxide synthase; IRS2, insulin receptor substrate 2; LPL, lipoprotein lipase; P13K, phosphoinositide 3 kinase; PKB, protein kinase B; PKC, protein kinase C; SPT, serine palmitoyl tranferase; UCP3, uncoupling protein 3.

and glucose intolerance to frank diabetes (Butler et al., 2003). Thus, the possible role of membrane sphingomyelin in pancreatic-cell destruction is another key element in the multiple ways in which membrane lipids influence expression of the metabolic syndrome.

Finally, on this point of ceramide metabolism, cytokines such as TNFα are known to have very deleterious effects on a range of metabolic syndrome parameters. It is interesting that they are potent activators of sphingomyelinase and thus play a role in generation of ceramides, yet another way in which lipid membrane components must be considered, and important in the context of our understanding of the importance of chronic low-grade inflammation in the metabolic syndrome.

The relationships between membrane lipid components and fatty acid patterns within them, to obesity and insulin resistance as key elements of the metabolic syndrome are well described. There are many ways to account for these relationships mechanistically. The examples chosen above are illustrative and not exhaustive. However, they should serve to illustrate the importance of understanding the factors, both genetic and lifestyle, which regulate membrane lipid composition in our efforts to combat the current major epidemic of obesity and type-2 diabetes.

21.2.6 Summary of Dietary Lipids

There is an increasing body of knowledge on the role of dietary fat in metabolic syndrome, justifying further research to test the efficacy of food interventions with idealized fat profiles, but also considering the total diet context, including possible synergistic effects from the presence of other macronutrients such as carbohydrate.

21.3 CARBOHYDRATES AND LIPID BALANCE

As mentioned previously, population studies and NHANES data show a reduction in the percentage of dietary calories from fat during the 1990s but an increase in the prevalence of obesity (Prentice and Jebb, 1995 and www.cdc.gov/nchs/nhanes.htm). This reduction in the percentage of the diet from fat calories was despite an overall increase in caloric consumption so the absolute amount of fat in the diet was relatively constant. Also, physical activity levels declined over the same period which may also be contributing to the increasing rate of obesity. Nonetheless, these data, in conjunction with prospective weight loss studies showing that low-fat diets are no more effective for weight loss than high-fat diets, sparked interest in other macronutrients, mainly carbohydrate, as significant factors in the development of obesity and metabolic dysregulation that leads to the metabolic syndrome particularly because by reducing fat in the diet there is a subsequent increase in dietary carbohydrate (CHO). The type of CHO may be important. In particular, carbohydrate quality rather than carbohydrate quantity in the diet has been the focus of intense investigation.

21.3.1 Carbohydrate Classification

Carbohydrates are the most important food energy provider among the macronutrients and accounts for between 40% and 80% of the total energy intake. The FAO/WHO

report in 1998 (FAO/WHO, 1998) recommended the consumption of at least 55% of total energy from a variety of carbohydrate sources. Carbohydrates may be classified according to their degree of polymerization and may be divided initially into three principal groups: sugars, oligosaccharides, and polysaccharides (see Table 21.1).

Each of these groups can be subdivided by the monosaccharide composition of the individual carbohydrates. Sugars comprise monosaccharides, disaccharides, and polyols; oligosaccharides include malto-oligosaccharides, other oligosaccharides, and fructo-oligosaccharides; the last group are polysaccharides which are divided into starch and nonstarch polysaccharides (Asp, 1996; Cummings et al., 1997; Englyst and Hudson, 1996).

The effects of dietary carbohydrates have been studied for many years with this broad range of compounds showing both beneficial and detrimental physiological actions. This conundrum can be explained by the examination of carbohydrate subtypes. The effects of different carbohydrates are often directly related to (1) the type of carbohydrate, (2) their rate of digestibility and the products produced, (3) their physical function in the gastrointestinal tract, and (4) their ability to act as substrates for fermentation by the microflora present in the colon. The major source of carbohydrate in the diet is starch and materials derived from starch. Starch is a complex energy storage structure found in many of the higher plants species and is composed mainly of glucose. However, the digestibility of starch by humans is affected by a myriad of factors including the type of starch, how food is prepared, and the physiological status of the person consuming the starch (Birkett and Brown, 2007, Table 21.2).

In recent times, simple sugars such as glucose, which are rapidly digested and absorbed, have been implicated in the initiation and promotion of diseases and conditions associated with issues of global public health concern, such as diabetes and the "obesity epidemic." Total sugar intake (g/day) has been positively correlated

TABLE 21.1
Major Dietary Carbohydrates

Class (DP[a])	Subgroup	Components
Sugars (1–2)	Monosaccharides	Glucose, galactose, fructose
	Disaccharides	Sucrose, lactose, trehalose
	Polyols	Sorbitol, mannitol
Oligosaccharides (3–9)	Malto-oligosaccharides	Maltodextrins
	Other oligosaccharides	Raffinose, stachyose, fructo-oligosaccharides
Polysaccharides (>9)	Starch	Amylose, amylopectin, modified starches
	Nonstarch polysaccharides	Cellulose, hemicellulose, pectins, hydrocolloids, gums

Source: Adapted from Cummings, J.H. et al., *Eur. J. Clin. Nutr.*, 51, 417, 1997. With permission.

[a] DP, degree of polymerization.

TABLE 21.2
Food and Physiological Factors Affecting the Rate and Extent of Starch Digestion

Category	Detail
Food behavior	Type of starchy food eaten
	Amount of starchy food eaten
	Customs of food preparation and consumption
Nature of the starch eaten	Amylose content
	Granularity
	Conformation
Food processing	Loss of cellular and native plant structure
	Processing conditions
	Extent of gelatinization
	Particle size
	Other food components—antinutrients, viscous fiber, fat
Physiology	Health status
	Individual physiological differences
	Extent of chewing
	Gastric emptying
	Viscosity
	Gastrointestinal transit time
	Enzyme inhibition

Source: Birkett, A.M. and Brown, I.L., *Resistant Starch*, Henry, C.J.K. (ed.), Woodhead Publishing Limited, Cambridge, U.K., 2007, 174. With permission.

with BMI and total fat mass in overweight children, but negatively correlated with insulin sensitivity (Davis et al., 2007). Another simple sugar, fructose, has been linked to promotion of liver dysregulation, the accumulation of visceral adiposity, and the development of the metabolic syndrome relative to either glucose or a number of complex carbohydrates. This area has been well studied (Gaby, 2005; Kabir et al., 2005).

21.3.2 Absorption of Dietary Carbohydrate—Influence on Insulin Sensitivity

The absorption rate of glucose from starch or other sources may have important ramifications in terms of insulin sensitivity. Postprandial hyperglycemia and concomitant hyperinsulinemia have been implicated in the development of insulin resistance in both humans and rats. In humans, consumption of foods which cause a large rise in postprandial plasma glucose concentrations is associated with an increased concentration of free fatty acids in the plasma (Ritz et al., 1991; Vanamelsvoort and

Weststrate, 1992). This increase in plasma free fatty acid concentration causes a decrease in glucose oxidation (Ritz et al., 1991), which may impair insulin sensitivity (Yki-Jarvinen, 1990). Elevated plasma free fatty acid concentrations also act to increase the concentration of mitochondrial acetyl CoA (Belfiore and Iannello, 1998) which inhibits pyruvate dehydrogenase thereby decreasing glucose oxidation. Postprandial hyperinsulinemia and hyperglycemia have also been shown to decrease glucose uptake through a decrease in GLUT4 mRNA and protein abundance (Cusin et al., 1990; Petersen et al., 1991).

In an attempt to define and predict the physiological effects of different carbohydrates, the glycemic index (GI) was developed. GI refers to the postprandial area under the glucose curve for a given food, expressed as a percentage of that for glucose or white bread which are defined as 100%. The GI is a measure for predicting the relative rate of glucose absorption from a food and, therefore, the rate of glucose appearance in the bloodstream. It should be noted however that although all CHO are glycemic, foods containing CHO have various levels of glycemic response depending on portion size and amount of CHO. The GI does not always reflect the true effect because it is based on a standard amount of CHO (50 g of glycemic CHO). Also, glycemic response is dependent on the type of CHO consumed. According to the AOAC method, glycemic carbohydrate (also referred to as readily available) is measured as total carbohydrate minus dietary fiber. Since this method does not include resistant starch (RS) when it is present, it will be mistakenly included as glycemic carbohydrate, leading to an overestimation of available CHO. Further to this, Robertson et al. (2003) showed that prior consumption of RS can modify the GI response to subsequent meals thereby limiting their accuracy. Therefore, when dealing with RS it is more appropriate to refer to excursions in glucose in terms of glycemic response (GR), instead of GI. In rats, a high GR diet has been demonstrated to increase GLUT4 expression in adipocytes and stimulate lipogenesis (Kabir et al., 1998a,b). In addition, high GR diets cause higher postprandial plasma triglyceride levels. These data are paralleled by human studies which show that consumption of high GR foods is associated with an increased concentration of free fatty acids in the plasma (Ritz et al., 1991). A high GR diet also acts to increase serum triglyceride concentrations not only postprandially but also in response to subsequent meals (Liljeberg and Bjorck, 2000). That is, triglyceride concentrations at lunch are significantly higher if a high GR breakfast is consumed relative to a low GR breakfast. Thus, slowly absorbed starches which have been shown to decrease peak glucose and insulin concentrations and total area under the curve for glucose and insulin, such as those from whole grains, may prove beneficial in the prevention of insulin resistance and associated disorders.

Resistant starch (RS) comprises starch, or materials derived from starch, that resist digestion in the small intestine and arrive in the large bowel (Asp et al., 1987). RS has been divided into four subcategories (numbered from 1 to 4) which reflect the mechanism by which the digestibility of the starch has been reduced (Brown et al., 1995). RS has demonstrated a variety of beneficial physiological effects that can be summarized from the observations made as a result of studies of one particular type, specifically the starch derived from high amylose maize or corn (a type of RS2) which is currently the most extensively researched. These effects are listed in Table 21.3.

TABLE 21.3
Potential Health Benefits of RS2 from High Amylose Corn

Reduced glycemic response	Lower postprandial glucose response
	Lower insulin response
	Improved insulin sensitivity
	Delayed onset of insulin resistance
Reduced energy	Fewer available calories
Improved intestinal health	Fermentable substrate (controlled)
	Cecal and fecal bulking
	Increased production of short-chain fatty acids
	Increased butyrate concentration
	Reduced intestinal pH
	Reduced levels of secondary bile acids
	Reduced levels of ammonia
	Reduced symptoms of diarrhea
	Increased mucosal barrier strength
	Improved immune response
Improved colonic tissue health	Increased apoptotic index
	Decreased DNA damage
	Decreased levels of cytotoxic compounds
Prebiotic	Selectively utilized by bifidobacteria
	Promotes growth of beneficial indigenous microbes (lactobacilli and bifidobacteria)
	Promotes probiotic growth and activity (bifidobacteria)
	Reduced pathogenic bacteria levels
	Increased butyrate production
Culture protagonist	Improves yield of probiotic cultures during growth
	Improves survival of probiotic cultures during processing, in foods, in vivo
Increased mineral absorption	Calcium
	Magnesium
Metabolic effects	Increased lipid metabolism
	Decreased lipogenesis
	Increased lipid oxidation
	Decreased body fat accumulation
	Decreased adipocyte volume
	Possible contribution to satiety
Tolerance	Tolerated at high levels

Source: Adapted from Birkett, A.M. and Brown, I.L., *Resistant Starch and Health*, Hamaker, B. (ed), Woodhead Publishing Limited, Cambridge, U.K., 2007, 63. With permission.

It has been shown in rats that the long-term consumption of a low RS diet causes insulin resistance whereas consumption of a high RS diet maintains insulin sensitivity (Higgins et al., 1996). In addition, the degree of insulin resistance caused by chronic ingestion of digestible starch was as severe as that caused by feeding a diet in which 60% of the carbohydrate was glucose (Higgins et al., 1996). Kabir et al. (1998a,b) have demonstrated that chronic RS feeding in rats causes a decrease in adipocyte size and an increase in maximal insulin-stimulated glucose oxidation. In healthy adults, Robertson and coworkers have demonstrated that dietary supplementation with RS in the form of high amylose maize starch can improve insulin sensitivity 24 h after a meal (Robertson et al., 2003) and also after 4 weeks of chronic consumption (Robertson et al., 2005).

Here we have demonstrated that there are several mechanisms whereby hyperglycemia and hyperinsulinemia can cause insulin resistance, a recognized feature of many of the diseases comprising the metabolic syndrome. These data, in conjunction with the fact that slowly absorbed or resistant carbohydrates, such as RS, have been associated with decreased BMI and improved insulin sensitivity, suggest that long-term ingestion of slowly absorbed or resistant carbohydrates may aid in the prevention and/or treatment of the metabolic syndrome. Conversely, rapidly absorbed carbohydrates seem detrimental to insulin sensitivity and have been associated with the development of metabolic syndrome.

21.3.3 Fermentation of Dietary Carbohydrates—Influence on Insulin Sensitivity

As mentioned previously, RS, as well as dietary fiber (including fructo-oligosaccharides and inulin), and some lente carbohydrates are resistant to digestion in the small intestine and therefore pass to the large bowel for fermentation. This fermentation in the large bowel gives rise to an increased concentration of short-chain fatty acids (SCFA), a majority of which are utilized by the intestinal mucosa but, systemically, can improve insulin sensitivity (Robertson et al., 2003, 2005). This may be because elevated SCFA concentrations cause a decrease in circulating free fatty acid concentrations (Anderson and Bridges, 1984; Scheppach et al., 1988; Wolever et al., 1989). In addition, RS and dietary lipid can form a strong association in which the absorption of dietary fat will be delayed until the RS molecule is at least partially degraded. Thus, any lipid associated with RS will be absorbed more slowly acting to decrease the postprandial circulating concentration of free fatty acids which are detrimental to insulin sensitivity (as discussed previously).

RS assists in the development and maintenance of a healthy large bowel through both physical effects and the beneficial stimulation of the colonic microflora, in particular by increasing the numbers of beneficial bifidobacteria and lactobacilli and leading to the decreased presence of pathogenic bacteria, and the chemical compounds that they produce (Bird et al., 2000; Brown, 2006; Birkett and Brown, 2007). The composition of the colonic microflora can have important impacts on health including providing protection against the initiation of certain types of cancer (Le Leu et al., 2003), increased absorption of micronutrients such as minerals

(Lopez et al., 2001) and improving energy recovery (FAO/WHO, 1998). It has been observed that there is a significant difference in the relative abundance of certain types of colonic bacteria in obese and lean humans (Turnbaugh et al., 2006). In particular obese mice were found to have increased numbers of Firmicutes relative to those of Bacteroidetes when compared to lean mice. How this change in the microbiota contributes to the manipulation of energy metabolism and lipid utilization in the body is unknown but it has now been shown that dietary fermentable carbohydrates, such as RS, can affect the expression of genes associated with peripheral lipid regulation (Keenan et al., 2007).

