Nutrition and Health
Series Editor: Adrianne Bendich

Giamila Fantuzzi
Carol Braunschweig *Editors*

Adipose Tissue and Adipokines in Health and Disease

Second Edition

Humana Press

Nutrition and Health

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

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Preface

In the last 10 years, adipose tissue and adipokines—messenger proteins produced by adipocytes—have become the focus of extensive investigation as a result of the recognition of the health problems associated with the ever-expanding worldwide obesity problem that affects both children and adults. Numerous advances have been made since publication of the first edition of *Adipose Tissue and Adipokines in Health and Disease*, in terms of basic adipocyte biology, understanding of the determinants of obesity, distribution of body fat and weight loss, as well as the mechanisms linking excess adiposity to various comorbidities. The second edition of *Adipose Tissue and Adipokines in Health and Disease* appears 5 years after the initial volume of the Nutrition and Health series on the same topic. The aim of the current edition remains to provide comprehensive information regarding adipose tissue, its physiological functions and its role in disease, collecting in one place updated information spanning the range of adipose tissue studies, from basic adipocyte biology to epidemiology and clinical aspects.

The volume is divided in four parts: the first two deal with basic adipose tissue and adipokine biology, while the last two address the problem of obesity and alterations in adipose tissue function from an epidemiological and clinical standpoint.

The chapters that compose Part 1, *Adipose Tissue*: *Structure and Function*, provide an overview of the evolution and biology of adipose tissue and adipokines as well as a state-of-the-art discussion about different types and function of adipose tissue and its distribution in the body. Part 2, *Adipose Tissue Inflammation and Adipocyte Dysfunction in Obesity*, tackles the topic of mechanisms linking expansion of adipose mass to disease pathogenesis by way of inflammation, dysfunctional cellular responses as well as alterations in micronutrient metabolism. Part 3, *Obesity*, addresses epidemiological, genetic and epigenetic aspects of obesity as well as both positive and negative outcomes of rapid weight loss. Finally, the chapters collected under Part 4, *Adipose Tissue and Disease*, explain mechanisms by which obesity and adipose tissue dysfunction increase risk of various pathologies, from diabetes to cancer.

This volume is expected to serve as a useful resource not only for physicians interested in adipose tissue biology but also for basic scientists who want to know more about applied aspects of the field. The book specifically targets endocrinologists, residents and fellows, internists, nutritionists and general practitioners who are exposed to an ever-expanding obese population and need access to relevant, updated research results collected in one place.

Chicago, IL, USA

Giamila Fantuzzi, Ph.D.
Carol Braunschweig, Ph.D.

Series Editor Page

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

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

Adipose Tissue and Adipokines in Health and Disease, *Second Edition*, edited by Giamila Fantuzzi, Ph.D. and Carol Braunschweig, Ph.D. clearly exemplifies the goals of the Nutrition and Health Series. The major driver of this unique and timely Second Edition is to provide the reader with the most recent objective, data-driven summaries of the current scientific understanding of the biochemical, physiological, and pathological relationships between the bioactive molecules synthesized by adipose tissue and their effects on other cells and tissues in the body. Within the past 5 years, since the first edition was published, there has been intensive interest in the obesity epidemic and numerous laboratory experiments and clinical studies published in scientific publications have linked the increased risk of obesity to the actions of the adipokines. Thus, it is of great value to the scientific community, health practitioners, graduate, and medical students to now have a volume that examines the totality of the evidence and also suggests avenues where future clinical studies can provide more definitive answers to questions concerning the role of adipose tissue and adipokines in human health and disease.

Adipose Tissue and Adipokines in Health and Disease, *Second Edition* represents the most comprehensive compilation of recent data on the critical drivers of caloric intakes in children and adults and the potential consequences of biochemical signals that result in overconsumption. The volume chapters examine the adverse effects to the heart, kidneys, brain, and overall metabolism as well as the potential risk of cancer associated with increased bodyweight. The expertise of the volume's editors

Giamila Fantuzzi and Carol Braunschweig helps the reader to understand the relevance of the endocrine functions of adipose tissue and the complex biochemical interactions associated with maintaining the "ideal" body weight.

Dr. Giamila Fantuzzi is a Professor in the Department of Kinesiology and Nutrition at the University of Illinois at Chicago. From 2000 to 2004 she was an Assistant Professor in the Department of Medicine at the University of Colorado Health Sciences Center. Dr. Fantuzzi is a graduate of the University of Milano, Italy, where she obtained a Ph.D. in Experimental Endocrinology. She completed her postdoctoral fellowships in the laboratory of Neuroimmunology at the Mario Negri Institute for Pharmacological Research in Milano, Italy and in the Division of Geographic Medicine and Infectious Diseases at the New England Medical Center of Tufts University in Boston, MA. Dr. Fantuzzi has published extensively on the role of cytokines, adipokines, and adipose tissue in the regulation of inflammation. Her current research focuses on the role of adipose tissue in regulating inflammation in the pancreas and the gastrointestinal tract. Her research is currently funded by the National Institutes of Health and she received past funding from the Cystic Fibrosis Foundation, the Crohn's and Colitis Foundation of America, the Broad Medical Research Program, and the National Pancreas Foundation. Dr. Fantuzzi is an active member of several scientific societies and serves as an appointed member of the Tumor Microenvironment Study Section of the US Center for Scientific Review.

Dr. Carol Braunschweig is a Professor and Associate Head in the Department of Kinesiology and Nutrition at the University of Illinois at Chicago. Dr. Braunschweig received her BS and MS in Nutrition at Michigan State University and her Ph.D. in Epidemiology at the University of Michigan. Prior to her work in academia she was a nutrition support specialist in the Department of Pharmacy at the University of Michigan. Dr. Braunschweig's current research focuses on the role of nutritional intake and body composition on disease risks and outcomes in diverse populations including minority, the disabled, children, and hospitalized patients. Her research is currently funded by the National Institutes of Health and she received past funding from the American Cancer Society, Center for Disease Control, and the Department of Health and Human Services. She is an active member of several scientific societies and is also the Director of Clinical Nutrition at the University of Illinois Center for Clinical and Translational Sciences in Chicago, IL.

This 22 chapter volume is organized into four parts including six chapters within the first part on the basics of adipose tissue structure and function; four chapters in the second part on adipose tissue inflammation and adipocyte dysfunction in obesity; four chapters in the third part on obesity; and a final part that includes eight chapters that review the role of adipose tissue in chronic diseases.

The overview section on the structure and function of adipose tissue begins with a chapter that examines the relevance of the development of adipose tissue in evolution. This is especially important as the chapter helps us to understand the recent global trend towards obesity in humans. We learn that our closest primate relatives also have the potential to store fat in adipose depots for later utilization, and there is strong evidence that captive primates become fat when too much food and not enough exercise are provided. The importance of fat for the development of the human brain, reproduction, and immune system function is reviewed. The second chapter describes the development of white adipose tissue, its formation in adulthood, the process of differentiation of the adipocyte stem cells, and the new data that suggests that there are physiological as well as genetic differences in the adipose tissue that is deposited in regional fat depots throughout the body. The role of adipose tissue in the development of obesity is also reviewed in this chapter that includes more than 150 relevant references. The next chapter describes the metabolic events within white adipose tissue including the formation and breakdown of triglycerides and cholesterol, lipid droplet metabolic activities, microvesicles, and the other factors that stimulate white adipose tissue metabolism. There is an in-depth review of the lipases and other enzymes and bioactive molecules involved in fat metabolism in adipose tissue. The chapter includes over 200 recent references. The fourth chapter in this section examines the

concept of metabolically healthy obesity and reviews the relevant animal models used to describe the distribution of fat and its metabolic consequences that are associated with reduced risk of inflammatory responses associated with obesity. The authors describe the epidemiology of this population of apparently metabolically healthy obese individuals and their significantly lower incidence of cardiovascular disease and diabetes. Chapter 5 examines the important changes in white adipose tissue with aging and its consequences to fat metabolism and overall health in the elderly. The chapter includes a detailed description of the age-associated decrease in subcutaneous white adipose tissue and the consequent alteration in the balance between it and the inflammation-provoking visceral adipose tissue. The final chapter in this section describes the two best characterized adipokines, leptin and adiponectin, their synthesis in adipocytes, the stimuli for their synthesis, their actions, and the consequences of imbalance in the face of obesity. The chapter emphasizes the role of these two key adipokines in regulating inflammatory and immune responses that act as critical mechanisms to link nutritional status and adiposity to several pathologies including infectious and autoimmune diseases, diabetes, cardiovascular disease, and cancer.

The second part contains four chapters that explore adipocyte dysfunction and adipose tissue inflammation in the obese individual. Chapter 7 examines the sources of inflammatory molecules and cells associated with the inflammation that include adipose tissue and other tissues that are activated in response to obesity. A number of intracellular organelles participate in the synthesis of adipokines and other cell regulators. The endoplasmic reticulum is one of the critically important organelles involved in lipid synthesis, glucose metabolism, and protein processing. The next two chapters describe the role of the endoplasmic reticulum and effects of endoplasmic reticulum stress that may cause an aberrant unfolded protein response in adipose tissue. Recent data point to a link between adipocyte maturation and control of fat deposition. The next chapter describes the process known as autophagy which can be initiated when there is an increase in endoplasmic stress. Autophagy is a mechanism for intracellular degradation of cytoplasmic components including macromolecules and organelles. The chapter includes a detailed description of the core processes and genetic factors involved in adipocyte autophagy. There is a review of the recent data that suggest that normal adipose cell formation and adipocyte homeostasis are dependent upon autophagy. The last Chapter 10 describes the consequences of micronutrient deficiencies on adipose tissue and includes over 250 references. Micronutrient deficiencies in obese individuals may be due to an increased requirement related to the increased body size; there may be decreased absorption; metabolism may be altered as a result of the ongoing inflammatory process; and there may be increased deposition and sequestration of especially fat-soluble essential nutrients in the larger than normal mass of adipose tissue. The chapter reviews the resultant physiological changes that have the potential to promote increased fat deposition and increased risk of chronic diseases including cardiovascular disease, type 2 diabetes, and cancer. Specific micronutrients that are reviewed include vitamins A, C, and D, iron, selenium, and zinc.

The third part contains four chapters that examine the broad field of obesity with emphasis on its epidemiology in children, the genetics of obesity, and a separate chapter on the epigenetics and a final chapter on the physiological consequences of rapid weight loss. Chapter 11 describes the different reference standards used by nations to classify children as obese. Childhood obesity is considered a global public health crisis, and the prevalence is increasing in many parts of the world. Globally, the prevalence of overweight and obesity has increased in preschool age children, from approximately 4 % in 1990 to 7 % in 2010. The authors provide a strong rationale for the need for national policies and programs to combat the obesity epidemic. The genetics of obesity is complex and continues to include a host of genetic factors such as discrete genetic defects or chromosomal abnormalities that are both autosomal and X-linked disorders, multiple gene involvement, and alterations to small and large segments of the genome. The chapter includes a detailed description of polygenic obesity studies that include three main approaches: candidate gene studies, genome-wide linkage, and genome-wide association studies. The authors indicate that about 20 loci are consistently associated with

obesity-related traits in obese adults. In children, the most important locus discovered was in the "fat mass and obesity-associated" gene. In addition to the complexities associated with genetic effects on the risk of obesity, there are environmental factors that can affect genetic functions. The next chapter examines several nutritional factors that are known to influence epigenetic phenomena including DNA methylation, histone modifications, noncoding RNA expression, and chromatin remodeling mechanisms. These epigenetic factors include transcriptional regulatory pathways and phenotypic plasticity. The evidence concerning the role of epigenetic phenomena in obesity development and early life exposure to environmental/nutritional factors is reviewed. Chapter 14, the final chapter in this section on obesity discusses the overall effects of weight loss for the obese patient. The chapter reviews both nonsurgical and surgical strategies for slow and rapid weight loss, respectively. The chapter emphasizes the beneficial effects of bariatric surgery including rapid and significant weight loss and improvements in metabolic diseases including diabetes in a relatively short time period. Detailed descriptions of the many other potential benefits of bariatric surgery are included, and an equally detailed discussion of potential adverse effects of the surgery itself as well as the gastrointestinal changes that can affect nutritional status is also reviewed.

The fourth and last part in the volume examines the importance of adipose tissue and its effects in disease states. There are eight chapters that include an examination of the effects of human lipodystrophy, adipose tissue's role in type 2 diabetes, adipokines, and nonalcoholic fatty liver disease, and other chapters on cardiovascular disease, kidney disease, joint disease, and finally, cancer. Lipodystrophy is a disease characterized by the lack of adipose tissue. The cause is usually due to genetic defects; however recently HIV-infected patients who have been treated with protease inhibitors have also shown symptoms of this disease. Chapter 15 includes a detailed description of the genetic lipodystrophies that are inherited as autosomal dominant or recessive traits. The genetic loci that affect differentiation of adipose tissue or lipid storage are reviewed and illustrated in relevant figures. The clinical effects of the lack of adipose tissue include insulin resistance, hypertriglyceridemia, fatty liver, and diabetes. The author explains that this disease has provided evidence of a role for adipose tissue at the center of energy homeostasis and has helped to identify a number of new genetic loci which affect adipogenesis and/or lipid storage. The next chapter examines the effects of obesity and how these may worsen the adverse effects and/or hasten the development of type 2 diabetes. The risk of type 2 diabetes is increased exponentially when body mass index is above the overweight category. However, not every patient with type 2 diabetes is obese or vice versa. The authors indicate that obesity is associated with insulin resistance as is type 2 diabetes. Obesity results in increased production of adipokines/cytokines, excess nutrient consumption, ectopic fat deposition, mitochondrial dysfunction, and impairment of certain brain functions involved in the regulation of energy homeostasis. Obesity can also adversely affect insulin sensitivity in the liver and result in chronic inflammation. In type 2 diabetes, there is a destruction of beta cell function. Another serious disease that is associated with obesity and worsened by obesity's proinflammatory adipokines is nonalcoholic fatty liver disease. The authors of Chap. 17 indicate that nonalcoholic fatty liver disease represents a spectrum of pathological conditions characterized by significant lipid deposition in the liver of patients who do not consume excessive amounts of alcohol. The disease includes steatosis and the most severe forms: nonalcoholic steatohepatitis and related cirrhosis, and hepatocellular cancer. The link between nonalcoholic fatty liver disease and obesity is described in detail and extensively referenced in over 250 citations. Major factors include the decreased production of adiponectin and increased and/or ineffective production of leptin, other adipokines, and inflammatory cytokines from adipose tissue such as TNF-α that are seen in obesity. The next chapter on the association between obesity and cardiovascular disease reminds us that obesity is a leading modifiable risk factor of cardiovascular disease. The authors indicate that obesity is associated with premature atherosclerosis, increased myocardial infarction, hypertension and heart failure risk, and cardiovascular deaths. A number of factors that contribute to cardiovascular disease in obesity including insulin resistance, hypertension, lipid

abnormalities, and premature coronary artery disease are reviewed. The proinflammatory state seen in obesity is considered to have a pathological role in cardiovascular disease progression. New weight-loss drugs, their mode of action, and effects on cardiovascular function are described. The next chapter examines the effects of obesity on the respiratory system and its consequences. Obesity is a major risk factor for the development of asthma; is associated with increased health care utilization in chronic obstructive pulmonary disease; is associated with decreased response to influenza vaccine; and is a significant risk factor for mortality from H1N1 influenza. Obesity is associated with pulmonary hypertension which may be linked to breathing at low lung volumes which increases airway resistance and predisposes to airway closure and expiratory flow limitation. Sleep apnea is also reviewed as there is increased risk with obesity.

Chapter 20 reviews the effects of obesity on kidney function as well as the effects of kidney function loss on the obese patient. As reviewed in detail in other chapters in this section, obesity directly affects the development and progression of type 2 diabetes, hypertension, and dyslipidemia; diabetes and hypertension are the two most common causes of renal impairment. Diabetes is considered a major causative factor in almost half of all cases of end-stage renal disease and need for kidney dialysis treatment. There is an in-depth review of the mechanisms involved in obesity's adverse effects on kidney function. Another major adverse effect of obesity is joint pain and osteoarthritis that are both examined in the next chapter. Obesity results in joint loading and obesity-related inflammatory processes, and obesity plays a major role in the pathogenesis of osteoarthritis in both weight-bearing and nonweight bearing joints. The knee, hand, and hip joints are particularly affected in obese women and are discussed in detail. Data are presented that link adipokines to osteoporosis development and progression. There is also a discussion of the benefits of weight loss. The last chapter in the volume reviews the data associating obesity with significant cancer risk. Obesity is a major risk factor for colon, esophageal, pancreatic, endometrial, kidney, and postmenopausal breast cancer. In addition, obesity significantly increases the cancer mortality rates of both men and women. The chapter describes the epidemiologic studies associating obesity with colon and breast cancer risk and the mechanisms by which adipose tissue and adipokines increase these risks. The link between the location of adipose tissues, waist circumference, waist-to-hip ratio, and specific adipose depots on cancer risk in the colon and breast are described and over 150 relevant references are included in the chapter.

The logical sequence of the sections enhances the understanding of the latest clinical and laboratory studies of adipocytes and their bioactive secretions as well as related cytokines and their functional effects on human metabolism. This unique volume serves as a critical resource for practice-oriented physicians, integrative health care practitioners, researchers involved in the genetics of adipose tissue, molecular and cellular biologists, physiologists and other academicians involved in the education of graduate students and postdoctoral fellows, medical students, interns and residents, allied health professionals, and nutritionists who are actively involved in providing data-driven recommendations on the role of adipose tissue in the health of their students, patients, and clients. The volume is of great importance as it contains balanced objective evaluations of the pathology of obesity and the newest data on the effects of the major adipokines including leptin and adiponectin as well as the more recently discovered adipokines and related cytokines that can affect the ability to lose weight either from dieting or bariatric surgery.

Adipose Tissue and Adipokines in Health and Disease, *Second Edition*, contains over 50 detailed tables and figures that assist the reader in comprehending the complexities of the interactions between the involuntary synthesis of adipokines and the consequences of the proinflammatory effects of the majority of these molecules. There are chapters that review in detail the chronic effects of obesity in children, teens, and adults who are at great risk for developing diabetes, nonalcoholic liver disease, bone and joint inflammation and deterioration, kidney disease as well as cardiovascular diseases, and certain cancers. There are in-depth discussions of the genetic aspects of fat metabolism and laboratory

animal models that help to elucidate the epigenetic factors that influence the risk of obesity-related adverse effects. Health professionals involved in the care of obese and overweight patients are provided balanced documentation and awareness of the newest research on the critical importance of maintaining optimal body weight throughout life. Hallmarks of the 22 chapters include keywords and bulleted key points at the beginning of each chapter, complete definitions of terms with the abbreviations fully defined, and consistent use of terms between chapters. There are over 2,300 up-to-date references; all chapters include a conclusion to highlight major findings. The volume also contains a highly annotated index.

This unique text, with chapters written by well-recognized, practice and research oriented investigators, provides practical, data-driven resources based upon the totality of the evidence to help the reader understand the basics of fat metabolism, adipokine biochemistry, and the consequences of acute and chronic dietary overconsumption in young children through adolescence and adulthood. The overarching goal of the editors is to provide fully referenced information to practicing health professionals and educators so they may have a balanced perspective on the value of assuring the best nutritional quality for their patients and clients.

In conclusion, *Adipose Tissue and Adipokines in Health and Disease*, *Second Edition*, provides health professionals in many areas of research and practice with the most data-driven, up-to-date, well-referenced and comprehensive volume on the current state of the science and medical practice with regard to the nutritional care of patients and clients who want to understand the rationale behind the increased health risks associated with being overweight or obese. The volume will serve the reader as the most authoritative resource in the field to date and is a very welcome addition to the Nutrition and Health Series.

Adrianne Bendich, Ph.D., F.A.C.N., F.A.S.N.
Series Editor

About the Series Editor

Dr. Adrianne Bendich, Ph.D., FASN, FACN has served as the "Nutrition and Health" Series Editor for over 15 years and has provided leadership and guidance to more than 100 editors that have developed the 60+ well respected and highly recommended volumes in the Series.