The colonic microflora can be influenced by the consumption of beneficial bacteria or probiotics. Many probiotics are available commercially, including lactobacilli and bifidobacteria, and are routinely incorporated in supplements and foods such as yoghurt. Many of the fermentable carbohydrates (FOS, inulin, RS resistant maltodextrin galactooligosaccharides, etc.) promote the growth of beneficial bacteria in the colon and are called "prebiotics." The consumption of a specific probiotic, a *Bifidobacteria lactis*, in combination with a particular RS (this is referred to as a synbiotic) has shown a protective effect by increasing apoptosis colon cancer model in rats (Le Leu et al., 2005). Using this approach, it may be may be possible to adjust an individual's microbiota to favor a lean and healthy body composition.

21.3.4 Carbohydrates and Lipid Metabolism

Among the complex carbohydrates, resistant starch (RS), lente carbohydrate and whole grains have demonstrated some ability to positively impact on lipid metabolism and reduce the incidence of conditions such as metabolic syndrome (Deroos et al., 1995). In rats, high RS intake causes a significant decrease in adipocyte cell size and total fat pad weight relative to low RS intake (Dedeckere et al., 1993; Lerer-Metzger et al., 1996; Kabir et al., 1998a,b). This change is associated with decreased expression of fatty acid synthase, the rate limiting step in fat synthesis, and GLUT4 (Yunes et al., 1995; Kabir et al., 1998a,b). It has also been demonstrated that the rate of de novo lipogenesis, as measured by incorporation of ^{14}C-glucose into adipocyte triglyceride, was higher for rats fed a low RS diet than those fed a high RS diet (Kabir et al., 1998a,b). In humans, there is also evidence for an increase in fat oxidation in response to RS intake (Higgins et al., 2004).

At a genetic level RS has been shown to upregulate genes involved in lipid oxidation, synthesis, and storage, as well as production of a variety of peptides, such as glucagon-like peptide 1 (GLP-1) and peptide tyrosine tyrosine (PYY), that are associated with increasing satiety, reducing appetite, and stimulating insulin release (Watford, 2002). This effect appears to be mediated via the colonic production of bacterially produced short-chain fatty acids. The data demonstrate that RS consumption significantly lowers plasma cholesterol and triglyceride concentrations (Dedeckere et al., 1993; Yunes et al., 1995; Fukushima et al., 2001; Lopez et al., 2001; Kishida et al., 2002). This effect is also evident in diabetic rats (Kim et al., 2003). However, data in humans is less clear, with no change in fasting or postprandial plasma triglycerides in response to RS ingestion (Jenkins et al., 1998) but a small decrease later in the day (Liljeberg and Bjorck, 2000). However, in a population-based study, high

whole grain intake was associated with lower total cholesterol, LDL cholesterol, and 2 h glucose concentration. This data needs to be verified by controlled clinical trials.

21.3.5 Summary of Dietary Carbohydrates

There are a number of mechanisms whereby dietary carbohydrate quality can influence weight gain, fat oxidation, and insulin sensitivity. These effects are generally mediated either by the rate of absorption or the degree of fermentation of carbohydrates. As a whole, these data show that the type of dietary carbohydrate can be a key factor in the development or prevention of insulin resistance, obesity, and the metabolic syndrome and should be carefully considered in terms of lifestyle recommendations aimed at both the prevention and treatment of this condition.

21.4 INTERACTION BETWEEN DIETARY FAT AND CARBOHYDRATE SUBTYPES

As summarized above, there is an extensive body of evidence supporting the beneficial effects of both fat and carbohydrate subtypes on various mechanisms of energy balance, metabolism, and disease. However, very little has been published on the combined effects that these macronutrients may have when given as an integrated diet. Unfortunately, this is an area that remains poorly investigated.

As mentioned earlier, RS promotes large bowel function through fecal bulking and the production of SCFA through bacterial fermentation, which increases insulin sensitivity. It has also been noted that $n-3$ PUFAs have an anti-inflammatory effect and can also increase insulin sensitivity through changes in plasma membrane fatty acid composition and fluidity. Therefore, it would stand to reason, as an example, that a diet combining these two macronutrients would have an additive effect toward improving bowel function and insulin sensitivity.

Regarding bowel function, one study by Patten et al. (2006) investigated the effects that RS and $n-3$ PUFA, separately and in combination, had on bowel function, specifically ileal tissue. They concluded that there were significant independent effects in SCFA production (most probably due to the RS), fatty acid composition, and ileal contractility, but there were few interactive effects. Other studies (Coleman et al., 2002; Conlon and Bird, 2003) have looked at the combined effects of these diets on markers for colon cancer and on genetic colon damage, with much the same outcomes. More investigation needs to be done on the mechanisms surrounding this lack of synergy between RS and $n-3$ PUFA.

In contrast to these negative findings, unpublished data by Brown et al. showed significant differences in body weight and in glucose excursions between diets of different starch (RS vs amylopectin, a more readily digestible starch) and lipid composition ($n-3$ PUFA vs more saturated fat). While the potent variable was RS, the combination RS and $n-3$ PUFA diet was superior to the individual effects, resulting in significantly lower body weights and postprandial glucose levels than three other combination diets. It must be mentioned however that most of the difference was due to the independent effect of RS. To explain this effect, one could postulate that RS and $n-3$ PUFA bind as they travel through the bowel. Lipid and RS can form

a strong bond in which the lipid is sequestered in the core of the RS molecule. In this case, it is likely that the absorption of dietary fat will be delayed until the RS molecule is at least partially degraded. Thus, from a positive perspective, any lipid bound with RS will be absorbed more slowly, acting to decrease the postprandial circulating concentration of free fatty acids which are detrimental to insulin sensitivity. This is of course, at present, pure speculation but the very limited data available point out a strong need for studies at the interface of fat and carbohydrate subtypes where significant health benefits might accrue.

21.5 CONCLUSION

Both fat and CHO subtypes influence outcomes in metabolic syndrome, but in reality people eat foods and whole diets, not just nutrients. Emerging mechanistic theories on how certain types of lipids and CHO interact may begin to provide a basis for a framework in which knowledge of this synergy might be built (Jacobs and Tapsell, 2007). In turn, food product development may draw in this knowledge to create novel foods with particular health benefits.

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22 Food Intake and Obesity: The Case of Fat

Jennifer T. Smilowitz, J. Bruce German, and Angela M. Zivkovic

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22.1 INTRODUCTION

The prevalence of overweight and obesity today is unprecedented and is steadily and globally rising (Balkau et al., 2007). Why are so many apparently healthy individuals consuming more calories than they need? Variations in the development and consequences of obesity have been proposed to depend on genetic predisposition combined with various environmental factors that lead to a chronically unbalanced energy intake relative to its expenditure. The variables that could contribute to fluctuations in body weight and composition have been proposed to include genetics (Corella and Ordovas, 2005; Warensjo et al., 2007; Benzinou et al., 2008; Masuo et al., 2008; Pichler et al., 2008); activity (Hill and Melanson, 1999), including nonexercise activity thermogenesis (Levine et al., 1999); diet composition (Labayen et al., 2003; Layman et al., 2003; Meckling et al., 2004; Cornier et al., 2005; Capel et al., 2008) and structure (Brand-Miller et al., 2002; McMillan-Price et al., 2006); and metabolic phenotype (Cornier et al., 2005). There is also evidence that today's "obesogenic environment," which includes easy, 24 h access to high-energy foods, large portion sizes, and a social environment that promotes a sedentary lifestyle, is contributing to obesity (Poston and Foreyt, 1999). Yet, not all individuals become obese in response to this obesogenic environment, indicating that there is a strong genetic component involved in the development of obesity (Wardle et al., 2008). Individuals are different, and the causes and solutions for their overweight condition are therefore necessarily also different.

Because of the inherent biological differences between people the solution to the obesity epidemic will not be easy. In fact, it is unlikely that there will be "one" solution. The failure of dietary modification approaches for obesity treatment and prevention has been illustrated clearly in weight loss studies time after time, leading to the frustrating conclusion that diet-based weight loss approaches are largely unsuccessful in the long-term. Even more frustrating is the fact that the failure of dietary approaches to weight loss is consistent even across different types of diets, from national guidelines to popular or fad diets. Researchers found that after 1 year of self-help vs. a commercial program (in this case weight watchers), study participants' weight change ranged from a loss of 28 kg to a "gain" of 12 kg on the commercial program, and a loss of 26 kg to a "gain" of 15 kg on the self-help program (Heshka et al., 2003). After 2 years, the ranges were from −23 to +21 kg, and −26 to +30 kg respectively. Over the 2 year period as many as 27% of participants dropped out of the study. Another recent study showed even lower adherence rates, with up to 42% of participants dropping out of their weight loss program by year 1 (Dansinger et al., 2005). Furthermore, regardless of diet type (Atkins, Ornish, weight watchers, and zone diets) as early as month 1 of the study, participants' adherence levels based

on 3-day food records and telephone interviews were at 50%, meaning that they adhered to the diet recommendations only about half the time. By the end of year 1, adherence levels were as low as 30% with an average weight loss of just 2–4 kg. Only about 25% of the 160 participants were able to sustain the recommended minimum 5% weight loss over the year-long period, which is associated with improved metabolic markers of disease risk. Even fewer participants, just over 10%, maintained the recommended 10% weight loss.

The evidence clearly indicates that a one-size-fits-all approach is inconsistent with a condition as refractory to dietary intervention as obesity. Even with the current array of available diets from low-carbohydrate to low-fat diets, from commercial prepared-meal approaches to Internet-based programs, the successful maintenance of weight loss has been an elusive target.

A logical conclusion in the face of high variability among individuals and in light of recent advancements of "omic" technology is to pursue the identification of genotype as a way to identify biochemical differences in response to dietary intervention. Identifying genes responsive to nutrients and their polymorphisms that relate to varying health outcomes is already being used for drug discovery. These same relationships may lead to the eventual use of dietary prescriptions to specific individuals. Yet genotype has proven to be difficult to assign directly to variations in phenotype. Genes do not change in response to diet and environment, but phenotype does. The span between genes and metabolites involves many biochemical steps, the sensitivities and specificities of which respond to diet and the environment. Metabolites are the functional outcomes of the interactions between genotype and environment. Hence, the clinical practice of assigning individual health status and risk will likely require direct measures of metabolism rather than genetic predispositions for errors.

Studies that simultaneously quantify the lipid metabolites—substrates and products of biochemical pathways—in tissues and biofluids have proven to be extremely valuable in revealing dysregulation in biochemical pathways associated with other metabolic diseases such as atherosclerosis. This chapter describes the use of comprehensive analysis of lipids associated with various biochemical pathways combined with specific dietary challenges to reveal the dynamic nature of an individual's metabolic phenotype (German et al., 2007). Circulating lipids are derived from both diet and endogenous metabolism. These lipids are highly dynamic, interactive biological molecules that make up most cellular components and signaling molecules, and they dictate energy partitioning and control of food intake.

Remarkably, although food intake is central to the problem of obesity, the vast majority of studies attempting to explain the variations in metabolism that could account for excess intake and for its metabolic consequences have examined individuals and their various physiological, metabolic, and endocrine characteristics in the fasted condition. Furthermore, studies to date have examined only a subset of the metabolites representing the various biochemical pathways that are both responsive to dietary intake and associated with energy metabolism. Both of these decisions—to largely avoid examining the fed state and to constrain metabolic interrogation to a small subset of metabolites—have severely limited the ability of studies of energy metabolism to clarify precisely how diet as a variable impacts weight regulation.

Clinically, lipids are measured in the fasted condition, yet this is the period when most indices of diet and its effects on lipid metabolism are minimal. In this chapter, the principles of metabolomics are extended into two directions—input variables as food metabolite composition and output variables as the subsequent effects on postprandial metabolism within individual humans. This approach is providing insights into the metabolic regulation associated with energy balance and obesity.

The practical application of a challenge approach that measures the fluxes through specific biochemical pathways is the ability to personalize dietary recommendations based on an individual's metabolic phenotype. We propose that this approach would have a profound impact on the long-term success of diet and lifestyle-based interventions. Not only would metabolically appropriate diet and lifestyle modification be more effective in producing measurable improvements in health, perhaps even more importantly, it would increase patient acceptance and long-term adherence. Currently, people are wary of dietary recommendations because they seem to be changing every day. One day it is "beneficial" to consume eggs, the next day it is "deleterious" to consume eggs. The truth is that for some individuals eggs are beneficial while for others the cons outweigh the pros and for them egg consumption is a net negative. If we measure with accuracy and specificity the metabolic responses of individuals to specific meals and food items, and provide clear evidence that specific dietary components are causing harm whereas others are beneficial, the acceptance of recommendations will be much higher. Instead of rigidly imposed levels of acceptable intake of foods and food components that are deleterious to the health of "the average person" individuals would be free to choose foods that are palatable and enjoyable to them in doses that are metabolically appropriate for them. The success of long-term dietary and lifestyle approaches that prevent obesity and produce weight loss will ultimately depend on the acceptability of those regimens to individuals living their normal lives.

22.2 FAT METABOLISM

22.2.1 Fat Digestion

The successful digestion of fat is one of the most impressive biological feats of animals. Humans, in particular, are genuinely remarkable in their ability to absorb energy dense but highly insoluble triglycerides (TG) from complex food matrices, to deliver these lipids to adipose tissue for storage, and thence to mobilize from storage and distribute them to peripheral tissues according to varying energy requirements. The processes of digestion, absorption, transport, and distribution are highly involved and utilize a wide spectrum of active molecules. The highly interactive nature of digestion begins almost immediately after commencing to eat. The digestive system itself is complex, with many structural, chemical, and enzymatic systems combining to successfully digest and absorb impressive quantities of lipids.

The importance of lipids to the signaling of metabolic regulation was generally ignored in early research largely because lipids were not considered to stimulate insulin, and it was thought that there were no taste receptors and sensory responses to lipids or their digestion products. However, the importance of fats to food intake

regulation from hunger to satiety and metabolic processes from fuel prioritization to energetic efficiency is being increasingly recognized (Feinle-Bisset et al., 2005).

It is well known that the sensory qualities of foods are critical to dietary preference and total food consumption. Taste, among other variables that influence food intake, is an important determinant of what and how much we eat. Until recently, fats were thought to influence taste perception only on the level of texture and consistency, since pure, fresh fatty acids (FA) are flavorless (Mizushige et al., 2007). However, new evidence suggests that a fat-sensing mechanism separate from the detection of viscosity exists in the mouth, and is responsible for "tasting" fat (Garcia-Bailo et al., 2008). The putative fat taste receptor is the integral membrane protein CD36, which by unknown sensory mechanisms detects free FA released by lingual lipase and involves the prolonged depolarization of potassium channels on taste receptor cells (Houdali et al., 2003; Steer et al., 2003; Fukuchi et al., 2004; Gilbertson et al., 2005; Laugerette et al., 2005; Garcia-Bailo et al., 2008). There is also evidence that β-endorphin, an opioid peptide, and dopamine, a neurotransmitter, are released in the brain following fat ingestion, suggesting that fat perception in the mouth may bypass traditional flavor mechanisms and instead stimulate directly the brain's reward system through signaling networks that have yet to be worked out (Mizushige et al., 2007).