In addition to "Adipose Tissue and Adipokines in Health and Disease," edited by Giamila Fantuzzi and Carol A. Braunschweig, major new editions in 2013–2014 include:

1. *Integrative Weight Management* edited by Dr. Gerald E. Mullin, Dr. Lawrence J. Cheskin, and Dr. Laura E. Matarese, 2014
2. *Nutrition in Kidney Disease, Second Edition* edited by Dr. Laura D. Byham-Gray, Dr. Jerrilynn D. Burrowes, and Dr. Glenn M. Chertow, 2014
3. *Handbook of Food Fortification and Health, volume I* edited by Dr. Victor R. Preedy, Dr. Rajaventhan Srirajaskanthan, and Dr. Vinood B. Patel, 2013
4. *Handbook of Food Fortification and Health, volume II* edited by Dr. Victor R. Preedy, Dr. Rajaventhan Srirajaskanthan, and Dr. Vinood B. Patel, 2013
5. *Diet Quality*: *An Evidence-Based Approach, volume I* edited by Dr. Victor R. Preedy, Dr. Lan-Ahn Hunter, and Dr. Vinood B. Patel, 2013
6. *Diet Quality*: *An Evidence-Based Approach, volume II* edited by Dr. Victor R. Preedy, Dr. Lan-Ahn Hunter, and Dr. Vinood B. Patel, 2013

7. *The Handbook of Clinical Nutrition and Stroke*, edited by Mandy L. Corrigan, MPH, RD; Arlene A. Escuro, MS, RD; and Donald F. Kirby, MD, FACP, FACN, FACG, 2013
8. *Nutrition in Infancy, volume I* edited by Dr. Ronald Ross Watson, Dr. George Grimble, Dr. Victor Preedy, and Dr. Sherma Zibadi, 2013
9. *Nutrition in Infancy, volume II* edited by Dr. Ronald Ross Watson, Dr. George Grimble, Dr. Victor Preedy, and Dr. Sherma Zibadi, 2013
10. *Carotenoids and Human Health*, edited by Dr. Sherry A. Tanumihardjo, 2013
11. *Bioactive Dietary Factors and Plant Extracts in Dermatology*, edited by Dr. Ronald Ross Watson and Dr. Sherma Zibadi, 2013
12. *Omega 6/3 Fatty Acids*, edited by Dr. Fabien De Meester, Dr. Ronald Ross Watson, and Dr. Sherma Zibadi, 2013
13. *Nutrition in Pediatric Pulmonary Disease*, edited by Dr. Robert Dumont and Dr. Youngran Chung, 2013
14. *Magnesium and Health*, edited by Dr. Ronald Ross Watson and Dr. Victor R. Preedy, 2012
15. *Alcohol, Nutrition and Health Consequences*, edited by Dr. Ronald Ross Watson, Dr. Victor R. Preedy, and Dr. Sherma Zibadi, 2012
16. *Nutritional Health, Strategies for Disease Prevention, Third Edition*, edited by Norman J. Temple, Ted Wilson, and David R. Jacobs, Jr., 2012
17. *Chocolate in Health and Nutrition*, edited by Dr. Ronald Ross Watson, Dr. Victor R. Preedy, and Dr. Sherma Zibadi, 2012
18. *Iron Physiology and Pathophysiology in Humans*, edited by Dr. Gregory J. Anderson and Dr. Gordon D. McLaren, 2012

Earlier books included "*Vitamin D, Second Edition*" edited by Dr. Michael Holick; "Dietary Components and Immune Function" edited by Dr. Ronald Ross Watson, Dr. Sherma Zibadi, and Dr. Victor R. Preedy; "Bioactive Compounds and Cancer" edited by Dr. John A. Milner and Dr. Donato F. Romagnolo; "Modern Dietary Fat Intakes in Disease Promotion" edited by Dr. Fabien De Meester, Dr. Sherma Zibadi, and Dr. Ronald Ross Watson; "Iron Deficiency and Overload" edited by Dr. Shlomo Yehuda and Dr. David Mostofsky; "Nutrition Guide for Physicians" edited by Dr. Edward Wilson, Dr. George A. Bray, Dr. Norman Temple, and Dr. Mary Struble; "Nutrition and Metabolism" edited by Dr. Christos Mantzoros; and "Fluid and Electrolytes in Pediatrics" edited by Leonard Feld and Dr. Frederick Kaskel. Recent volumes include "Handbook of Drug-Nutrient Interactions" edited by Dr. Joseph Boullata and Dr. Vincent Armenti; "Probiotics in Pediatric Medicine" edited by Dr. Sonia Michail and Dr. Philip Sherman; "Handbook of Nutrition and Pregnancy" edited by Dr. Carol Lammi-Keefe, Dr. Sarah Couch, and Dr. Elliot Philipson; "Nutrition and Rheumatic Disease" edited by Dr. Laura Coleman; "Nutrition and Kidney Disease" edited by Dr. Laura Byham-Gray, Dr. Jerrilynn Burrowes and Dr. Glenn Chertow; "Nutrition and Health in Developing Countries" edited by Dr. Richard Semba and Dr. Martin Bloem; "Calcium in Human Health" edited by Dr. Robert Heaney and Dr. Connie Weaver; and "Nutrition and Bone Health" edited by Dr. Michael Holick and Dr. Bess Dawson-Hughes.

Dr. Bendich is President of Consultants in Consumer Healthcare LLC, and is the editor of ten books including "Preventive Nutrition: The Comprehensive Guide for Health Professionals, Fourth Edition" coedited with Dr. Richard Deckelbaum (www.springer.com/series/7659). Dr. Bendich serves on the Editorial Boards of the *Journal of Nutrition in Gerontology and Geriatrics and Antioxidants*, and has served as Associate Editor for *Nutrition* the international journal; served on the Editorial Board of the *Journal of Women's Health and Gender-Based Medicine*, and served on the Board of Directors of the American College of Nutrition.

Dr. Bendich was Director of Medical Affairs at GlaxoSmithKline (GSK) Consumer Healthcare and provided medical leadership for many well-known brands including TUMS and Os-Cal. Dr. Bendich had primary responsibility for GSK's support for the Women's Health Initiative (WHI)

intervention study. Prior to joining GSK, Dr. Bendich was at Roche Vitamins Inc. and was involved with the groundbreaking clinical studies showing that folic acid-containing multivitamins significantly reduced major classes of birth defects. Dr. Bendich has coauthored over 100 major clinical research studies in the area of preventive nutrition. She is recognized as a leading authority on antioxidants, nutrition and immunity and pregnancy outcomes, vitamin safety, and the cost-effectiveness of vitamin/mineral supplementation.

Dr. Bendich received the Roche Research Award, is a *Tribute to Women and Industry* Awardee, and was a recipient of the Burroughs Wellcome Visiting Professorship in Basic Medical Sciences. Dr. Bendich was given the Council for Responsible Nutrition (CRN) Apple Award in recognition of her many contributions to the scientific understanding of dietary supplements. In 2012, she was recognized for her contributions to the field of clinical nutrition by the American Society for Nutrition and was elected a Fellow of ASN. Dr. Bendich is Adjunct Professor at Rutgers University. She is listed in Who's Who in American Women.

About the Volume Editors

Dr. Giamila Fantuzzi, Ph.D., is a Professor in the Department of Kinesiology and Nutrition at the University of Illinois at Chicago. From 2000 to 2004 she was an Assistant Professor in the Department of Medicine at the University of Colorado Health Sciences Center.

Dr. Fantuzzi is a graduate of the University of Milano, Italy, where she obtained a Ph.D. in Experimental Endocrinology. She completed her postdoctoral fellowships in the laboratory of Neuroimmunology at the Mario Negri Institute for Pharmacological Research in Milano, Italy and in the Division of Geographic Medicine and Infectious Diseases at the New England Medical Center of Tufts University in Boston, MA.

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Dr. Carol Braunschweig, Ph.D. is a Professor and Associate Head in the Department of Kinesiology and Nutrition at the University of Illinois at Chicago. Dr. Braunschweig received her BS and MS in Nutrition at Michigan State University and her Ph.D. in Epidemiology at the University of Michigan. Prior to her work in academia she was a nutrition support specialist in the Department of Pharmacy at the University of Michigan. Dr. Braunschweig's current research focuses on the role of nutritional intake and body composition on disease risks and outcomes in diverse populations including minority, disabled, children, and hospitalized patients. Her research is currently funded by the National Institutes of Health and she received past funding from the American Cancer Society, Center for Disease Control, and the Department of Health and Human Services. She is an active member of several scientific societies and is also the Director of Clinical Nutrition, UIC Center for Clinical and Translational Sciences.

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We would also like to thank Series Editor Dr. Adrianne Bendich for her light, but firm, touch that has been instrumental in keeping us on track.

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Part I
Adipose Tissue: Structure and Function

Chapter 1
Adipose Tissue in Evolution

Karin Isler

Keywords Primates • Adaptation • Evolution • Adipose depots • Body fat content

Key Points

- To understand the recent global trend toward obesity in humans, we need an evolutionary perspective on the costs and benefits of storing fat in adipose depots. In general, wild-living monkeys and apes appear to be relatively lean compared to humans, but in captivity, some species tend to accumulate fat depots from lack of exercise and too much food. In short, there are two strategies to survive lean periods: cognitive flexibility (and thus a large brain) or physiological flexibility through storing fat. Humans combine these two usually exclusive strategies, which may have evolved together with our unique form of locomotion, a striding bipedal gait.

Introduction

For storage and later utilization of ingested energy, fat is the most efficient form, because 1 g of fat contains nine calories, whereas 1 g of protein or carbohydrates contains only four calories. Ever since Neel's [60] concept of a "thrifty genotype," the human ability to store fat in adipose depots has been related to our species' potential to withstand periodic famines. This ability, while being adaptive for subsistence cultures in a rough and highly variable Pleistocene environment, has become a maladaptation in modern societies due to the continuous availability of the preferred sweet and fatty foods. To understand the recent global trend toward obesity in humans, we need an evolutionary perspective on the costs and benefits of adipose depots (cf. [10, 68, 84]), which can be gained by looking at other mammals and, in particular, primates. Many animals living in natural habitats manage to meet the challenge of alternating periods of food scarcity and abundance by storing fat. Others rely more on skillful retrieval and extraction of hidden high-quality foods, and thus follow a strategy of cognitive instead of physiological flexibility. In this chapter, I will present comparative evidence for an almost unique human strategy to combine physiological and cognitive buffering of lean periods.

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Adipose Depots in Primates and Humans

In contemporary humans, the amount of body fat accounts for about 14 % of the total body weight in a healthy man and 24 % in a woman [61]. This high amount of stored fat is not entirely due to modern, industrial lifestyle, as even in hunter-gatherer populations, or subsistence cultures inhabiting harsh environments, body fatness in women is around 20 % [47]. The published information on body fat content of wild mammals is limited (Fig. 1.1), but it is evident that humans lie in the upper range of the distribution. In general, large animals store more fat relative to their energy throughput, as metabolic rates exhibit a negatively allometric relationship with body mass [44]. Large animals can not only thrive on foods of low energy density (e.g., herbivore ungulates, elephants) but also tolerate fluctuations of energy input better than small animals (cf. [23]).

We humans belong to the order of Primates, and we are most closely related to monkeys, and in particular to apes such as chimpanzees, bonobos, gorillas, and orangutans. Pond [64] demonstrated that nonhuman primates and humans share a unique distribution of fat deposits, in that they accumulate a "paunch," which consists of subcutaneous and intra-abdominal deposits of fat in the anterior abdominal region. Both deposits increase disproportionately with adiposity through adipocyte proliferation [66]. The relative amount of body fat is known for some monkeys and lemurs [59, 63, 66], but only very few ape specimens [88–90], as complete cadavers of apes are very difficult to obtain. Postmortem examinations, although crucial for the welfare of these valuable animals in captivity, usually destroy the most information on abdominal fat deposits [90]. Therefore, we cannot infer whether the last common ancestor of African apes and humans already possessed an increased ability to store fat [64]. Nevertheless, although the amount of adipose tissue in captive apes may be a gross overestimation of even the fattest individuals in wild settings (cf. [90]), the potential to store fat in adipose depots for later utilization is clearly present in our relatives. There is ample evidence of captive primates becoming fat with too much or the wrong sort of food and not enough exercise [21, 65, 89, 90]. To assess whether or not it is feasible to use this potential in the wild, indirect measures of fatness such as variation in body mass or the excretion of ketone bodies in the urine are more readily available than direct data obtained from dissections.

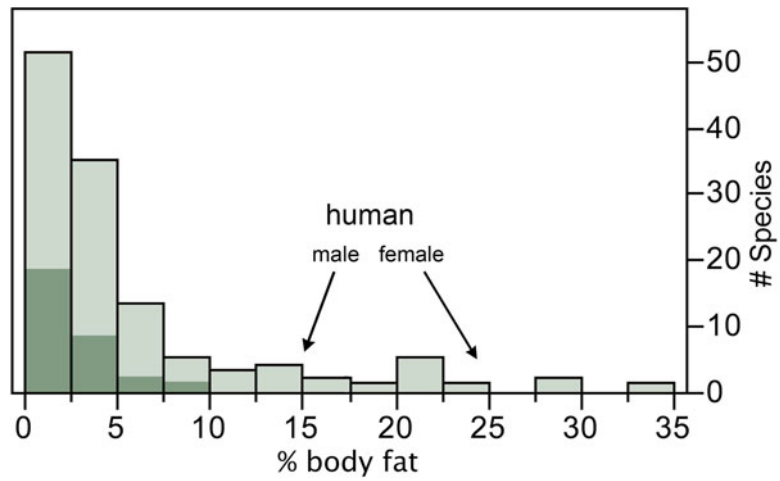

Fig. 1.1 Adipose depots mass as percentage of total body mass in a sample of 123 species of mammals (data from [59], complemented with data from [65] for large mammals). Nonhuman primates are *dark shaded*. Human males and females are indicated with an *arrow*

In general, wild-living monkeys and apes appear to be relatively lean compared to humans [2]. But wild baboon females feeding from human garbage dumps had 23.2 % body fat compared with 1.9 % for their wild-feeding counterparts [6]. Inferred by differences in body mass between wild and captive anthropoid primates [49], some species seem to have a higher potential to store fat in adipose depots than others. Bornean orangutans suffer from periodic food shortages due to unpredictable El Niño events in some years. During these lean periods, they are in negative energy balance, as measured by the presence of ketones in their urine [45]. In captivity, Bornean orangutans are most prone to becoming obese, unless put on a very strict high-fiber low-calorie diet [19, 89]. In wild chimpanzees, urinary C-peptide measurements revealed also a fluctuating metabolic activity [22], although the presence of ketones was not found to be linked to food shortages [48]. Similarly, some baboon and macaque species inhabit very seasonal habitats such as the Atlas Mountains in Morocco, and develop obesity and diabetes much like humans if subjected to a nutrient-dense food regime (e.g., [13, 15, 33, 74]). On the other hand, many Malagasy lemurs exhibit strong seasonal changes in their body mass [58], as they live off stored fat during extended periods of torpor during the dry season. One species is even named "fat-tailed dwarf lemur," as the storage is mostly located in the root of their tails. In sum, the potential to store fat in wild primates is most expressed in species that live in a highly seasonal habitat.

Fat storage serves a different purpose in males and females. In species with high male-male competition, males store more fat before the breeding season, in order to be able to forego feeding during the strenuous period of concerted estrous of the females. It has been shown that fatter males have more opportunities to mate than their thinner competitors [75]. Females, on the other hand, store fat mainly for reproduction, when they are in negative energy balance during the strenuous lactation period (in humans, e.g., [23, 55]). In smaller animals that complete one breeding cycle in less than a year, this amounts to a capital breeding strategy [80]. But if the breeding period is extended as in larger monkeys and especially in apes that exhibit very prolonged development periods compared to most other animals, a rapid utilization of adipose depots in females could be potentially disastrous. For example, orangutan mothers rear one offspring only every 7–9 years [79, 85], and lactation persists over the major part of this period. Offspring must grow very slowly to avoid starvation in lean periods. Therefore, the selective benefit of a higher amount of adipose depots, which is also more stable during short periods of reduced energy input, is most pronounced in females. These different functions may explain why retention of the breast, hip, and thigh depots in women is greater than that of the metabolically more active abdominal fat depots of men.

Cognitive Versus Physiological Buffering of Starvation

Beyond energy storage, fat is also likely to play a role in many other body functions, such as hormones and the immune system (e.g., [78]). Compared to most other organs, the human brain contains a large proportion of fat [86]. In particular, increased incorporation of long-chained poly-unsaturated fatty acids (LCPUFAs) has been associated with brain growth in human infants [26]. However, there are no consistent differences in the amount of LCPUFAs in breast milk between gorillas and humans [57]. To see whether human brain size may have been coevolving with genes promoting increased energy efficiency [16, 24], we must look for comparative evidence from brains of other species.

Within the animal kingdom, brain size varies tremendously between species for any given body size. As we humans arguably are the most encephalized species, i.e., exhibit the largest brains relative to the expected size for our body mass, explaining this variation in brain size has been a focus of scientific research for a long time. Numerous hypotheses have been put forward to explain why the human brain evolved to a size nearly three times as large as that of our closest living relatives, the chimpanzees (for an energetic viewpoint, see, e.g., [2, 50]). The critical issue, however, is how to test these hypotheses, as an experimental approach is not feasible for this kind of ultimate questions.

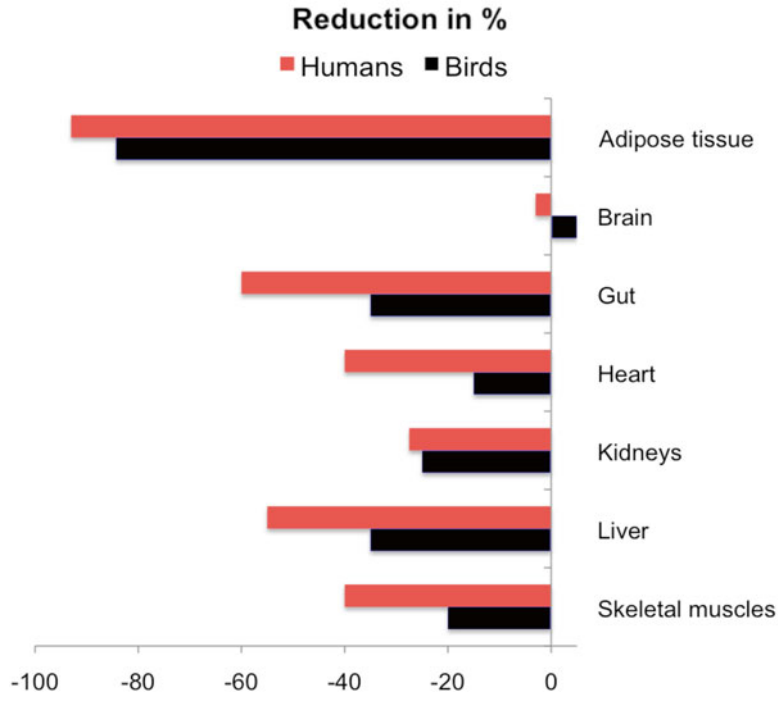

Fig. 1.2 Reduction of tissue mass during long-distance migration in birds (*Calidris tenuirostris*, [9]) and during famine in humans (after data from [71])

In particular, proximate mechanisms of links between traits may be quite different from the trends observed on an evolutionary timescale, as selection may favor trait combinations with a high fitness benefit regardless of any direct causal links on the proximate level. Moreover, selection experiments are restricted to short-lived model organisms such as *Drosophila* or small rodents, and are therefore likely to yield only limited insight in the evolution of long living, cognitively advanced species such as primates. As a consequence, broad-scale comparative studies on correlates of brain size evolution are needed, although such studies must be carefully validated (for a critique, see [31]).