The stomach plays a significant role in the overall digestive process by acting as a strong homogenizer as the fat exits through the muscular pyloric valve into the duodenum. This emulsifying action of the pylorus dramatically increases the surface area of the emulsified oil, affording much greater access to lipolytic enzymes. The emulsified oil exits the stomach into the small intestine, and with the arrival of the bile and pancreatic secretions, the process of fat digestion and absorption ensues.

Bile contains primarily bile acids, cholesterol, and phospholipids in an aqueous micellar mixture produced in the liver and accumulated in the gall bladder. Release of bile into the intestine is stimulated by the intake of food. Bile itself is regulated via a variety of factors that are now emerging as among the most important biochemical steps in human physiological regulation. Bile acids, derived from cholesterol, are now known to constitute one of the central signaling systems in the fasted–fed transition. Their abundance is sensed by multiple nuclear receptors in many tissue types and is controlled by a wide variety of genetic, physiologic, and dietary determinants (Thomas et al., 2008). Bile acid production, release, recirculation, and conjugation are stimulated in response to and regulated by the quantity and composition of dietary lipids (Costarelli and Sanders, 2001).

These various predigestive and digestive events then cascade into a wide range of physiologic, metabolic, and endocrine processes (Feinle et al., 2001) that can influence whole body metabolism. The diversity of metabolism within individuals can be considered their metabolic phenotype.

22.2.2 Metabolic Phenotype

Metabolic phenotype has been described in relation to the sum of an individual's genetic blueprint and environment and their interaction to manifest physical and biochemical characteristics (German et al., 2003). Metabolic phenotype is influenced

in part by developmental plasticity and imprinting early in life, and in part by the interactions of multiple influential factors over time (Figure 22.1). Both intrauterine signaling and early childhood influences determine the full expression of genotype, which in turn establishes the foundation of metabolic phenotype. For example, polymorphisms in FA desaturase genes involved in long-chain polyunsaturated fatty acid (PUFA) synthesis are associated with increased benefit from breastfeeding on IQ, illustrating the crucial effects of early imprinting (Caspi et al., 2007). The epigenetic control of gene expression by dietary and environmental factors *in utero* determines lifelong health trajectories through DNA methylation and chromatin remodeling (reviewed in Nafee et al., 2008).

Over time, the cumulative and immediate effects of lifestyle (diet, exercise, smoking, alcohol intake, micronutrient supplementation, etc.), health state, chronic as well as acute diet, extent of irreversible tissue damage, toxic exposure, mental, and emotional health, fitness level, and doubtless other factors all influence the current metabolic phenotype of an individual at any point in time. Major dietary modulations such as switching from a vegetarian diet to a low-meat diet or a high-meat diet (Stella et al., 2006) and metabolic status such as insulin sensitivity coupled to macronutrient composition (Cornier et al., 2005) produce measurable differences in metabolic phenotype. Even presumably minor dietary preferences such as the consumption of chocolate affect plasma metabolic profiles (Rezzi et al., 2007). Metabolic phenotypes differ and are measurable among cultural and ethnic groups as well as among

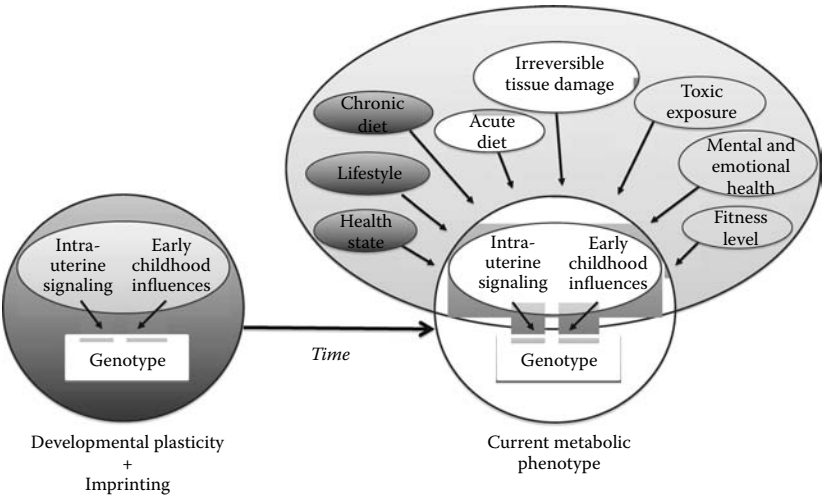

FIGURE 22.1 Determinants of metabolic phenotype. Metabolic phenotype is determined by a wide variety of factors including genotype, environment, developmental plasticity, and imprinting early in life. Both intrauterine signaling and early childhood influences determine the full expression of genotype, which in turn determines the foundation of metabolic phenotype. Over time, the cumulative and immediate effects of diet, lifestyle, health state, chronic as well as acute food intake, the extent of irreversible tissue damage, toxic exposure, mental and emotional health, fitness level, and other factors influence the phenotype of an individual at any point in time.

populations with widely different dietary patterns (Holmes et al., 2008). Gut microbiome differences can also be detected by metabolic phenotyping (Li et al., 2008).

22.2.2.1 Measurement of Metabolic Phenotype

An individual's current metabolic phenotype can be assessed and evaluated by a variety of complementary approaches, each of which is designed to reveal an important aspect of overall metabolic functioning at a given time (Figure 22.2). The evaluation of symptoms in a clinical setting has been widely used in a variety of diagnoses. Clinical assessments of biochemical functions include the measurement of blood glucose, insulin, lipids and other biomarkers, anthropometric measurements such as body mass index (BMI, kg/m^2) and waist to hip ratio, and physiological measurements such as blood pressure. Imaging technology provides the ability to visualize function and includes radiology, magnetic resonance imaging (MRI), computerized axial tomography scans, dual energy x-ray absorptiometry, and others. Functional assessment of metabolic phenotype employs *in vivo* techniques that reveal the functioning of a system in real time, which include real-time MRI, stress

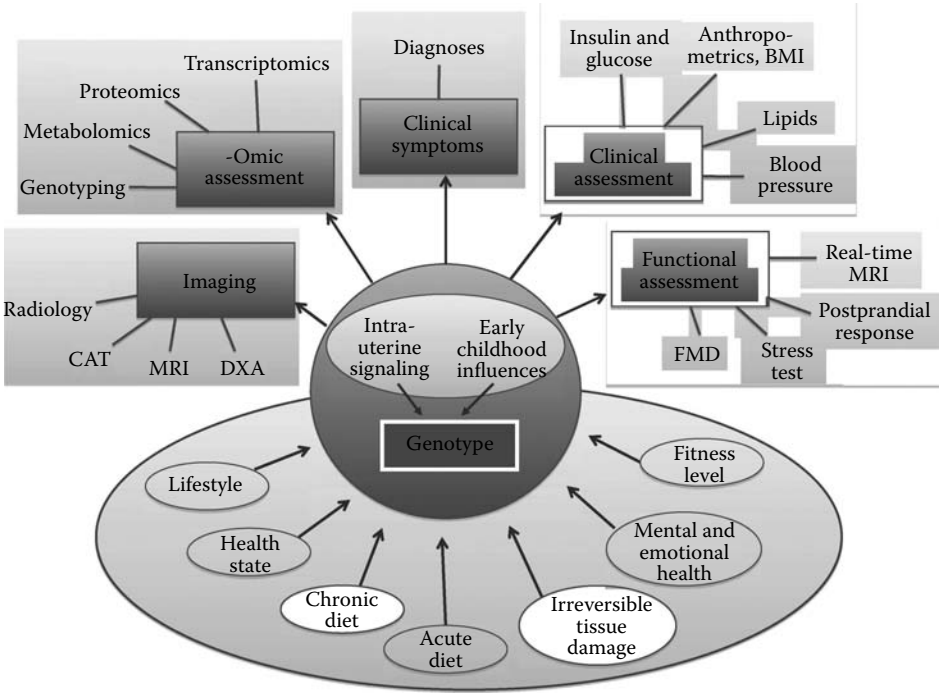

FIGURE 22.2 Measurement of metabolic phenotype. Current metabolic phenotype can be measured in a number of ways. Clinical evaluation includes a variety of scales, assessors, and diagnoses. Clinical measures of biochemical function include the measurement of blood glucose, insulin, lipids, pH, anthropometric measurements such as BMI, kg/m^2 and blood pressure. Imaging technology includes radiology, MRI, computerized axial tomography (CAT) scans, dual energy x-ray absorptiometry (DXA), and others. Functional assessments include real-time MRI, stress testing, FMD, and measurement of postprandial response, among others.

testing, flow-mediated vasodilation of the brachial artery (FMD), and measurement of postprandial response, among others.

22.2.2.2 Functional Assessment of Metabolic Phenotype

Real-time and *in vivo* measures of metabolic function are a logical next step for informative assessment methodologies to understand the dynamic nature of metabolism. There are theoretical advantages of assessing health as the progressive metabolism of nutrients by measuring their flux dynamics in the blood after a meal. But are they practical? This concept is being applied using "omic" technologies to measure various processes in response to pharmacologic stimuli over time. Plasma FA profiles are associated with disease risk such as the risk of developing insulin resistance (reviewed in Steer et al., 2003). We propose that the multifactorial nature of the long-term and short-term influence of diet on the metabolic phenotypes of different individuals is reflected in the relative and absolute composition of plasma lipids measured comprehensively as FA within separated lipid classes (Figure 22.3).

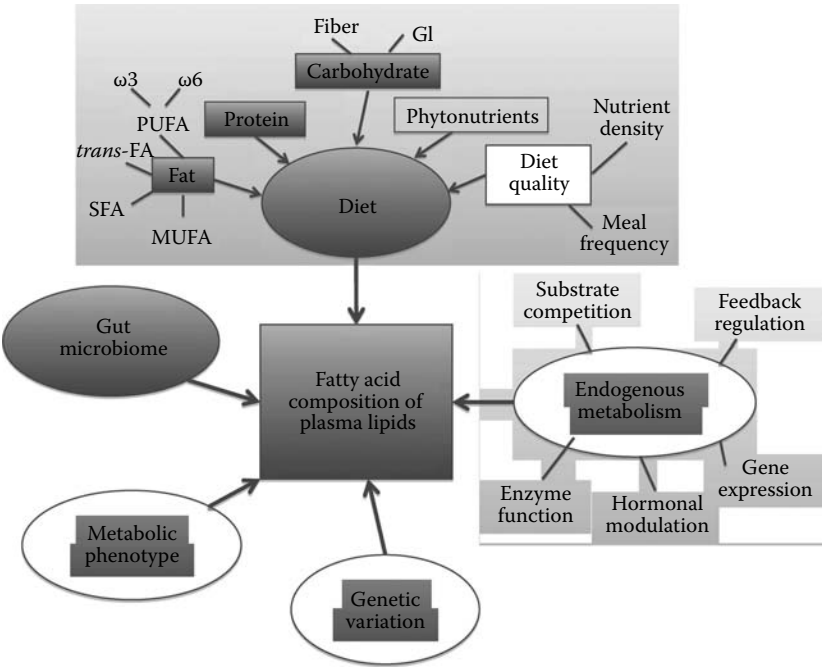

FIGURE 22.3 Determinants of plasma FA composition. The determinants of plasma FA composition include metabolic phenotype, genetic variation, endogenous metabolism, intestinal microflora or gut microbiome, and diet. Gene expression and enzyme function are influenced by both genotype and endogenous metabolic function including hormonal modulation, feedback regulation, and substrate competition. The relative intakes of protein, carbohydrate, and fat, as well as their specific composition (e.g., SFA, MUFA, *trans*-FA, PUFA, omega-3 (n3) fatty acids, omega-6 (n6) fatty acids, glycemic index (GI), fiber, diet quality (e.g., nutrient density, meal frequency) and phytonutrients all contribute to the effect of diet on plasma lipid composition.

22.2.3 Determinants of Plasma Lipid Composition

As key regulators in integrative lipid anabolic and catabolic pathways, FA composition of circulating lipid metabolites are influenced by diet (Houdali et al., 2003; Fukuchi et al., 2004; Ntambi and Miyazaki, 2004), genetics (Schaeffer et al., 2006; Baylin et al., 2007; Warensjo et al., 2007), pharmacologic (Kim et al., 2000), hormonal (Ghebremeskel et al., 2004; Thomas et al., 2005; Bitsanis et al., 2006), metabolic status (Sjogren et al., 2008), and environmental triggers (Kis et al., 1998). The determinants of plasma FA composition include metabolic phenotype (Figure 22.1), genetic variation, endogenous metabolism, intestinal microflora or gut microbiome and, of course, diet (Figure 22.3). Gene expression and enzyme function are influenced by both genotype and endogenous metabolic function, including hormonal modulation, feedback regulation, and substrate competition. The influence of chronic and short-term diet includes the effects of macronutrients and micronutrients as well as diet quality. The relative intakes, composition, and structure of protein, carbohydrate, and fat, are important. Saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), *trans*-fatty acids (*trans*-FA), and PUFA all modulate plasma lipids, as does the relative intake of ω3 and ω6 FA. The digestibility of carbohydrates, which influences glycemic index, the absolute intakes of fiber—both soluble and insoluble—and other characteristics of starches such as viscosity all have important effects on metabolism and lipid status. The intake of essential micronutrients, the nutrient density of food, and other aspects of diet quality such as meal frequency also play important roles in diet-mediated modulation of plasma FA composition.

22.2.4 Postprandial Response to Challenge

The importance of postprandial lipid metabolism was first reported in 1979 (Zilversmit, 1979), when the hypothesis was proposed that plasma chylomicrons, their remnants, and very low-density lipoprotein (VLDL) remnants, together termed TG-rich lipoproteins (TGRL), are mediators of atherogenesis. The term postprandial lipemia was coined and studies examining the impact of high-fat meals on plasma lipoproteins and TG excursions ensued. Around the same time, the effects of FA composition on the endothelial function of arteries was under investigation (Nordoy, 1979). Today the postprandial literature continues to discuss primarily the effects of varying the relative fat content and FA composition of meals on postprandial lipemia and endothelial function (de Koning and Rabelink, 2002). Postprandial lipemia is associated with an increased risk for myocardial infarction (Stampfer et al., 1996) and is increased in patients with cardiovascular disease (Karpe, 1997).

Research on postprandial response has expanded to include a variety of approaches for using challenge meals to investigate acute metabolic and physiological responsiveness to meals. These include the use of oral fat tolerance tests (OFTT), oral glucose tolerance tests, and tests of response to different types of meals (Rudolph et al., 2007), food components such as polyphenols (Papamichael et al., 2008), diet quality such as the use of oils that had been previously used for deep frying (Williams et al., 2001), medications (Boquist et al., 1998; Wilmink et al., 2001; Boquist et al., 2002;

Ceriello et al., 2002), lifestyle factors such as exercise (Silvestre et al., 2008; Weiss et al., 2008), and meal frequency (Murphy et al., 1996). Postprandial responsiveness has been used both in a long-term sense to investigate the effects of modulating chronic diet and lifestyle (Fuentes et al., 2008) and in an acute sense to investigate the immediate effects of varying meal composition on postprandial metabolism (Chong et al., 2007).