If we aim to find patterns of correlated evolution in a broad array of species, we must take the different degrees of relationship between taxa. Otherwise, species-rich taxa groups or taxa with special adaptations can exert too much influence on the overall relationship. "Phylogenetically informed" methods have been refined in recent years [62], and allow us to remove the influence of similarity in traits due to shared ancestry through statistical procedures. Regarding brain size evolution, we have presented a coherent framework that covers all aspects of energetic costs of the brain, the Expensive Brain framework [36]. Brain tissue is among the most metabolically costly tissues in the body [72]. In particular, brains continuously use approximately the same amount of energy even during rest and REM sleep [54], and while during starvation almost all other organs shrink, brains remain spared (Fig. 1.2). During ontogeny, severe and permanent damage may result if the brain is undernourished (e.g., [52]).

Therefore, evolution of a relatively larger brain than its ancestor in a species requires that the increase in costs due to encephalization is met by some combination of the following two responses: an increase in total metabolic throughput or a redirection of the allocation of energy from other body functions. The first pathway to evolve a relatively large brain, stabilizing the amount of available energy on a higher level, has been shown to play a role at least in some lineages. Mammals and especially primates exhibit a positive correlation between basal metabolic rate (BMR) and brain size (controlling for effects of body mass and phylogenetic relatedness [34, 35]). Moreover, relatively large-brained anthropoid primates

experience less seasonality in their dietary consumption than would be expected from the seasonality of their habitats [83]. The second pathway, a redistribution of energy from other costly body functions, has only partly been confirmed by comparative studies. On one hand, a trade-off between production and brain size is confirmed by the tight correlations of various life history traits with brain size (e.g., [8, 36]), and by a relaxation of these correlations if energy subsidies from nonmothers are available for offspring production [37]. On the other hand, another widely accepted idea, the Expensive Tissue Hypothesis by Aiello and Wheeler ([3], see also [4]), proposed a trade-off between the energy consumption of the digestive tract and the brain. Originally, the hypothesis was put forward to explain human brain size within anthropoid primates, but later it was argued that such a trade-off between brain and digestive tract or other expensive organs should be found in other lineages as well [34, 35]. Supportive results were however found only in a study on three species of distantly related fish [42], but not in birds [34, 35], bats [39], and platyrrhine or strepsirrhine primates [7, 29]. We have recently been able to reject the validity of the Expensive Tissue Hypothesis as a general principle in mammals or primates with a new collection of mammalian organ mass data [59], but some argue that in the human case such a redirection may still apply [77]. Resolution of the remaining issues will require new data on the digestive tract of extant apes, as the currently available measurements are not conclusive with regard to the question whether the human gut is reduced in size compared to our relatives.

However, although costs of brain tissue must not be neglected, potential benefits of being large-brained and thus of having increased cognitive abilities [17] also play a role in explaining interspecific differences in relative brain size. To outweigh the costs of a relative increase in brain size, a larger brain must confer a fitness benefit to its bearer. This could be either due to a direct reduction of mortality by cognitive means (and thus ultimately a prolonged lifespan, e.g., [28]), an increased chance to reproduce [12, 43], or the production of more surviving offspring. Independent variables, such as niche characteristics and the inherited morphological or behavioral traits of a species, determine whether mortality can or cannot be reduced or avoided by cognitive means, and thus whether an increase in brain size and cognitive abilities can affect survival and reproduction, and become a selective advantage [81]. In lineages that pass this "life-history filter" of unavoidable mortality, such selective forces could apply to both the ecological and the social domain.

At present, the overlap between datasets on the various variables of interest is not sufficiently large to allow testing a model combining all energetic constraints with fitness benefits of brain size in mammals. The only studies so far that included both aspects investigated the effect of seasonality on brain size evolution in primates. In this study, energetic constraints and potential cognitive benefits of large brains were disentangled by looking at the difference between environmental and experienced seasonality [82, 83]. We found that catarrhine primates exhibit cognitive buffering [76], i.e., they exhibit a positive correlation between relative brain size and the difference between experienced seasonality and habitat seasonality [83]. Most primates change their diet composition in periods when preferred foods are scarce toward increased intake of so-called fallback foods (e.g., [45]), but relatively large-brained and thus cognitively more flexible monkeys and apes are still able to access some relatively high-quality foods, and thus counterbalance an energetic constraint of experienced seasonality on brain size by cognitive buffering.

In sum, there are two strategies to survive lean periods: cognitive or physiological flexibility. The second option is achieved through storing fat, reducing activity, and sometimes by decreasing metabolic rates as in hibernation or torpor (e.g., [32, 56, 73]). These two strategies are mostly exclusive, because the cost of transport of additional body fat is high in most animals. The costs are not only increased locomotor costs due to higher total body mass and a less optimal body geometry, but also increased predation risk (or decreased hunting success) due to less agility and speed.

We therefore would expect a negative correlation between relative brain size and the potential to store fat across mammals. Indeed, in a new dataset of mammalian organ mass and body composition data from dissection of 455 cadavers, we found a negative correlation between the amount of adipose depots and brain size, controlling for fat-free body mass and phylogeny [59]. The negative correlation was most pronounced in female specimens of wild origin (Fig. 1.3). In Primates, on the other hand, a significant negative correlation is found only in raw species means regression (Fig. 1.4), but not if methods to

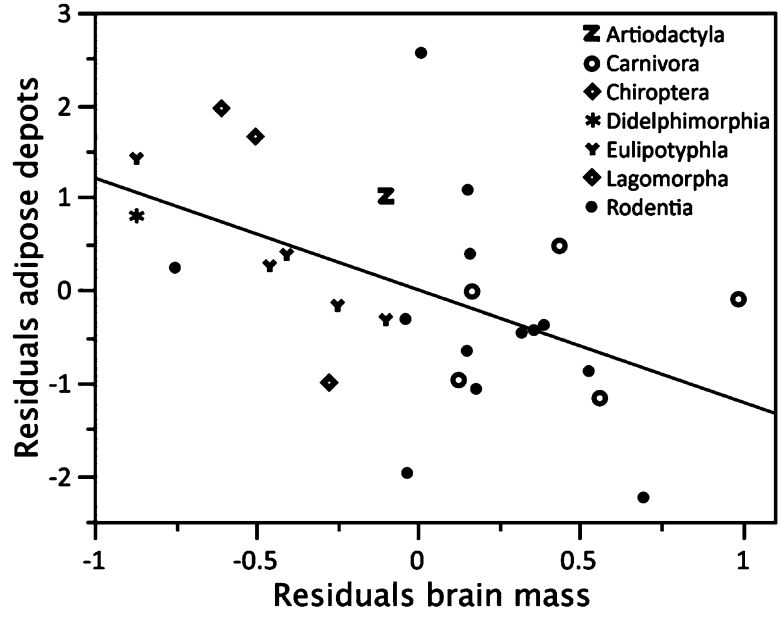

Fig. 1.3 Least-squares regression of adipose depots mass vs. brain size in wild adult female mammals, controlling for lean body mass. $N = 28$ species, $p = 0.006$, $r^2 = 0.26$. Data from Navarrete et al. [59]

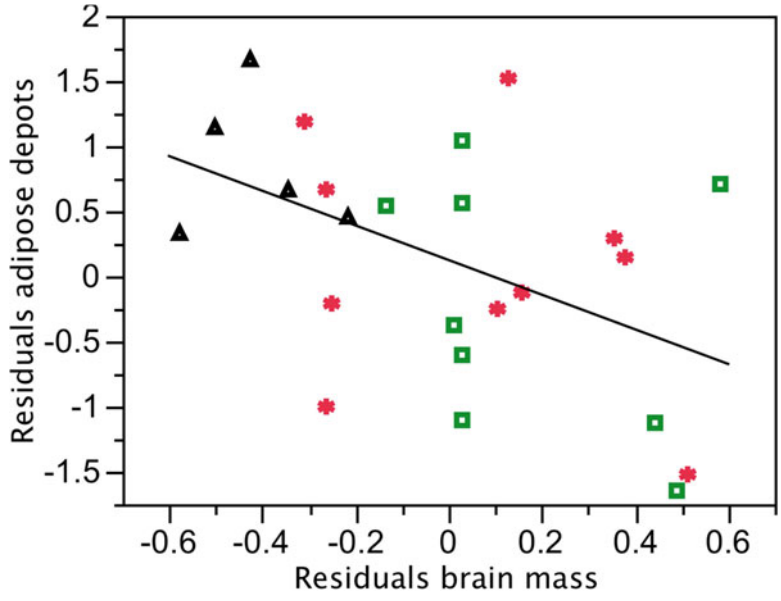

Fig. 1.4 Least-squares regression of brain size vs. adipose depots in primates, controlling for lean body mass. $N = 24$ species, $p = 0.018$, $r^2 = 0.23$. *Triangles*: lemurs, *squares*: platyrrhines, *stars*: catarrhines. Data from Navarrete et al. [59]

control for phylogenetic relationships are applied. We argued in the appendix of Navarrete et al. [59] that this merely reflects the insufficient quality of our sample. Due to practical limitations, we measured only abdominal depots in primates, and the number of specimens per species was usually only one, hinting at a problem with error variation in this group. Therefore, these results must be regarded as preliminary.

But how can humans combine the two usually exclusive strategies of physiological and cognitive buffering? Apart from humans, some marine or semiaquatic mammals exhibit both a relatively large brain and large adipose depots, such as whales and dolphins, seals, sea lions, and beavers. In aquatic locomotion, costs of transport do not increase with body mass if the body retains a geometrically similar, streamlined shape [5]. We hypothesized that costs of bipedal locomotion may increase less steeply with additional body mass than costs of quadrupedal locomotion [59], but this remains to be tested.

Developmental Aspects

In the evolutionary history of human adipose depots, developmental aspects should also be considered. In comparison with our closest living relatives, the great apes, human life history is characterized by relatively large, but immature neonates, a very short lactation period and interbirth interval, and a long postreproductive lifespan (e.g., [40]). Moreover, full-term human babies are born with relatively large adipose depots (15 % of total body mass), and continue to accumulate fat during early childhood [46]. Kuzawa [46] proposed that in addition to buffering against periods of food shortage, the increased amount of adipose depots is protecting human infants against alternating nutritional stress due to infections in the postweaning period. Catch-up recovery by rapid fat accumulation would be adaptive for small children, and be retained in adults.

However, new data on body fat content of neonate marmosets (15.2 %, [69]) call into question whether humans are really unique in this respect. Still, adipose depots of neonates seem to be positively correlated with relative brain size across mammals (Fig. 1.5), but the available data does not allow testing this correlation within orders. In primates, the high value of marmosets and humans hints at a potential role of allomaternal care for neonate fat depots, as callitrichid primates are the only

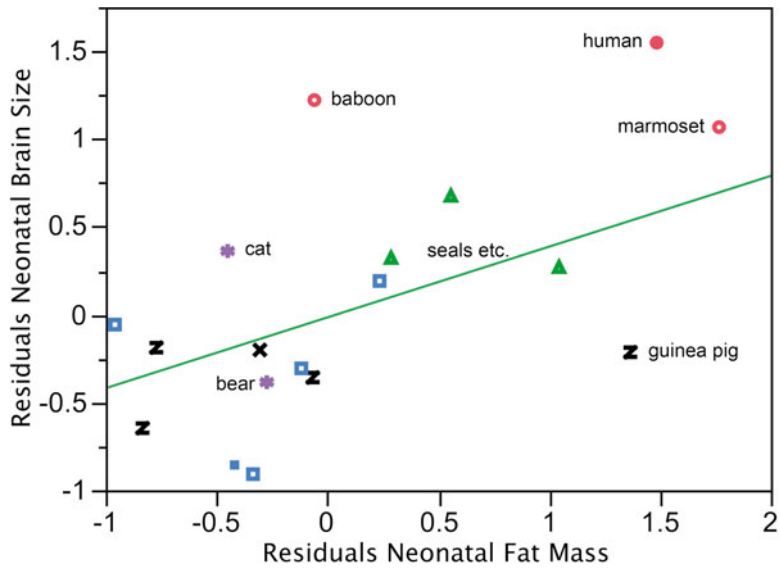

Fig. 1.5 Relative neonatal brain size correlates positively with relative neonatal fat mass ($N = 17$ species of mammals, least-squares regression, $p = 0.037$, $r^2 = 0.26$). Both variables are size-corrected by calculating their residuals against neonatal body mass from least-squares regressions. Humans were excluded from the calculation, but shown for comparison. Data of neonatal fat mass are from Kuzawa [46], Power et al. [69] for marmosets, and Mahan et al. [53] for pigs. Data for neonatal brain size are from Capellini et al. [14], and from DeSilva and Lesnik [18] for baboons

other true cooperative breeders within the order of primates. This means that, as in humans, both fathers and other group members, usually older siblings, help in carrying and provisioning the infants. In humans, postreproductive females also invest heavily in their grandchildren [30]. At the moment, it is however completely unclear why only human and marmoset mothers should be able to produce relatively fat offspring. Both buffering against famine and against periods of undernutrition due to illnesses should act as positive selective forces on infant fat mass also in other primates.

Conclusion

To sum up, comparative evidence suggests that we humans are, compared to most other monkeys and apes, well adapted for storing fat in adipose depots. However, it remains difficult to reconstruct the evolutionary history of this adaptation. The fossil record of early hominins has grown in the last years, and our family tree now comprises many species that were characterized by a mosaic of human-like and ape-like traits (e.g., [11, 51]). Paradoxically, reconstructing ancestral traits has become more ambiguous with the increasing number of fossil remains. One of the few unequivocal trajectories is the ever-increasing trend toward larger brains (Fig. 1.6). In early members of our own genus, *Homo*, the best known form of which is *Homo ergaster* from East Africa, brains were already so large that we must assume some form of allomaternal childcare [1, 38]. At the same time, the climate in Africa got more seasonal [20]. The ability to accumulate fat would have been a selective benefit in the more seasonal open environments occupied by *Homo ergaster* [2]. Moreover, several crucial components of human lifestyle such as cooperative hunting or defense, increased consumption of meat and bone marrow, extractive foraging techniques, and the use of fire evolved around that time (e.g., [27, 40, 87]).

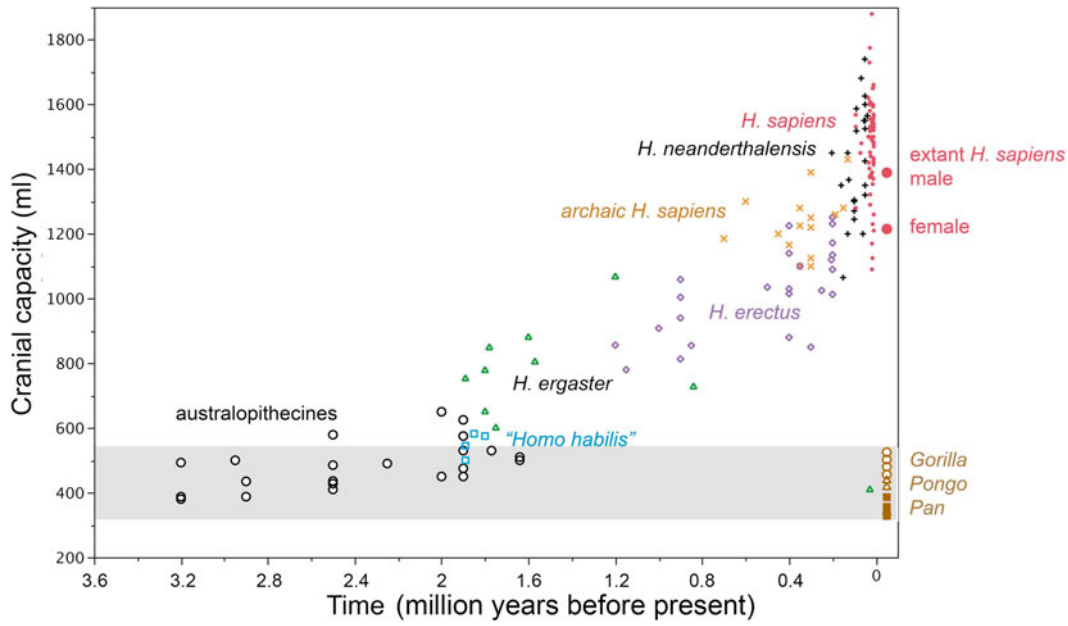

Fig. 1.6 Brain size as inferred from cranial capacity in fossil hominins. Data from Kappelman [41] and Rightmire et al. [70]. Note that Neanderthals and upper Paleolithic *Homo sapiens* on average had slightly larger brains than contemporary humans. The triangle next to the extant ape values represents the enigmatic insular taxon *Homo floresiensis* [25]

Early *Homo* ventured out of Africa for the first time, a fully modern striding gait evolved in *Homo* from earlier forms of bipedalism of australopithecines [67], and climbing trees was finally abandoned. It seems likely that the unique human combination of cognitive buffering of lean periods and increased potential to store fat in adipose depots evolved at this time, roughly two million years before present. Fat storage may have played an important role in the subsequent history of enormous brain expansion in Pleistocene *Homo*.

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Chapter 2
Adipose Stem Cells and Adipogenesis

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Keywords Adipose stem cells • White adipose tissue • Adipocytes • Adipogenesis • Obesity • Adipose tissue depots • Transcriptional control of adipocyte development

Key Points

- Although most development occurs during prenatal and early postnatal life, white adipose tissue retains the ability to expand during adult life, especially to accommodate energy surplus. Adipose tissue expansion occurs by increase of existing adipocytes' size or by recruiting new fat cells. Evidence in human subjects suggests that obesity complications result from the inability of subcutaneous adipose tissue to expand and safely store lipids, which leads to ectopic deposition in other tissues and insulin resistance due to lipotoxicity. This impaired expandability is due to the limited ability of adipose tissue progenitor cells to supply new adipocytes through their differentiation into specialized cells (adipogenesis). Therefore, understanding the mechanisms regulating adipogenesis is important not only for gaining insight into the pathogenesis of metabolic diseases but also for identifying targets for pharmacological interventions.
- Mature adipocytes develop from committed preadipocytes through a process termed terminal differentiation. The molecular regulation of white adipocyte terminal differentiation is extensively characterized via utilization of cell lines. However, the preceding process involves commitment of adipose stem cells (ASCs) to the adipocyte lineage with the loss of capacity to differentiate into other cell types, known as determination. Little information is known about the mechanisms that regulate the adipocyte commitment phase. Current investigations are focused on elucidating this poorly characterized step in adipocyte development. This chapter summarizes recent findings regarding the role of ASCs in adipogenesis.
- Convincing evidence for distinct depot-dependent populations of ASC pools is emerging, as adipocyte progenitors may contribute to regional variation in white adipose tissue function and development. Thus, a summary of depot-dependent differences in the gene expression patterns and cellular dynamic properties of adipocyte progenitor cells is presented.
- Finally, new lines of evidence analyzing how obesity impacts ASC abundance and functional potential are included.

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Abbreviations

ASCs	Adipose stem cells or adipose-derived stem cells
WAT	White adipose tissue
MSCs	Mesenchymal stem cells
SVF	Stromovascular fraction
ESCs	Embryonic stem cells
iPSC	Induced pluripotent stem cells
hMADS	Human multipotent adipose-derived stem
APCs	Adipocyte precursor cells
VAT	Visceral adipose tissue
SAT	Subcutaneous adipose tissue
Pref-1	Preadipocyte factor 1
COL6A2	Type VI collagen alpha 2 chain
FRP2/SFRP2	Frizzled-related protein 2
DIPA	Delta-interacting protein A
Zfp423	Zinc-finger protein 423
LXRα	Liver X receptor alpha
BMP(s)	Bone morphogenetic proteins
IGF-1	Insulin-like growth factor-1
FGF	Fibroblast growth factors
Lox	Lysyl oxidase
Gpc4	Glypican 4
Nr2f1	Nuclear receptor subfamily 2 group F member
Shox2	Short stature homeobox 2
En1	Engrailed 1
PBX1	Pre-B-cell leukemia transcription factor

Introduction

Adipocytes are highly specialized cells that form and store fat in adipose tissue and play a major role in energy homeostasis in vertebrate organisms. Obesity results from an energy surplus and is characterized by an increased storage of lipid and expansion of adipose tissue. Obesity modifies the endocrine and metabolic functions of adipocytes and is a risk factor for many other metabolic diseases, including type II diabetes, cardiovascular ischemic disease, atherosclerosis, and hypertension.