22.2.5 Postprandial Assessment

In addition to the effects of varying diet, lifestyle, and meal composition on postprandial lipid metabolism and endothelial function, the challenge approach has been used to investigate physiological effects on satiety and gastric emptying (Hlebowicz et al., 2007), appetite and energy intake (Bowen et al., 2007), substrate oxidation (Roberts et al., 2008; Stiegler et al., 2008), glycemic response (Tuomasjukka et al., 2007; Leeman et al., 2008), insulin response (Milton et al., 2007; Poppitt et al., 2007; Maki et al., 2008), energy expenditure (Smeets et al., 2008), enzyme activity (Chong et al., 2008), nitrogen utilization (Juillet et al., 2008), colonic fermentation (Nilsson et al., 2008), and absorption of lipid peroxidation products (Gorelik et al., 2008) as well as carbohydrates with varying digestibility characteristics (Nilsson et al., 2008).

22.2.5.1 Oral Fat Tolerance Tests

The concept of a nutritional challenge to model metabolism is not new. The oral glucose tolerance test, used since the 1950s (Mosenthal and Barry, 1950), is a standardized test involving the administration of 75 g of glucose in a liquid solution, followed by the measurement of blood glucose and insulin over 120 min. Yet, the OFTT is not standardized as to amount, composition, and structure of the fat used, or even the name used for the challenge. Some studies administer 80 g fat (Murphy et al., 1996; Cortes et al., 2006), others 50 g fat (Gudmundsson et al., 2000; Jackson et al., 2005), still others administer fat as a percentage of energy relative to body size (Shimabukuro et al., 2007). The term "high-fat meal" is used throughout the literature, yet the composition of the high-fat meal varies widely from study to study and spans the spectrum from dairy fat high in SFA to extra-virgin olive oil enriched in MUFA, to safflower oil enriched in PUFA. The OFTT is also administered under various names, including "oral fat load," "oral fat-loading test," "high-fat meal," and "high-fat mixed test meal."

This lack of standardization in the fat challenge model has made it impossible to compare across studies to conclude consistently any biological discoveries generated from this method. There is an obvious need to standardize the OFTT so that the acute metabolic responsiveness of different groups of individuals on different treatments can be compared. Only by standardizing the challenge can the variable in question be studied without potential confounding effects from the variation in the challenge itself. With such standardized experimental protocols in place, it is possible to take advantage of the substantial knowledge of lipid biochemistry and the analytical tools of metabolomics to study the variation in metabolic responses to a dietary lipid challenge in individual humans.

22.2.6 Challenge Design

The dietary challenge approach, coupled to lipid metabolomics, is an experimental instrument for measuring the dynamic and interactive changes of lipid pathways in the postprandial state. In the near term, designing dietary challenges will depend on the targets of biochemical pathways and the responsiveness of physiological outcomes in question such as blood pressure and insulin sensitivity. The complexity of lipid pathways is mediated by diet in several ways: (1) as substrates and products of these pathways, (2) as modulators of enzymatic activities, (3) as stimulators of hormonal regulation of enzymatic activities, and (4) as effectors of gene expression regulation. Developing a standardized challenge to scrutinize lipid metabolism combines lipid biochemistry and effects from food, points of regulation at various transcriptional and posttranslational levels, cellular lipids and abundance, and interactions between organ systems and the plasma compartment.

22.2.6.1 Fasted vs. Fed

Using a response-to-challenge model adds several dimensions to existing methods for revealing lipid metabolism. The fasted condition is the metabolic state when the influence of diet is least detectable. In the fasted condition of a healthy individual, insulin's counterregulatory hormones—glucagon and epinephrine—peak while circulating insulin concentrations are low. As a result, free fatty acids (FFA) are liberated from adipose tissue and are oxidized by extra-hepatic tissues: the liver and kidneys are gluconeogenic and hepatic glycogenolysis is active. During this state, energy partitioning shifts to the catabolic, oxidative state. Conversely, in the fed state, insulin peaks, glucagon and epinephrine are suppressed, and energy partitioning shifts to the anabolic state. Circulating lipids change in response to both of these states, yet the fasted condition has received the most attention. To achieve a complete picture of lipid metabolism, both states need to be rigorously studied and compared. Until now the fasted state has been the focus of investigation because the fed state was thought to be a confounding factor for understanding metabolism. However, if an individual is fasted overnight and then subjected to a well-defined meal, their metabolic responses to that specific meal can be quantified. Rather than being a confounding factor, the fed state becomes a window into the functioning of metabolism following a metabolic challenge—the meal.

There are several advantages to measuring lipids in the fed condition to discriminate individual variation as metabolic phenotypes. A diet challenge augments not only substrates, products, and intermediates of existing pathways, but also metabolic, hormonal, intestinal, and stress signals, all of which are points of potential individual discrimination. When designed appropriately, the challenge can be a tool to perturb specific pathways of interest. A challenge in which all lipid pathways are interrogated and measured simultaneously would be ideal, but this feat would require formulation of dietary components that are substrates to all pathways in question, and not abundant in the food supply and circulation. Assessment of pathway activities can be done for isolated pathways with ease. Intermediates of pathways that are not highly regulated such as stearidonic acid (SDA, 18:4n3) or γ-linolenic acid and odd-chain FA may serve as valuable assessors of pathway activity.

22.2.6.2 Inclusion of Time

The concentrations of circulating lipids are dynamic, and actively respond to environmental stimuli and diet. One time point only reveals a snapshot, whereas metabolites measured over a time course provide a short film of the dynamic interactions between lipid pathways. Measuring changes of lipid metabolism provides a valuable assessment of metabolic regulation. For example, postprandial circulating TG is proving to be independent predictor of cardiovascular disease risk when compared with measurements in the fasted condition (Bansal et al., 2007). In the future, as tools develop to control the composition and structure of food coupled to more comprehensive measurements of repeated time samples using lipid metabolomics, investigators and clinicians will gain a powerful diagnostic tool to understand an individuals' postprandial lipid metabolism. Metabolic outcomes of interest will determine the appropriate time intervals and frequency of blood draws (Cohn et al., 1988).

22.2.7 Crossover vs. Placebo-Controlled Trials

The high interindividual variability in postprandial lipid metabolism as well as the multifactorial and complex dynamic nature of metabolism both require that the study of postprandial response to challenge use an adequate study design capable of detecting signal in an environment that is prone to high noise. In other words, because small variations in the exact composition of meals result in variable responses and because the influence of diet is variable depending on the individual, studies must be designed to capture both variables. Thus, researchers have used the crossover study design in order to increase statistical power. The traditional approach of using a randomized, placebo-controlled design, in which half of the participants are randomized to treatment and the other half are randomized to the control arm, fails when the aim is to understand the phenotypic differences among individuals. The crossover design is powerful when each participant acts as his or her own control, undergoing both the treatment and control arms repeatedly to obtain estimates of intraindividual variation.

22.2.7.1 Interindividual Variation in Postprandial Metabolism

Early postprandial research revealed that the interindividual variation in response to meals and diets is high. Many components contribute to an individual's postprandial response (Figure 22.1). Differences between people in the various aspects of postprandial response are due to multifactorial interactions involving genetic background, intrauterine and early childhood imprinting (which comprise developmental plasticity), the chronic effects of diet and lifestyle, exposure to toxins and other environmental factors, as well as recent short-term and acute aspects of diet and lifestyle. Gender and BMI influence peak/nadir and time courses of postprandial hormonal responses (Carroll et al., 2007). Metabolic status such as obesity determines circulating postprandial leptin, which in turn depends on the macronutrient composition of the challenge (Romon et al., 2003). Gender also influences lipid metabolism in a variety of ways, including greater VLDL secretion rates and lower postprandial TG concentrations in women compared with men (reviewed in Mittendorfer, 2005). Differences in postprandial lipid metabolism are also caused by normal dynamic

fluctuations in metabolism as part of diurnal rhythms and other cyclical events. For example, in women, lipid metabolism is affected by the phase of the menstrual cycle (Gill et al., 2005). Other determinants include health status (e.g., diabetic vs. healthy) (Nappo et al., 2002), age (Lin et al., 2007), and even place of residence, which reflects cultural differences as well as differences in food availability and composition (van Oostrom et al., 2004).

Some aspects of interindividual variation in lipid metabolism and its responses to diet have been assigned to specific genetic variations. For instance, polymorphisms in the FADS1 and FADS2 genes for desaturases, which metabolize essential FA, contribute not only to differences in membrane FA composition, but also the susceptibility to inflammatory disease (Schaeffer et al., 2006). The interaction of genes and diet determines the phenotypic outcome of a genotype (Ordovas et al., 2002). For example, in 6% of 470 healthy women and men, the combination of a variant lipoxygenase genotype and increased dietary intake of the ω6 FA resulted in increased intima-media thickness, a measurement of atherosclerosis progression (Neufeld et al., 2004), whereas, the intake of ω3 FA was negatively associated with intima-media thickness. On the other hand, in people without the lipoxygenase genotype variant, dietary FA intake did not influence the extent of atherosclerosis in the carotid artery.

The developing field of nutrigenomics has not yet had the same success with postprandial variations, yet the data from existing postprandial studies imply that this will be a fertile field of investigation. Strikingly, Burge et al. (2003) recently found coefficients of variation for plasma TG concentrations and areas under the curve over 6 h in response to a lipid challenge as high as 98% (Table 22.1). This study included six similarly healthy,

TABLE 22.1
Coefficients of Variation (CV) for Plasma TG Metabolites

Metabolite[a]	Mean	SD	CV (%)
Total TG AUC[b] Day 1	0.91	0.47	52
Total TG AUC Day 2	0.51	0.5	98
Total TG AUC Day 3	0.55	0.47	85
[^{13}C]-TG at 2 h	48	28.5	59
[^{13}C]-TG at 10 h	50	49	96
TG at 2 h	0.8	0.4	50
TG at 4 h	0.72	0.43	50

[a] Six subjects were assessed in response to the same mixed macronutrient meal at breakfast followed by lunch (once with [^{13}C]-labeled tripalmitin at breakfast, and once at lunch), or a prolonged fast followed by lunch (with [^{13}C]-labeled tripalmitin) on three separate days. CV were calculated from mean ± SD. (Data from Burdge, G.C. et al., *Eur. J. Clin. Nutr.*, 57, 1536, 2003.)

[b] AUC, area under the curve.

young, white, male subjects, indicating that even within a very narrowly defined group of individuals, variation in postprandial responses is high. Such high signals within healthy individuals provide a significant opportunity for nutrigenomic research, which is typically frustrated by small variations in most metabolic outcomes.

22.2.7.2 Intraindividual Variation in Postprandial Lipid Metabolism

Both interindividual variation and intraindividual variation are assumed to contribute to the observed variability of response to challenge. However, this issue has not been adequately addressed. The crossover design in and of itself allows researchers to better account for inter- and intraindividual variation, but it does not distinguish between them. Statistical methods are employed to normalize response to the individual. For example, repeated measures ANOVA takes into account intraindividual variation to estimate responses. However, this essentially eliminates the intraindividual variation rather than addressing it directly. We investigated the contribution of inter- vs. intraindividual variation in postprandial lipid metabolism to resolve whether the variability that is observed in response to a meal results from noise or true biological variation (Zivkovic, 2008).

Three normal individuals were tested for their responses to the same challenge meal by three discrete tests over a period of several months. To capture the full extent of inter- and intraindividual variations, the subjects were explicitly instructed "not" to control diet and lifestyle during that time period. The three individuals tested were all considered metabolically healthy and had normal clinical chemistries (TG < 150 mg/dL, total cholesterol < 200 mg/dL, glucose < 126 mg/dL) and were all normal weight. Despite the lack of dietary and lifestyle control, the three individuals were consistent within their own responses and yet sufficiently different from each other that their lipid responses were all distinguishable, meaning that the interindividual variation among the subjects was significantly greater than the intraindividual variation within each subject over the three test days. The implications of these findings are first, that the high variability in postprandial response is true biological variability among individuals rather than noise or random fluctuation in metabolites in response only to short-term dietary changes. Second, the details of an individual's metabolic phenotype can be uncovered through the approach of combining "omic" technology with the *in vivo* assessment of metabolic function (e.g., the response to challenge approach).

22.2.8 Lipid Biochemical Pathways

The complex structures and composition of cellular lipids are determined by diet, endogenous metabolism through the FA synthase (Maier et al., 2008), desaturation, and elongation pathways (Nakamura and Nara, 2004), and mono- and peroxisomal β-oxidation (Wang et al., 2006). There are three endoplasmic reticulum membrane-bound desaturases in humans: stearoyl CoA desaturase (SCD), catalyzing the synthesis of MUFA from SFA; Δ6-desaturase and Δ5-desaturase, involved in synthesizing highly unsaturated FAs from dietary PUFA (Nakamura and Nara, 2004). Seven distinct human elongase subtypes—based on endoplasmic reticulum tissue expression—are involved in FA chain elongation of SFA, MUFA, and PUFA (Wang et al., 2006).

22.2.8.1 MUFA

SCD is a lipogenic enzyme that catalyzes the synthesis of MUFA, integral components of membrane phospholipids, TG, wax esters, and cholesterol esters (Ntambi et al., 2002). Endogenous MUFA are synthesized via the insertion of a *cis*-double bond by SCD on SFA with 12–19 carbons in length (Nakamura and Nara, 2004). SCD expression and activity are regulated by diet, hormones, pharmacology (reviewed in Nakamura and Nara, 2004), and development (Zhang et al., 2005).

SCD is predominately expressed in liver, adipose (Nakamura and Nara, 2004; Sjogren et al., 2008), pancreas and brain (Zhang et al., 2005), skeletal muscle (Le et al., 2008), testis, and epididymis. This enzyme is regulated dietarily through induction by carbohydrates (Saether et al., 2003b; Wang et al., 2006), fructose (Le et al., 2008), vitamin A (Miller et al., 1997), PUFA (Nakamura and Nara, 2004; Wang et al., 2006), protein type (Tovar et al., 2005), chronic alcohol (Umeki et al., 1984; Pawlosky and Salem, 2004), and plant sterols (Leikin and Brenner, 1989); developmentally (Zhang et al., 2005); pharmacologically through peroxisome proliferator-activated receptor (PPAR)-α agonists (Riserus et al., 2005; Wang et al., 2006) and PPAR-γ agonists (Takasawa et al., 2008); hormonally by insulin (Wang et al., 2006; Flowers et al., 2007), leptin (Wang et al., 2006); glucocorticoids (Marra and Alaniz, 1995; Saether et al., 2003b); estrogen (Lippiello et al., 1979; Marra and Alaniz, 1995; Hermier et al., 1996); mineralcorticoids, testosterone (Marra and Alaniz, 1995; Saether et al., 2003b); follicle-stimulating hormone (Saether et al., 2003b); dehydroepiandrosterone in rats and mice, but not in guinea pigs (Imai et al., 2001); thyroid hormone (Waters et al., 1997); and exercise (Ikeda et al., 2002).

Based on animal data, SCD has received much attention as an important regulator of diet-induced obesity and insulin resistance (Flowers and Ntambi, 2008). Global deletion of SCD1 in mice results in resistance to high-fat diet-induced obesity and glucose intolerance (Ntambi et al., 2002), yet recently, leptin-deficient mice also deficient in SCD were reported to develop severe diabetes (Flowers et al., 2007).

The practical application of metabolite profiling to describe metabolic function of SCD activity in humans has been attempted (Warensjo et al., 2006, 2007). Currently, an index of SCD activity is used as the ratio of its products to precursors in circulation and tissues. A case-cohort study demonstrated a higher ratio of 18:1n9/18:0 in adipose of insulin resistant compared with healthy controls. Yet, the ratio of adipose 16:1n7/16:0 was not different between these two groups (Sjogren et al., 2008). Because 18:1n9 is abundant in the food supply, the application of the SCD index is confounded by habitual and acute diet. Furthermore, the SCD index in plasma does not necessarily accurately reflect the activity among various organ systems that contribute to the net flux of lipids into and out of plasma. In obese individuals, SCD is up-regulated in skeletal muscle associated with low rates of FA oxidation, increased TG synthesis and increased MUFA in muscle lipids. This phenotype of coexisting dysregulation of muscle metabolism was mimicked by overexpressing human SCD in myotubes of lean individuals (Hulver et al., 2005). An SCD activity index in plasma and expression in adipose was increased in insulin-resistant individuals in response to insulin-sensitizing therapy with a PPAR-α agonist (Riserus et al., 2005). The use of animal models and estimates of circulating

FA ratios have not clarified the metabolic function and clinical relevance of SCD in humans.

22.2.8.2 PUFA

PUFA are essential for maintaining membrane composition, the various structural properties of membranes and regulation of transcription factors for energy metabolism (Jump et al., 2005), immune function (Brassard et al., 2007), growth and development (Williard et al., 2001), visual and neuronal development (Salem et al., 2001; Kuperstein et al., 2005), and eicosanoid synthesis (Schmitz and Ecker, 2008). The mammalian PUFA pathways consist of a series of desaturation and elongation reactions that convert 18-carbon essential FA in the diet to 24-carbon intermediates, followed by a peroxisomal retroconversion reaction that forms the final 22-carbon product (Williard et al., 2001) (Figure 22.4).

The Δ5- and Δ6-desaturase enzyme systems are most highly expressed in liver, followed by brain, skeletal muscle, lung, heart, placenta, kidney, pancreas (Cho et al., 1999a,b), intestine (Garg et al., 1992; Dias and Parsons, 1995), mammary tissue (Rodriguez-Cruz et al., 2006), testis, and epididymis (Saether et al., 2003b). The varying activities of these pathways are still being recognized and their diversity of functions is remarkable. For example, Δ6-desaturase expressed in sebaceous glands in human skin catalyzes an unexpected "sebaceous type" reaction converting

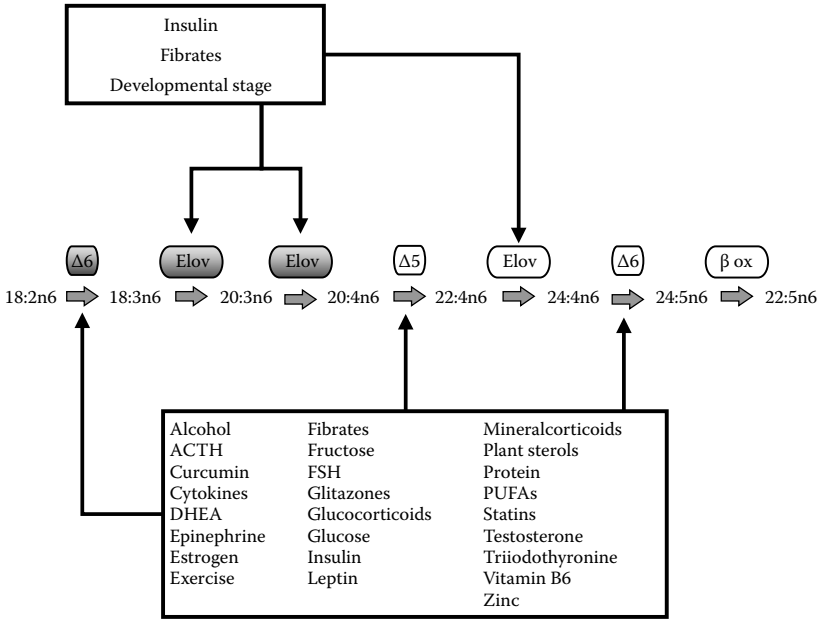

FIGURE 22.4 Regulation of PUFAs using the omega-6 pathway as a model. Nutritional, hormonal, pharmacological, and developmental regulation of expression, and activity of Δ5- and Δ6-desaturase and elongase enzymes.

palmitate into the MUFA, sapienate. Alterations in this pathway have been implicated in the pathogenesis of acne (Ge et al., 2003).

The Δ5- and Δ6-desaturases have essential roles in synthesizing PUFA, and the expression of both enzymes is regulated by nutrition (Cho et al., 1999a,b; Matsuzaka et al., 2002), hormones, metabolic status, pharmacology (Wang et al., 2006), and exercise (Zhang et al., 2005). The importance of the elongation reactions in regulating lipid metabolism and function was revealed only recently. Several distinct FA elongase subtypes are regulated by diet, drugs, hormones, postnatal development, and metabolic status, and they are present in human genomes with different tissue distribution (Wang et al., 2006). More specifically, carbohydrates (Wang et al., 2006), amino acid composition (Shimada et al., 2003), vitamin B6 (Bordoni et al., 1998), and even secondary plant metabolites such as curcumin-related compounds (Nakano et al., 2000) affect Δ6-desaturase expression. Drugs such as statins and PPAR-α agonists synergistically alter Δ5-desaturase expression (Rise et al., 2007), as do insulin (Brenner, 2003; Saether et al., 2003b; Wang et al., 2006), dietary lipid composition (Cho et al., 1999a,b), protein type (Tovar et al., 2005), plant sterols (Leikin and Brenner, 1989), zinc (Eder and Kirchgessner, 1996), adrenocorticotropic hormone (Mandon et al., 1987), epinephrine (Mandon et al., 1986), and PPAR-α-regulated (Wang et al., 2006) expression of Δ5- and Δ6-desaturases (Figure 22.5).

22.2.8.3 Omega-3

Omega-3 PUFA are now considered to be essential for the assembly of specific membrane compositions and presumably structures in neurological membranes. Higher intakes of ω3 PUFA than those minimally required have been documented to provide therapeutic benefit in reducing cardiovascular disease (Sun et al., 2008) and inflammation (Schmitz and Ecker, 2008). These therapeutic benefits are derived largely by the long-chain PUFA eicosapentaenoic acid (EPA, 20:5n3) and docosahexaenoic acid (DHA, 22:6n3) (Jump, 2002). α-Linolenic acid (LNA, 18:3n3), largely from plant sources, is a substrate in the desaturation and elongation pathway; however, conversion into highly unsaturated FA is inefficient and varies between the sexes. Twenty-one days after ingestion of [^{13}C]-LNA, interconversion into EPA and DHA, respectively, was 21% and 9% in females (Burdge and Wootton, 2002) and 7.9% and 0% in males (Burdge et al., 2002). Additionally, oxidation rates of [^{13}C]-LNA 24 h after ingestion was 50% greater in males compared with females (Burdge et al., 2002; Burdge and Wootton, 2002). The stark sex differences in rates of interconversion could stem from the difference in measurement outcomes. Labeled FA of plasma cholesterol esters, the predominant carriers of EPA, were measured in females but not in males (Burdge and Wootton, 2002; Burdge et al., 2002). Additionally, the sex difference in body composition, hormones, differential loss of label through feces, and habitual diet were not controlled or adjusted.

Intermediates in the ω3 FA pathway have been used in a double-blinded parallel group design. Males and menopausal females ingested encapsulated EPA, SDA, and LNA in doses of 0.75 g/day for 3 weeks followed by an increase in dose to 1.5 g/day for another 3 weeks. The relative effectiveness in increasing circulating phospholipid EPA among the EPA, SDA, and LNA groups was 1 to 0.3 to 0.07, respectively.

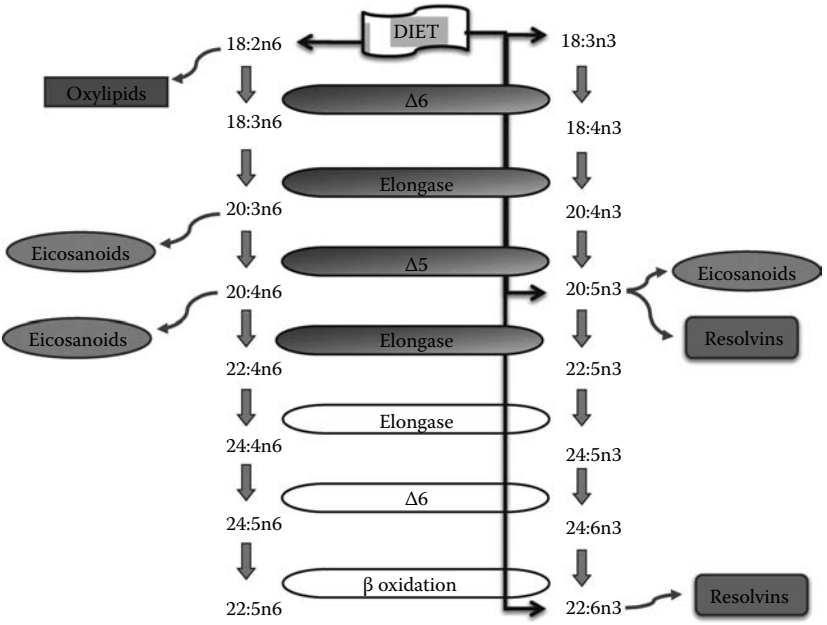

FIGURE 22.5 Omega-3 and omega-6 PUFA pathways. The metabolism of dietary fats and endogenously synthesized FA substrates, intermediates and products of omega-3 and omega-6 PUFA pathways. Linoleic acid, α-linolenic acid, and their metabolic products, arachidonic acid (AA), EPA, and DHA originate from diet. EPA and homo-γ-linolenic acid are precursors to eicosanoids with anti-inflammatory actions; arachidonic acid is a precursor to inflammatory eicosanoids; and both EPA and DHA are precursors to resolvins that exert anti-inflammatory actions. Δ6- and Δ5-desaturases catalyze the reaction for insertion of a double bond at the 6th and 5th carbon from the carboxyl end of the FA substrate, respectively. Elongase enzymes are condensing enzymes that transfer two carbons at the carboxyl end of the fatty acyl substrate. Prior to complete FA synthesis, FA intermediates are shuttled into cellular peroxisomes for removal of two carbons via β-oxidation.

None of the treatment groups increased circulating phospholipid DHA (James et al., 2003). DHA, a highly labile molecule, is strongly regulated in plasma, suggesting possible synthesis by precursors at targeted tissues such as the brain. Labeled studies in mice found that 85% of an administered dose of labeled DHA was recovered in circulating VLDL and low-density lipoprotein (LDL) fractions compared with 30% of labeled oleic acid 30 min after each injection. These data suggest an important role for highly selective transport processes for delivering FA such as DHA to target tissues (Polozova and Salem, 2007). It is yet unclear the extent to which dietary sources of LNA convert to DHA at targeted tissues *in vivo* and how this molecule is transported and regulated in the fed state.

22.2.8.4 Omega-6

Animal, mechanistic, and case-cohort studies have attributed variations in the essential polyunsaturated ω6 FA pathway with a host of diseases including inflammation

(Obukowicz et al., 1998), atopic eczema, allergic rhinitis (Schaeffer et al., 2006; Rzehak et al., 2008), and cardiovascular disease (Glew et al., 2004). Epidemiological case-cohort studies found associations between a common polymorphism in the promoter of the human Δ6-desaturase gene FADS2 and lower levels of very long-chain ω3 PUFA (Baylin et al., 2007). There were associations between variations in the gene cluster of the human Δ5- and Δ6-desaturase genes FADS1 and FADS2, respectively, and reduced levels of erythrocyte phospholipid arachidonic acid (Rzehak et al., 2008) and reductions in plasma phospholipid arachidonic acid (Schaeffer et al., 2006; Malerba et al., 2008; Rzehak et al., 2008), the direct precursor of inflammatory eicosanoids. Carriers of the rare alleles of several single nucleotide polymorphisms had a lower prevalence of allergic rhinitis and atopic eczema (Schaeffer et al., 2006). These studies reveal variation in nutritional genomics in the population, yet the direct effects of diet on these variations have not been measured.

Nutrient–gene interactions have been explored with the advancements of genomic technology coupled with the response-to-challenge model. Recently, de Vogel-van den Bosch et al. (2008) measured the global expression of intestinal barrier genes in response to challenges of PPAR-α (de Vogel-van den Bosch et al., 2008), EPA, DHA, and oleic acid. Out of 74 barrier genes that were upregulated 6 h after the PPAR-α challenge, 62%, 55%, and 26% of these genes were upregulated by EPA, DHA, and oleic acid treatments, respectively. These data demonstrate the indirect effects of dietary fat as important regulators of gut barrier function. The advances in genomics technologies coupled to the challenge model have led to the identification of interactions between nutrients and specific genes and with polymorphisms of dietary responsive enzymes.