Although most development occurs during prenatal and early postnatal life (reviewed in [1]), white adipose tissue (WAT) retains the ability to expand during adult life, especially to accommodate energy surplus. Adipose tissue expansion occurs in two ways—by increase of existing adipocytes' size (hypertrophy) or by recruiting new fat cells (hyperplasia). Accumulating evidence in human subjects suggests that obesity complications result from the inability of subcutaneous adipose tissue to expand and safely store lipids, which leads to ectopic deposition in other tissues and insulin resistance due to lipotoxicity. This impaired expandability is due to the limited ability of adipose tissue progenitor cells to supply new adipocytes through their differentiation into specialized cells (adipogenesis) (reviewed in [2]) [3–6]. Hence, in order to support expansion of adipose tissue mass and to maintain adipose dynamics in adults, proliferative adipocyte precursor cells (APCs) must exist to accommodate metabolic demands. Furthermore, recent studies by Spalding et al. suggest that approximately 10 % of the body's adipocytes

are regenerated each year [7]. In addition, adipocyte number can increase during the development of obesity, despite a higher rate of apoptosis [8]. Therefore, an adipocyte precursor pool is thought to remain present in adipose tissue during adult life and contribute to the renewal of new, mature adipocytes. Very few data is available regarding the nature of APCs, including commitment to the preadipocyte, as well as the processes that control adipose conversion and formation of new adipocytes in human adult adipose tissue. Understanding the origin of adipocyte precursors, as well as adipocyte differentiation, is relevant not only for gaining insight into the pathogenesis of metabolic diseases but also for identifying proteins or pathways which might be appropriate targets for pharmacological interventions. It is important to note that the developmental origin of white and brown fat is distinct, and different precursor cells are involved in the generation of these different types of adipose tissue (reviewed in [9]) [10].

The initial phase of white adipocyte differentiation is known as determination and involves the commitment of mesenchymal stem cells (MSCs) to the adipocyte lineage [11]. Determination results in the conversion of MSCs to preadipocytes, with the loss of capacity to differentiate into other cell types. Current investigations are focused on elucidating this poorly characterized step in adipocyte development. The second phase of adipogenesis is terminal differentiation, whereby preadipocytes assume the characteristics of mature adipocytes. Conversely, the molecular regulation of white adipocyte terminal differentiation is more extensively characterized via utilization of cell lines.

In recent years, much effort has been given to identify, isolate, and analyze APCs. Several laboratories have identified a source of multipotent stem cells, known as adipose-derived stem cells (ASCs) that are capable of proliferation and differentiation into multiple lineages in vitro and in vivo, including adipocytes, osteoblasts, chondrocytes, and myocytes [12–20]. ASCs have been defined by a variety of other terms, including the following: processed lipoaspirate cells, adipose-derived stromal cells, adipose-derived mesenchymal progenitor cells, and stromovascular fraction (SVF) (reviewed in [21]). Isolated ASCs have been shown to confer multiple lineages; however, the ability of ASCs to form tissues in vivo under specific experimental conditions may not accurately reflect their multilineage capacity in physiological contexts. Hence, it remains to be determined whether native ASCs within WAT behave in the same manner. In this chapter, we will review recent findings highlighting the role of ASCs in adipogenesis with a focus on the adipocyte commitment phase. We will also evaluate the influence of regional adipose tissue distribution as well as obesity on ASC biology.

Research Tools to Study Adipogenesis

Interestingly, the majority of studies that have identified molecular pathways and transcriptional regulators involved in adipogenesis have been performed in vitro using well-characterized cellular models. These studies have been primarily conducted in the 3T3-L1 or 3T3-F442A murine preadipocyte cell lines that were originally generated in the laboratory of Dr. Howard Green at Harvard University [22, 23]. In the last 37 years, these cells lines have been used by thousands of investigators worldwide. These clonal cell lines possess the properties of adipocytes in vivo and are homogeneous in regards to cellular population and differentiation stage, which allows a uniform response to treatments. In addition, these cells can be passaged indefinitely. The preadipocyte cell lines developed by Dr. Green have been extremely useful model systems for adipocyte biologists, and the data obtained in these cells have been validated from less mechanistic in vivo studies in the last decade.

Though cell culture systems have been useful to investigate adipogenesis, there are limitations of in vitro cellular models. In vivo adipocytes do not exist as a monolayer of identical cells, but in a complex environment comprised of various other cell types and influential factors within an extracellular matrix. In addition, cell lines are already committed to the preadipocyte lineage, and therefore cannot be

utilized to examine preadipocyte commitment phases. Despite substantial progress in defining adipogenic transcriptional control mechanisms, there is little in vivo information regarding the processes that regulate the commitment of adipose tissue-derived stem cells to a defined adipocyte lineage or the development of adipocyte progenitors into adipocytes.

An alternative approach for analyzing adipocyte commitment is the use of embryonic stem cells (ESCs) derived from the inner cell mass of mouse blastocysts. ESCs are able to differentiate into various lineages; therefore, pretreatment with retinoic acid (RA) is necessary to facilitate commitment to the adipose lineage and subsequent differentiation into adipocytes with adipogenic hormones [24]. Though ethical issues in extracting ESCs from human subjects limit their use in a clinical context, many laboratories utilize rodent ESCs to acquire valuable information regarding adipocyte development. Conversely, novel ESC-like pluripotent cells, termed induced pluripotent stem cells (iPSC), were generated from human skin fibroblasts by introducing various transcription factors (Oct3/4, Sox2, Klf4, and c-Myc) [25–27]. Hence, the generation of iPSC offers a method of analyzing human precursor cells by overcoming the immunological and ethical problems associated with ESCs. Although iPSC were shown to undergo adipogenesis [28], these induced cells are not a homogeneous adipocyte precursor population and have low adipogenic potential compared to other human adipose tissue-derived cells.

Additional stem cell lines have yielded valuable information regarding adipocyte development. The multipotent cell line of C3H/10T1/2 fibroblasts represents another good model to study adipocyte commitment, as in vitro exposure to 5-azacytidine, an inhibitor of methyltransferases, followed by adipogenic, chondrogenic, or myogenic stimuli can initiate differentiation into the respective mesenchymal cell type [29, 30]. Likewise, human multipotent adipose-derived stem (hMADS) cells are also a unique cell model to analyze adipocyte development [31], as they are isolated from the adipose tissue of young donors. These hMADS cells exhibit the characteristics of MSCs, i.e., the capacity to self-renew, as cells can be expanded in vitro for more than 160 population doublings (i.e., around 30 passages) while maintaining a normal diploid karyotype and multipotency at clonal level. These cells also have the capacity to differentiate into cells of the adipogenic, osteogenic, and myogenic lineages [19].

Primary preadipocytes, isolated and cultured from the SVF of adult adipose tissue explants, are able to proliferate and differentiate into mature adipocytes under appropriate adipogenic stimuli and can also be utilized for in vitro analysis of adipocyte development (reviewed in [32]) [33–36]. However, primary culture has disadvantages in that large amounts of adipose tissue are required, as preadipocytes comprise a small percentage of total fat tissue. Furthermore, preadipocytes are difficult to isolate from other fibroblast-like cells, and once isolated, have a limited life-span. Primary cultures also undergo a dramatic decrease in their ability to differentiate, and replicative senescence occurs with repeated subculturing. Nevertheless, primary preadipocyte cultures may more accurately represent adipose tissue function in vivo, as these cells are derived from an environment where various cell types and the natural milieu may influence differentiation and responsiveness. For instance, the proliferation and differentiation of both human and rodent primary preadipocytes have been shown to be influenced by the anatomic location of the depot as well as aging and gender [5, 33, 37–46].

Interestingly, several studies have demonstrated that mature adipocytes derived from adipose tissue have the ability to dedifferentiate in vitro into fibroblast-like stem cells by utilizing the ceiling culture technique [47–52]. Though dedifferentiated fat cells are a homogeneous mixture of adipocyte progenitors, it is unknown whether the level of dedifferentiation reaches that of native adipocyte progenitors or stem cells. Nevertheless, dedifferentiated fat cells can proliferate and differentiate into mature adipocytes both in vitro and in vivo [52] and, hence, can be a useful tool for studying in vivo adipogenesis.

The Characterization of Adipose Stem Cells

Characterization of ASCs has yielded conflicting findings, partially due to differences in isolation and culture techniques [53]. Notably, one cannot distinguish between ASCs and committed preadipocytes in culture, due to the lack of bona fide markers. Therefore, one of the greatest issues limiting the progression of ASC clinical research is the lack of consistency among research groups in defining the term "ASC." Likewise, many laboratories use the crude SVF, which is a mixture of all cells comprising the adipose tissue, such as endothelial cells, smooth muscle cells, various immune cell types (neutrophils, mast cells, and macrophages), and adipocyte progenitor cells. Yet, researchers classify these cells as ASCs. Conversely, others utilize the expanded and passaged adherent cell population derived from the SVF, which are enriched with adipocyte progenitor cells. Hence, the crude SVF cells, genuine ASCs, and committed preadipocytes may all exhibit different features and properties. Overall, a standardized ASC characterization will allow direct comparison of scientific results and clarify the potential clinical applications of ASCs.

Flow cytometry has been the most valuable in recent progress toward characterizing the cell populations of ASCs, and several laboratories have proposed cell surface marker expression profiles for ASCs (reviewed in [54]). More recently, cell surface markers have been identified to define adipocyte progenitor cell populations of ASCs that differentiate into adipocytes and form functional adipose tissue. Based on numerous observations, the surface marker expression pattern of adipocyte progenitor cells is believed to be Lin−, Sca-1+, CD34+, CD31−, CD45−, CD105−, CD24+, CD29+ [55–59]. Notably, CD34+ cells are distinct with regard to adipogenic progenitors and distinguish between different subgroups of ASCs, as they are more adipogenic than CD34− populations in vitro [55, 57, 59, 60]. One compelling study revealed that exclusion of CD34+ cells in human skeletal muscle studies inhibits ectopic adipose tissue formation in vitro and in vivo [61]. Recent work by Maumus et al. supports previous evidence that native ASCs are contained in the CD34+ cell population of WAT [62]. Additional markers have offered valuable information regarding preadipocyte commitment. Preadipocyte factor 1 (Pref-1) is an accepted marker of preadipocytes [63] but is also expressed in other cell types. Other preadipocyte markers include the type VI collagen alpha 2 chain (COL6A2) [64] and a secretory protein FRP2/SFRP2 [65]. All of these factors are highly expressed in undifferentiated preadipocytes and reduced in mature adipocytes; however, none are adipose tissue specific. Overall, the use of inconsistent surface markers for different experiments has made it difficult to compare results and draw definitive conclusions. Notably, some analyses of ASCs vary in the detection of CD133, a marker that is characteristic of stemness [3, 18]. Based on these data, it is suggested that identifying ASCs by the expression of a widely used set of cell surface markers will likely not be sufficient and proposed that identity should be established by physiological properties and function [66]. Therefore, ASCs have been characterized through functional assays, in which isolated cellular fractions are tested for proliferation and differentiation capacity in both in vitro cell culture and in vivo transplantation experiments. The limitation of these methods is that ASCs are removed from their natural cellular environment, which may alter their normal function.