22.3 FAT AND DIET

22.3.1 Lipids in Control of Food Intake

The association between excess energy intake and obesity is partly mediated through insulin sensitivity and leptin action. Control of food intake is a hypothalamic response to circulating concentrations of leptin and insulin that link energy intake and fuel stores (Ahima et al., 2006). Insulin is an important regulator of glucose homeostasis, modulator of energy partitioning (Schenck et al., 2008), PUFA synthesis (Brenner, 2003; Wang et al., 2006), FA transport, (Ghebremeskel et al., 2004; Thomas et al., 2005), and hypothalamic action on the control of food intake (Bruning et al., 2000). In the postprandial state, insulin enhances fuel storage, inhibits lipolysis (Unger, 2003), and is involved in leptin release by adipocytes (Cheng et al., 2000). Leptin mediates food intake through sympathetic outflow to the hypothalamus, but also exerts a direct effect on tissues by enhancing lipid oxidation and reducing lipogenesis (Unger, 2003). A state of chronic positive energy balance can lead to insulin resistance and complex etiologies including increased FA flux, nutrient overload, endoplasmic reticulum stress, chronic tissue inflammation (Schenck et al., 2008), lipotoxicity (Unger, 2008), and hyperleptinemia (Unger, 2003), all with potential consequences on the central nervous system (CNS). Insulin secretion influences the fate of lipids in the postprandial state. High FA turnover induces insulin resistance (Schenk et al., 2008) through lipotoxicity of nonadipose tissues (Unger,

2003). Although insulin regulates lipid metabolism and food intake directly at the CNS, lipids may also directly regulate food intake. Injected intracerebroventricularly, oleic acid exerted anorectic effects similar to those of insulin and leptin in rats 4 h after administration measured as a decrease in expression of neuropeptide Y, inhibition of hepatic glucose production, and concomitant reduction in food intake (Obici et al., 2002). With access to the CNS, circulating FFA (Miller et al., 1987) may exert a regulatory role on energy homeostasis in the brain as a signal of energy surplus. Moreover, FA composition (Feltrin et al., 2008), lipid load (Feltrin et al., 2007), and lipid structure (Little et al., 2007) exert different effects on the control of food intake. FA with ≥12 carbon atoms slowdown gastric emptying, modulate gastrointestinal hormone secretion, and suppress energy intake more than FA with <10 carbon atoms (Feltrin et al., 2008). In a double-blinded placebo-controlled study, intraduodenal administration of lauric acid (12:0), oleic acid, or saline, lauric acid suppressed energy intake; however, oleic acid increased peptide YY to the greatest extent (Feltrin et al., 2008). In a crossover study of men administered intragastrically with either 40 g of oleic acid as a FFA, 40 g of macadamia oil as a TG, or a control gastric emptying of FFA was slower than that of TG; hunger was less and fullness was greater with FFA compared with TG and control; increases of cholecystokinin and peptide YY were greater, and energy intake was less after administration of FFA compared with TG (Little et al., 2007).

22.3.2 Impact of a High-Fat Meal on Endothelial Function

The impact of high-fat meals on endothelial function in humans has been extensively studied and reviewed (O'Keefe and Bell, 2007). Within the literature, there is a lack of consistency in methods used to measure endothelial function; however, the use of the crossover study design has been consistently used. Postprandial hypertriglyceridemia-induced vasoconstriction measured as endothelium-dependent FMD quantifies percentage change in arterial diameter (Bae et al., 2003). Neither endothelium-independent vasodilation, as measured by responsiveness to nitroprusside (an endothelium-independent vasodilator), nor endothelium-dependent blood flow was impaired by high-fat meals in forearm resistance vessels, the small arteries and arterioles that are involved in the regulation of blood pressure (Gudmundsson et al., 2000). A reduction in endothelial function measured as forearm blood flow 2 and 4 h after consuming a high-fat meal was correlated with increased plasma FFA (Shimabukuro et al., 2007). Ingestion of a high-fat meal with or without 75 g glucose decreased endothelial function, measured as FMD, and increased nitrotyrosine, a marker of pro-oxidant formation (Ceriello et al., 2002).

The addition of dietary antioxidants to a high-fat meal partially attenuated endothelial dysfunction, as measured by change in blood pressure following L-arginine infusion (Nappo et al., 2002). The addition of vitamins E and C at dosages of 800 IU and 1000 mg, respectively, also prevented a high-fat meal-induced increase in the pro-inflammatory cytokines tumor necrosis factor-alpha (TNF-α) and interleukin-6, as well as adhesion molecules, which are known to mediate endothelial activation, an important component of endothelial dysfunction (Nappo et al., 2002). Timing of antioxidant ingestion had an impact on the adverse effects of high-fat meals, with

800 IU of vitamin E and 1000 mg vitamin C reducing C-reactive protein, a marker of systemic inflammation, to a greater extent when administered pre-supper compared with pre-breakfast, whereas only pre-breakfast antioxidant ingestion prevented the high-fat meal-induced elevation of plasminogen activator inhibitor-1, a prothrombotic molecule (Carroll and Schade, 2003).

22.3.3 Effect of Varying Dietary Fatty Acid Composition on Endothelial Function

22.3.3.1 MUFA

The effects of a large dietary load of SFA on endothelial function in the postprandial state have been reviewed (Botham et al., 2005). The effects of modifying the SFA to MUFA ratios have also been studied and have produced fewer consistent results. A Mediterranean diet style meal, rich in MUFA, increased endothelium-dependent vasodilation, as measured by laser Doppler, and decreased adhesion molecules compared with a SFA-rich meal (Fuentes et al., 2008). A meal enriched in MUFA from extra-virgin olive oil attenuated the increase in procoagulant factors relative to a SFA-rich meal (Delgado-Lista et al., 2008).

A high-fat test meal with a lower SFA:MUFA ratio (55:45) compared with a high SFA:MUFA ratio (70:30) did not attenuate the postprandial increase in IL-6, whereas it increased the level of plasma TNF-α (Poppitt et al., 2008). In type 2 diabetes patients, a meal consisting of skim milk plus 50 g of a MUFA-rich oil (high-oleic safflower oil and canola oil) was associated with impaired endothelial function (Hilpert et al., 2007). The modification of dairy fat to lower the SFA:MUFA ratio by increasing the content of 18:1n9 in the milk of cows on a modified feeding regimen had no effect on thrombogenic factors in the postprandial state after subjects ingested a challenge meal containing 1.2 g of the modified dairy fat/kg body weight (Tholstrup et al., 1999). A high-fat meal enriched in MUFA providing 80 g fat, of which 25 g was olive oil, caused a higher degree of endothelial dysfunction, as measured by FMD, compared with a high-fat meal enriched in walnuts; however, both meals reduced proinflammatory cytokines and adhesion molecules (Cortes et al., 2006). In normotensive and hypertensive patients with hypertriglyceridemia as well as in healthy subjects, a meal enriched in MUFA (refined olive oil) decreased adhesion molecules relative to a meal high in SFA (Pacheco et al., 2008).

22.3.3.2 PUFA

The effects of PUFA consumption on endothelial function may be divergent. A high-fat meal containing walnuts, which are rich in LNA, reduced endothelial dysfunction as measured by FMD compared with a similar meal enriched in MUFA, although both reduced postprandial inflammatory cytokines and adhesion molecules (Cortes et al., 2006). The addition of the long-chain ω3 FA EPA and DHA to a meal enriched in MUFA attenuated the impairment in endothelial function relative to MUFA alone (Hilpert et al., 2007). A meal enriched in walnuts lowered postprandial procoagulant activity relative to a SFA-rich meal (Delgado-Lista et al., 2008). When 9 g fish oil, which is rich in EPA and DHA, was added to a mixed-fat meal providing 40 g fat, endothelium-independent vasodilation was increased as measured by sodium

nitroprusside-induced reactivity (Armah et al., 2008). As measured by FMD, high-fat meals containing 3–5 g ω3 FA as either EPA + DHA or LNA increased endothelial function 50%–80% relative to MUFA-enriched meals (West et al., 2005).

22.3.4 Long-Term and Acute Effects of Dietary Fat Content and Composition on Postprandial Lipemia

Postprandial lipemia is an independent risk factor for heart disease (Karpe, 1997). Interestingly, a diet high in simple carbohydrates is generally associated with prolonged lipemia largely due to the production of VLDL in the liver. Studies attempting to assign the role of dietary fat to postprandial lipemia have yielded mixed outcomes. Saturated fat is associated with deleterious effects, whereas MUFA and PUFA are generally associated with beneficial effects on postprandial plasma lipid profiles; however, these associations have been inconsistent. Sixteen days of consuming a SFA-rich diet resulted in the accumulation of postprandial TGRL 7 h after a challenge meal, whereas consuming a PUFA-rich diet for 16 days attenuated this effect (Chung et al., 2004). Relative to SFA-rich meals, a PUFA-rich meal (ω6 FA from safflower oil) reduced postprandial TGRL-cholesterol and apolipoproteins E and C-III content (Jackson et al., 2005). A low-fat diet enriched in linoleic acid (LA, 18:2n6) attenuated the increase in average and maximal increments of TGRL-cholesterol postprandially relative to a low-fat diet enriched in oleic acid (18:1n9) (Higashi et al., 2001). Fish oil provided as TG oil or as plant sterol esters both reduced postprandial TG concentrations (Demonty et al., 2006). Relative to test meals enriched in palmitate (16:0) or LA, meals enriched in oleic acid and EPA + DHA reduced insulin response and nonsignificantly reduced postprandial TG in patients with type 2 diabetes mellitus (Shah et al., 2007). A meal containing MUFA and EPA + DHA attenuated the increase in postprandial TGRL relative to a meal containing MUFA alone (Hilpert et al., 2007).

Supplementation of hypertriglyceridemic men with 6 g/day fish oil providing 3 g/day EPA + DHA decreased postprandial plasma TG and FFA concentrations, and increased *ex vivo* LDL-oxidation and LDL-cholesterol relative to 6 weeks on 6 g/day olive oil (Leigh-Firbank et al., 2002). A MUFA-rich diet consumed for 3 weeks by patients with type 2 diabetes resulted in decreased small VLDL-TG; however, it also resulted in an earlier chylomicron peak in response to a high-fat meal relative to when a SFA-rich diet was consumed for 3 weeks (Rivellese et al., 2008). One study found no effect of modifying meal FA composition on any postprandial plasma lipid measure, finding that a meal rich in LA, a meal rich in MUFA, and a meal rich in EPA + DHA had no differential effect on areas under the curve for TG and cholesterol, TG and cholesterol peak values, or time to maximum concentration in healthy middle-aged men and women (Burdge et al., 2006).

22.4 CONCLUSIONS

Obesity is the result of chronic imbalance between caloric intake and caloric demands. This balance between intake and expenditure appears simple at first glance: calories in must equal calories out. Yet the metabolic regulation underlying

energy expenditure and food intake behavior is anything but simple, as is clearly illustrated by the failure of diet-based interventions to produce and sustain weight loss in most individuals.

The variation of dietary fat intake is one of the central controls on whole body metabolic dynamics. Future mechanistic studies that characterize the signals linking diet to endogenous metabolism are needed as caricatured in Figure 22.6. In such studies, appreciating all of the components of diet as input variables will be necessary for success. Furthermore, the variations between individuals with respect to the responses to dietary fat are a part of the causes in disparity in health among the population. This implies that as the science of metabolic regulation is brought to practice, assessing individual metabolism will need to include individual metabolic responses to standardized dietary challenge.

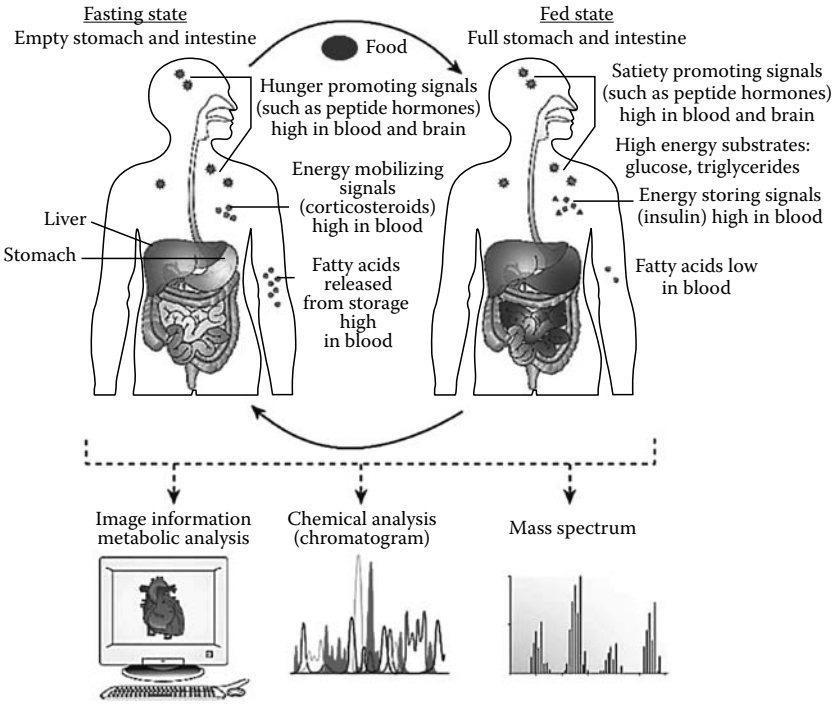

FIGURE 22.6 (See color insert following page 166.) Assessment of fasted and fed states. Metabolic regulation is a continuous process varying from fasted to fed states. Levels of metabolites rise and fall in response to the influx of nutrients from foods, as do the signaling systems that stimulate appetite and mobilize energy in times of need, and inhibit eating and direct energy-rich molecules into storage in times of surplus. The combined molecular-imaging and metabolic-profiling capabilities available today as a result of revolutionary advances in analytical sciences provide an unprecedented opportunity to accurately monitor these processes. If brought to practice as part of routine health care, these tools would similarly revolutionize the diagnosis, treatment, and prevention of metabolic diseases. (From Macé K. et al., *Nature*, 444 (7121), 854, 2006. With permission.)

In this chapter, we have detailed the biochemical and physiological rationale for a standardized dietary challenge approach as a means of assessing individual metabolism that is capable of assessing the response to diet. Primary in the list of reasons for this approach is to understand the function of metabolism in order to employ a functional assessment that includes diet rather than a static assessment based on the fasted condition. Measuring the flux of metabolites in response to a well-defined challenge meal provides a means to assess in real time the metabolic networks at work and how diet affects metabolism. Whereas measurements in the fasted state can offer only a limited perspective on the long-term effects of diet, measurements in the fed state, if carried out with an appropriate study design, can reveal a more complete picture of how an individual metabolizes a particular meal, as well as the long-term effects of a particular diet. Ultimately, the goal is not simply to produce weight loss at any cost but to improve health.

Establishing the parameters of a standardized fat challenge test will require a consensus of nutrition scientists and, once in place will provide a framework to a new dimension of human health assessment. Many questions remain unanswered and need to be resolved for the challenge approach to become useful and clinically applicable for metabolic assessment. The exact composition of challenge meals intended to uncover specific aspects of metabolism needs to be defined. For example, how much LNA should be included in a challenge meal designed to uncover an individual's ability to convert LNA to the longer chain EPA and DHA? The implication is that understanding an individual's capacity to convert LNA to EPA and DHA will guide recommendations for the consumption of plant-based sources of ω3 FA vs. fish oil. Another example is the influence of hormonal status on the effects of diet in women. Among the implications are: (1) that the assessment of metabolic status in menstruating women may need to be adjusted for the phase of the menstrual cycle or estradiol levels, which should be measured simultaneously in order to obtain an accurate representation of metabolism and (2) that different foods may be appropriate at different times of the month for optimal health in menstruating women. Many other questions include the timing of blood draws, the minimum set of phenotypic characteristics that should be accounted for in the analyses, and the number of replicates that is required to ascertain underlying metabolic response. Such issues need to be addressed in order to facilitate the assessment of metabolic phenotypes.

An additional challenge will involve building databases that reflect the boundaries of normal metabolic responses in which abnormal or dysregulated metabolism can be detected. Given the high degree of interindividual variability in responsiveness to diet within the population as discussed extensively in this chapter, how can a "normal" response be defined? The task of building comprehensively annotated databases that can be interrogated for similarities and differences among individuals will be crucial in understanding of how different individuals can be grouped together to derive a normal range of responses and to predict trajectories of varying long-term health outcomes. This, in turn, will have important implications for how we devise nutrition recommendations for individuals.

The current approach to tailor dietary recommendations by age, gender, weight, height, and activity level is the first step toward guiding personal health assessment

in practice. Obesity continues to be a major health concern in the United States, and increasingly around the world. Diet and lifestyle-based weight loss and obesity prevention approaches continue to have high failure rates. It is equally important to be able to assess the overall consequences of ongoing attempts and ultimately successful interventions to reduce adiposity. There is an urgent need to devise accurate and effective approaches for assessing the metabolic status of individuals.

The ultimate goal is to comprehensively assess individual responses to different diets and meals. By measuring individuals' responses it will not only be possible to determine the underlying metabolic mechanisms that are contributing to that individual's obesity-induced disease burden and therefore tailor dietary intervention appropriately to correct the metabolic dysregulation, but also to tailor diets and meals that will increase adherence because they will be personalized and thus optimized for that individual's metabolic phenotype.

ABBREVIATIONS

TG	triglycerides
FA	fatty acids
PUFA	polyunsaturated fatty acids
BMI	body mass index
MRI	magnetic resonance imaging
FMD	flow-mediated vasodilation of the brachial artery
SFA	saturated fatty acids
MUFA	monounsaturated fatty acids
trans-FA	*trans*-fatty acids
VLDL	very low-density lipoprotein
TGRL	triglyceride-rich lipoproteins
OFTT	oral fat tolerance test
FFA	free fatty acids
SDA	stearidonic acid
SCD	stearoyl CoA desaturase
PPAR	peroxisome proliferator-activated receptor
EPA	eicosapentaenoic acid
DHA,	docosahexaenoic acid
LNA	α-linolenic acid
LDL	low-density lipoprotein
CNS	central nervous system
TNF	α-tumor necrosis factor alpha

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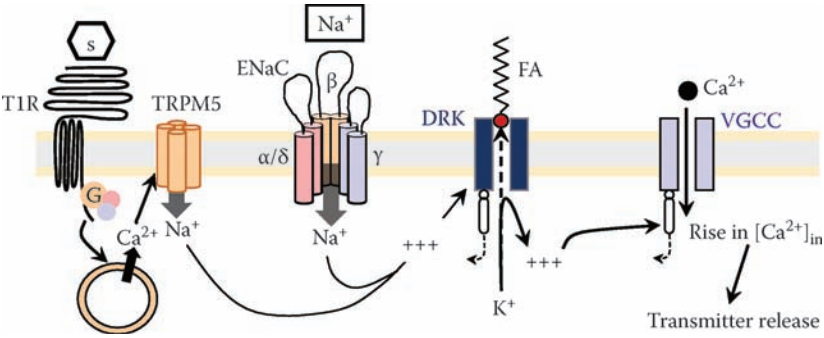


FIGURE 3.1 The ability of fatty acids to inhibit DRK channels is consistent with roles as either a taste primer or a taste modulator. Fatty acids (FA), like linoleic acid, inhibit DRK channels by acting as an open channel blocker. This inhibition would prohibit the efflux of K^+ ions, causing depolarization of the taste cell and subsequent activation of downstream signaling elements such as voltage-gated Ca^{2+} channels (VGCC). Alternatively, the depolarization elicited by other taste stimuli like sweet working through T1R GPCRs/TRPM5 pathway or the permeation of Na^+ ions though epithelial sodium channels (ENaC) in the case of salty taste could be enhanced and prolonged in the presence of FAs. This is diagrammatic only and not meant to imply that all these elements are in the same individual cells. Our expression data would argue, however, that there is overlap between FA-sensitive DRK channels and these other taste modalities.

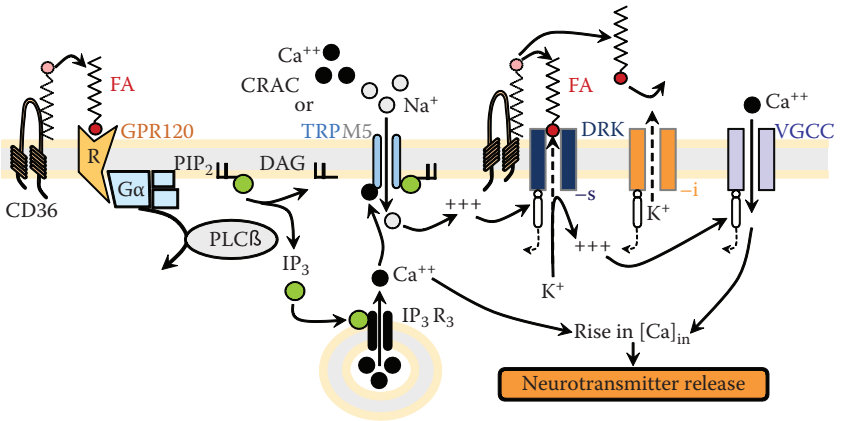


FIGURE 3.2 Putative transduction pathway for fatty acids in taste and trigeminal cells. Fatty acids (FA) delivered by the binding protein CD36 activate specific G protein–coupled receptors (like GPR120) to initiate a transduction cascade that in turn produces a second messenger leading to release of calcium from intracellular stores and activation of a store-operated ion channel (CRAC or TRPM-like channel) to produce a receptor potential. This potential opens fatty acid-sensitive DRK channels that are subsequently blocked by FA, leading to an enhanced and prolonged depolarization. The ratio of FA-sensitive:FA-insensitive DRK channels helps to determine the magnitude of the overall chemosensory response to FA stimulation. This depolarization is the impetus for the eventual release of neurotransmitter onto gustatory afferent fibers. It is not known whether the pathway downstream of DRK channels is in the same cell as the upstream elements or if this part of the pathway involves cell-to-cell signaling.

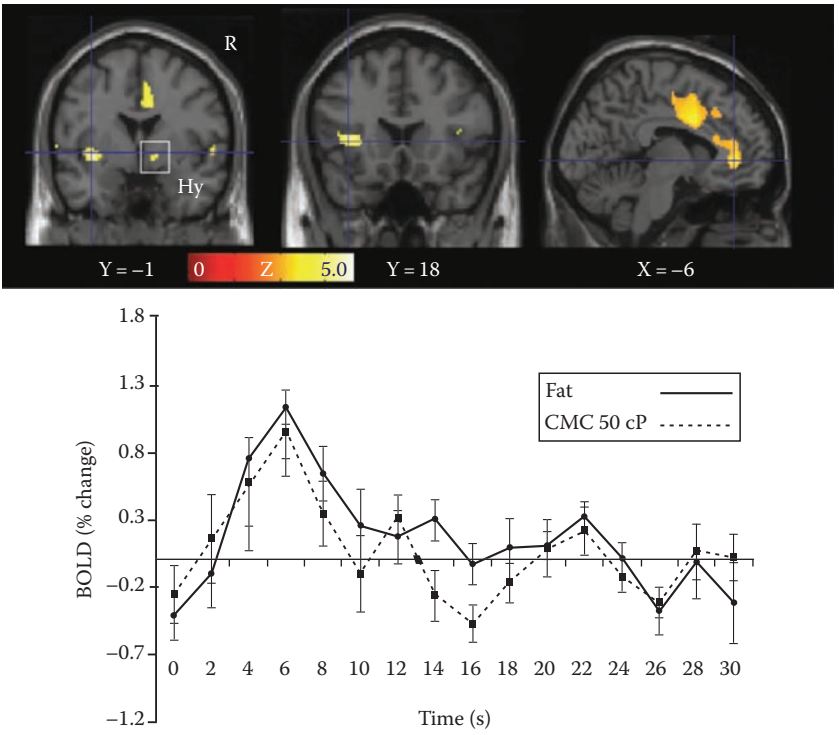


FIGURE 8.8 fMRI study of the responses to the oral delivery of fat as assessed by the comparison (fat-control). Activations were observed in the mid-insula and hypothalamus (Hy) (top row left), anterior insula (top row middle), and ACC (top row right). The average time-course data (across trials and subjects) from the mid-insular cortex (from the voxels marked by the crosshairs in the top row left) are shown in the bottom row for the conditions Fat and CMC 50 cP. (After de Araujo I.E.T. and Rolls E.T., *J. Neurosci*., 24, 3086, 2004. With permission.)

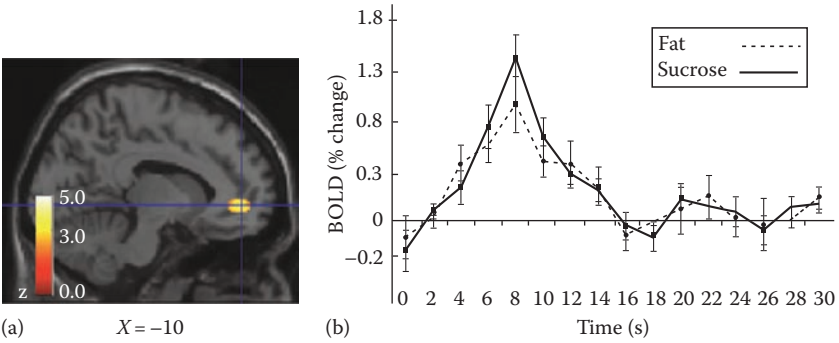
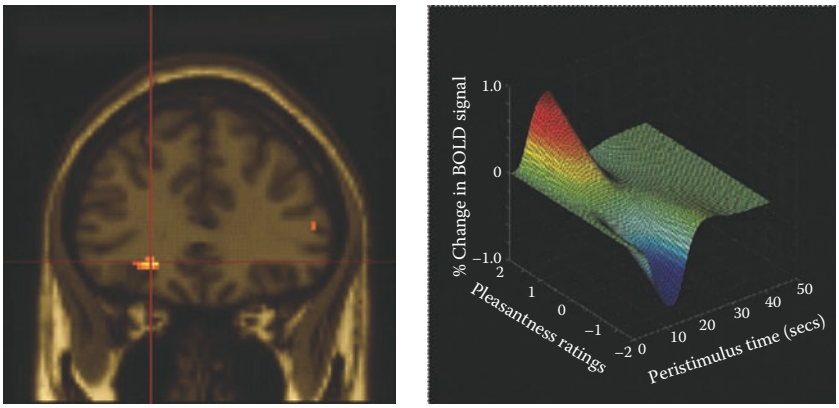
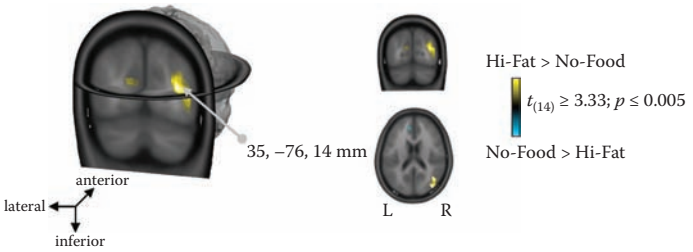


FIGURE 8.9 (a) Rostral ACC activation by fat-control and sucrose-control, as revealed by conjunction analysis. (b) The corresponding average time-course data (across trials and subjects) from the voxel marked by the crosshairs are shown. (After de Araujo I.E.T. and Rolls E.T., *J. Neurosci*., 24, 3086, 2004. With permission.)

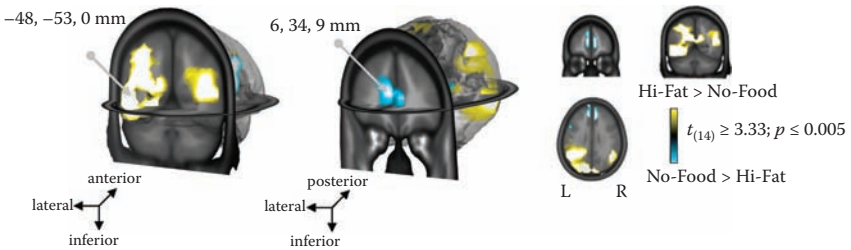


(a) (b)

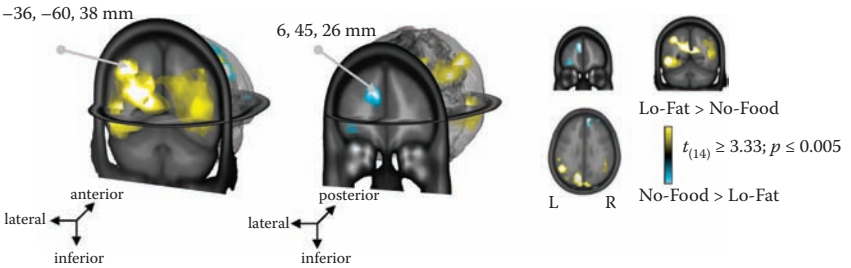
FIGURE 8.10 Areas of the human orbitofrontal cortex with activations correlating with pleasantness ratings for food in the mouth. (a) Coronal section through the region of the orbitofrontal cortex from the random effects group analysis showing the peak in the left orbitofrontal cortex (Talairach coordinates x, y, z = −22, 34, −8, Z-score = 4.06), in which the BOLD signal in the voxels shown in yellow was significantly correlated with the subjects' subjective pleasantness ratings of the foods throughout an experiment in which the subjects were hungry and found the food pleasant, and were then fed to satiety with the food, after which the pleasantness of the food decreased to neutral or slightly unpleasant. The design was a sensory-specific satiety design, and the pleasantness of the food not eaten in the meal, and the BOLD activation in the orbitofrontal cortex, were not altered by eating the other food to satiety. The two foods were tomato juice and chocolate milk. (b) Plot of the magnitude of the fitted hemodynamic response from a representative single subject against the subjective pleasantness ratings (on a scale from −2 to +2) and peristimulus time in seconds. (After Kringelbach, M.L. et al., *Cereb. Cortex*, 13, 1064, 2003. With permission.)



(a) Hi-Fat vs. No-Food (88–140 ms)



(b) Hi-Fat vs. No-Food (217–316 ms)



(c) Lo-Fat vs. No-Food (217–316 ms)

FIGURE 9.4 Statistical differences between the group-averaged LAURA source estimations ($N = 15$) displayed on a three-dimensional rendering of the MNI template brain with maxima indicated by arrows and Talairach coordinates. (a) Statistical difference between the source estimations for Hi-Fat vs. No-Food viewing over the 80–140 ms poststimulus period. (b) Statistical difference between the source estimations for Hi-Fat vs. No-Food viewing over the 217–316 ms poststimulus period. (c) Statistical difference between the source estimations for Lo-Fat vs. No-Food viewing over the 217–316 ms poststimulus period.

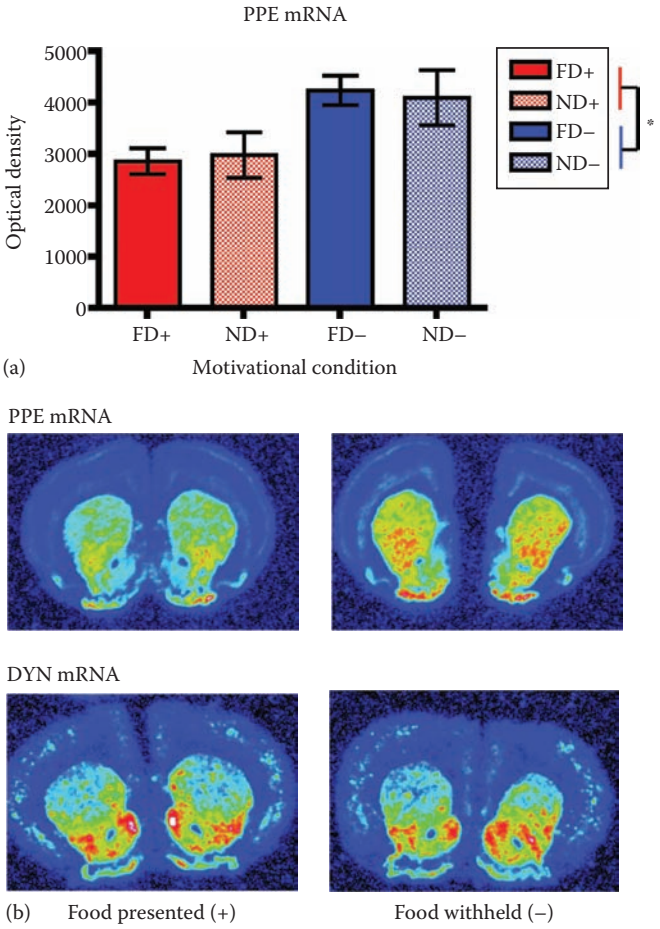


FIGURE 13.2 Striatal enkephalin (PPE) gene expression fluctuates with specific aspects of food intake history. Different groups of rats were either food restricted (FD, solid bars) or maintained ad libitum (ND, stippled bars). On the evening of the test day, some rats were given food as usual (red shade), but for others the food was withheld (blue shade). An example is shown of the marked downregulation of PPE within the lateral accumbens shell that resulted from food access relative to deprivation (a). As can be observed, PPE mRNA levels appear to track acute satiety state rather than longer term energy deficit, as decreases in PPE expression were seen in both food-restricted and nonrestricted animals following food availability. Asterisk indicates significant difference in gene expression between groups. (b) PPE, but not PPD, downregulation was observed across the entire striatum following access to food. (Adapted from Kelley, A.E. et al., *J. Comp. Neurol.*, 493, 72, 2005.)

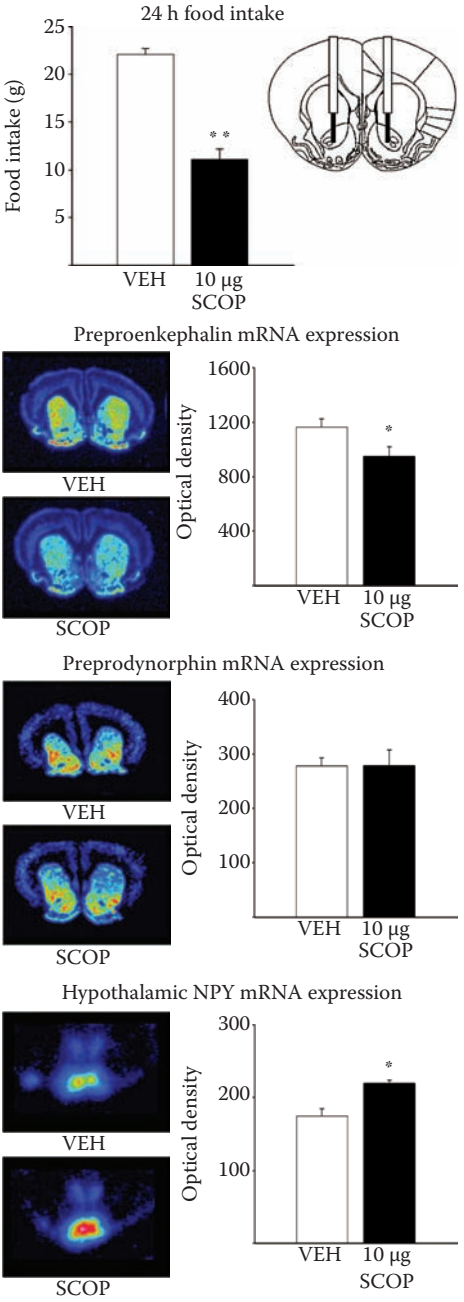


FIGURE 13.3 Cholinergic muscarinic receptors play a major role in food intake and enkephalin gene expression. A single infusion of scopolamine into the Acb reduces food intake by almost half over the following 24 h period. This behavioral effect is associated with a significant downregulation of striatal PPE mRNA, but does not influence levels of PPD mRNA. Note the quantitatively similar reduction in PPE expression compared to that following food access in Figure 13.2. Hypothalamic NPY is increased by scopolamine treatment, reflective of negative energy balance. Asterisks indicate significant difference between groups or significant change in gene expression. (Adapted from Kelley, A.E. et al., *J. Comp. Neurol.*, 493, 72, 2005.)

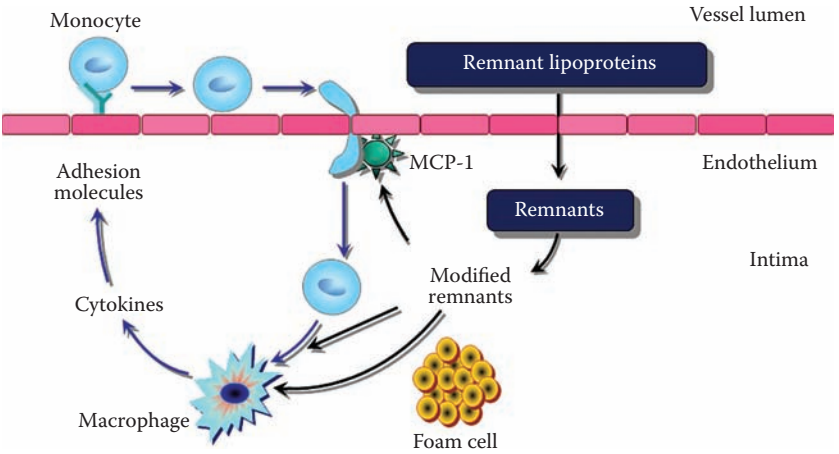


FIGURE 17.2 The effects of postprandial chylomicrons and VLDL on arterial endothelium. VLDL remnants and chylomicron remnants behave in much the same way as LDL. They enter the subendothelial space, where they become modified. The modified remnants stimulate MCP-1 thereby promoting the differentiation of monocytes into macrophages which are taken up by the macrophages to form foam cells. Like LDL, the remnant lipoproteins are proinflammatory and proatherogenic. (From Lopez-Miranda, J. et al., *Br. J. Nutr.*, 98(03), 458, 2007. With permission.)

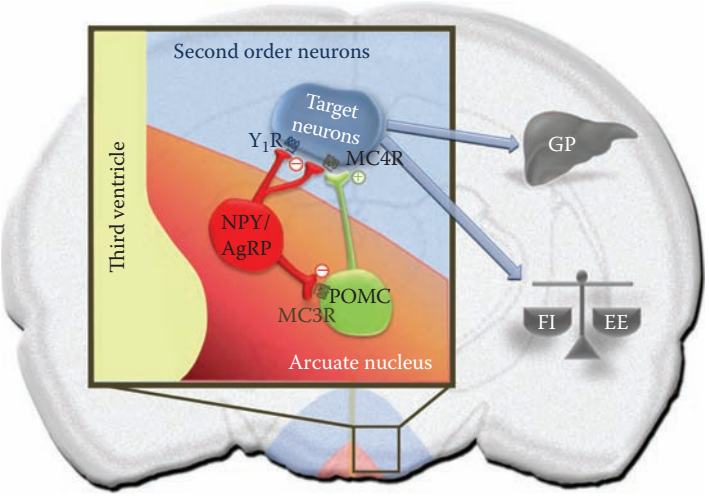


FIGURE 20.1 The hypothalamic melanocortin system and the regulation of energy and glucose homeostasis. The hypothalamic ARC is a critical mediator of energy and glucose homeostasis. Two groups of ARC neurons ("first-order" neurons), the NPY and AgRP coexpressing neurons and the POMC expressing neurons, are proposed to play a major part in mediating these regulatory effects. Projections from the ARC neurons reach adjacent hypothalamic nuclei ("second-order" neurons) including the PVN. The melanocortin signaling progressing along these nuclei and to downstream brain areas is mediated by the α-MSH, a cleavage product of POMC, which binds the melanocortin receptor MC4R. Inhibition of this "melanocortin tone" is mediated by (1) synaptic inhibition of POMC neurons by NPY/AgRP neurons (via MC3R) and of target second-order neurons by neuropeptide Y1 receptor (Y_1R) and (2) AgRP acting as an endogenous antagonist of MC4R. Insulin and leptin carry out their anorectic effects via inhibiting NPY/AgRP neurons and stimulating POMC neurons, while ghrelin activates NPY/AgRP neurons to promote feeding behavior. Nutrients such as LCFAs have been shown to downregulate the expression of AgRP to mediate the hypothalamic anorectic signal. GP, glucose production; FI, food intake; EE, energy expenditure.

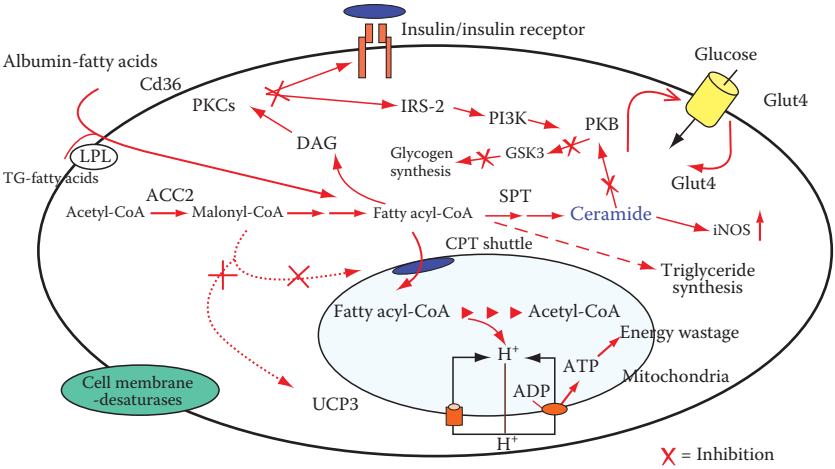


FIGURE 21.2 Some intracellular pathways of fatty acid handling which impinge strongly on insulin-stimulated glucose handling. Please see the text for further detail. ACC2, acetyl CoA carboxylase; ADP, adenosine diphosphate; ATP, adenosine triphosphate; CPT, carnitine palmitoyl tranferase; DAG, diacylglycerol; Glut4, glucose transporter 4; GSK3, glycogen synthase kinase 3; iNOS, inducible nitric oxide synthase; IRS2, insulin receptor substrate 2; LPL, lipoprotein lipase; P13K, phosphoinositide 3 kinase; PKB, protein kinase B; PKC, protein kinase C; SPT, serine palmitoyl tranferase; UCP3, uncoupling protein 3.

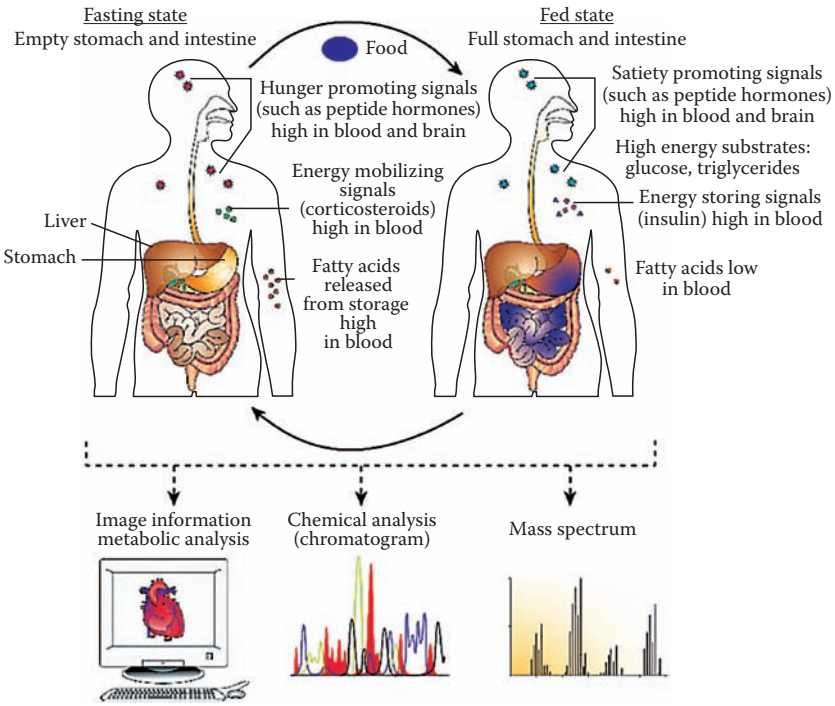


FIGURE 22.6 Assessment of fasted and fed states. Metabolic regulation is a continuous process varying from fasted to fed states. Levels of metabolites rise and fall in response to the influx of nutrients from foods, as do the signaling systems that stimulate appetite and mobilize energy in times of need, and inhibit eating and direct energy-rich molecules into storage in times of surplus. The combined molecular-imaging and metabolic-profiling capabilities available today as a result of revolutionary advances in analytical sciences provide an unprecedented opportunity to accurately monitor these processes. If brought to practice as part of routine health care, these tools would similarly revolutionize the diagnosis, treatment, and prevention of metabolic diseases. (From Macé K. et al., *Nature*, 444 (7121), 854, 2006. With permission.)

NEUROSCIENCE

A bite of cheese, a few potato chips, a delectable piece of bacon—a small taste of high-fat foods often draws you back for more. But why are fatty foods so appealing? Why do we crave them? ***Fat Detection: Taste, Texture, and Post Ingestive Effects*** covers the many factors responsible for the sensory appeal of foods rich in fat. This well-researched text uses a multidisciplinary approach to shed new light on critical concerns related to dietary fat and obesity.

Reflecting 15 years of psychophysical, behavioral, electrophysiological, and molecular studies, this book makes a well-supported case for an oral fat detection system. Using carefully designed behavioral paradigms, it explains how gustatory, textural, and olfactory information contribute to fat detection. The book also provides a detailed account of the brain regions that process the signals elicited by a fat stimulus, including flavor, aroma, and texture.

This readily accessible work also discusses:

- The importance of dietary fats for living organisms
- Factors contributing to fat preference, including palatability
- Brain mechanisms associated with appetitive and hedonic experiences connected with food consumption
- Potential therapeutic targets for fat intake control
- Genetic components of human fat preference
- Neurological disorders and essential fatty acids

Providing a comprehensive review of the literature from the leading scientists in the field, this volume delivers a holistic view of how the palatability and orosensory properties of dietary fat impact food intake and ultimately health. ***Fat Detection*** represents a new frontier in the study of food perception, food intake, and related health consequences.

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