Comprehensive gene expression studies have been carried out by various groups and reveal distinct genetic profiles for ASCs compared to other stem cell populations of different origins. Interestingly, ASCs and bone marrow-derived MSCs share many gene expression patterns and may be closely related [18, 67]. Likewise, a comprehensive proteomic analyses of the ASC secretome determined that cytokine secretory profiles are similar to that of bone marrow-derived MSCs (reviewed in [68]). Epigeneomic analyses of ASCs have been also performed during the last decade and have revealed that DNA methylation and posttranslational histone modifications greatly influence gene activity (reviewed in [69–72]). Epigenetic studies of human ASCs have located a large number of transcriptionally repressed hypermethylated gene promoters, primarily of genes encoding proteins involved in signaling and developmental functions pertaining to early fetal development.

However, promoter methylation changes after adipogenesis of ASCs are specific but did not correlate with their differentiation, suggesting that the adipose-tissue specific combinatorial changes of the DNA methylation and the histone code may contribute to the transcriptional regulation of genes involved in adipogenesis. Notably, many of these hypermethylated promoters are also found in stem cells from other tissues, supporting the view of common ontogeny of MSCs.

An interesting novel approach that characterizes the electrophysiological properties of the ion channels of ASC using a whole-cell voltage clamp technique has been recently established [73–76]. These studies detect high levels of mRNAs of various ion channel subunits and also identify Ca^{2+}-activated K^+ outward currents, characterized by rapid or slow activation, with an insignificant contribution from inward currents. Importantly, they demonstrate that these functional ion channels may contribute to the regulation of proliferation and differentiation. In addition, the depot-dependent differences in the membrane potential and electrophysiological properties of ASCs reflect their adipogenic potential and could thus be used as markers of adipogenesis [74]. Additional studies have shown that the activity of the large conductance K^+ channels in smooth muscle cells is modulated by phosphorylation via specific receptor-mediated signaling cascades [77], suggesting the possibility that the ion channels in ASCs could be effectors of receptor-dependent pathways of adipogenesis regulatory factors. The molecular mechanisms that underlie the link between ion conductance and ASCs require further analysis. Lastly, it is also hypothesized that ASC mechanical biomarkers can be used to identify cell types as well as predict tissue-specific lineage differentiation potential for ASCs [78].

Development of ASCs into the Adipocyte Lineage

Though controversy surrounds the developmental origin of ASCs and their association with adipocyte development, numerous studies have shown that ASCs can undergo adipogenesis in vitro and form adipose tissue in vivo, following culturing and adipogenic induction in vitro [79–81]. Novel data by both Rodeheffer et al. and Tang et al. highlight the detection and origin of white ASCs. Using cell surface markers (flow cytometry) or lineage tracing, they identified and isolated a population of murine undifferentiated APCs resident within the adipose tissue SVF cells that is capable of in vitro adipogenesis as well as proliferating and differentiating into a functional adipose tissue depot in vivo in rodents [57, 58]. This was evidence that WAT contains adipocyte precursors.

Significant advances toward understanding the regulatory processes involved in adipogenesis have largely been made by the identification of transcription factors and pathways that contribute to the adipogenic process (reviewed in [9]). The adipogenic cascade centers on the expression and activation of PPARγ, the master transcriptional regulator of adipogenesis. Three members of the C/EBP family (α, β, δ) also play important roles in differentiation and act in a feedback loop to regulate PPARγ expression. In addition to these central players, Krox20 (also known as early growth response gene 2, or Egr2), several members of the KLF family, STAT5, and SREBP-1c have been reported to promote adipogenesis, while GATA2/3, ETO/MTG8, CHOP10, GILZ, Delta-interacting protein A (DIPA), KLF2, FoxO1, and TCF/LEF are inhibitory (reviewed in [82]). The expression and activity of these transcription factors play an important role in modulating a variety of target genes that are important in conferring lipid accumulation, insulin sensitivity, and endocrine properties in mature adipocytes.

Though poorly understood, novel transcriptional regulators and factors that modulate WAT preadipocyte commitment are being identified. Studies by the Spiegelman laboratory have identified two transcription factors, PPARγ and zinc-finger protein 423 (Zfp423), that are expressed in adipogenic fibroblast cells, as opposed to nonadipogenic cells [83]. This evidence supports previous studies that establish PPARγ as a marker of preadipocytes [58]. Conversely, this report identifies Zfp423 as a novel transcriptional regulator of preadipocyte commitment, as exogenous expression of Zfp423 in nonadipogenic cells is sufficient to increase PPARγ expression and their adipogenic potential and knockout or knockdown of this transcription factor inhibits in vitro adipogenesis [83].

Recent work characterizes Zfp467 as another potential transcriptional regulator of preadipocyte commitment [84]. Likewise, exogenous expression of Zfp467 enhances the cells' adipogenic potential and upregulates PPARγ, adiponectin, and C/EBPα, while knockdown of this transcription factor impairs adipogenesis.

Recently, a study identified a novel transcription factor *Ets2*, a member of the ETS transcription factor family, which coordinately regulates expression of genes altered during different time points of pre- and postnatal adipose tissue development in mice [85]. Experiments in differentiating 3T3-L1 preadipocytes show that *Ets2* stimulates mitotic clonal expansion during the adipocyte commitment phase [85]. Interestingly, another member of the ETS domain-containing transcription factors from the PEA3 subgroup, ETV4, has been reported as one of the mediators of the adipogenic effect of a small molecule phenamil, which acts as an upstream inducer of the PPARγ expression [86].

Additional candidates that could be involved in adipocyte commitment have been identified using a comprehensive transcriptional analysis of in vitro differentiating hMADs [87]. A computational analysis of transcription binding sites in their promoters identifies a potential role for regulation by the nuclear hormone receptors, including liver X receptor alpha (LXRα), PPARγ, and COUP-TF1, an orphan nuclear receptor acting predominantly as a transcriptional repressor. In addition, several laboratories have investigated other potential transcriptional and paracrine regulators of preadipocyte commitment utilizing gene expression profiling of both adipogenic and nonadipogenic cells [58, 83], such as Gsc, Twist2, Mmp3, Egfr, Fgf10, Efemp1, Lgals3, Igfbp4, and Lpl.

Multiple signaling factors have been shown to influence the development of ASCs into adipocytes by an autocrine and/or paracrine mechanism, such as bone morphogenetic proteins (BMPs) [88], transforming growth factor β (TGFβ) (reviewed in [89]), insulin-like growth factor-1 (IGF-1) (reviewed in [90]), fibroblast growth factors (FGF) 1 and 2 [91, 92], and activin [93]. Various studies have also revealed negative regulators of adipocyte development, such as Hedgehog signaling [94] and WNT signaling, whose suppression in both in vitro and in vivo adipocyte development is essential for adipogenesis (reviewed in [95]). Additional intracellular signaling pathways have also been implicated in the adipogenic cascade, whose functions are continuously revealed (reviewed in [96]). Limited studies have shown that cell shape as well as extracellular matrix components may also influence adipocyte lineage commitment (reviewed in [97, 98]).

Members of the TGF-β superfamily, notably BMP-2 and BMP-4, have been shown to stimulate commitment toward the white adipocyte lineage [88, 99–101]. Specifically, BMP-4 upregulates PPARγ expression and enhances adipogenesis both in vitro and in vivo after implantation into mice [101]. Moreover, BMPs have been shown to exert their proadipogenic effects through the intracellular proteins Smads, which may also be important for preadipocyte commitment. Notably, both Zfp423 and Schnurri-2 are BMP-dependent transcriptional coactivators of Smad proteins [102], which confer their proadipogenic effects [83, 103]. Likewise, expression of BMP-4, BMP-4 receptors, and Smads is elevated in a cell line of MSCs that have increased adipogenic potential [100]. Lysyl oxidase (Lox) is another BMP-dependent transcriptional target of Smad 1/4 that is important for preadipocyte commitment; as knockdown of Lox impairs the commitment of MSCs to the adipocyte lineage and inhibits the adipogenesis of murine fibroblasts [88]. Collectively, these studies highlight the importance of BMP-2/4, Smads 1/4/5/8, and Lox as positive regulators of white preadipocyte commitment in rodents.

In recent years, activins, which are secreted proteins of the TGFβ family, have emerged as regulators of the ASC pool as well as the function of mature adipocytes (reviewed in [104]). They represent dimers composed of various combinations of four inhibin β subunits, βA, βB, βC, and βE. Adipocytes and ASCs express homodimers of βA and βB, named activin A and activin B respectively, as well as the heterodimer βA and βB named activin AB. Activin A is highly expressed in human ASCs and displays proliferative and antiadipogenic effects via the Smad 2 pathway. In contrast, activins B and AB are highly expressed in mature adipocytes, particularly in obesity, and contribute to their insulin resistant and inflammatory state. The activity of activins is controlled by a binding protein follistatin, which is decreased in obesity. Thus, the ratio of the follistatin/activin complex appears to be an important regulator of the ASC pool and adipocyte function that requires further investigation.

Studies have also identified FGFs as positive regulators of preadipocyte commitment. Exposure of cultured rat MSCs or human ASCs to FGF2 leads to increased expression of PPARγ and enhanced adipogenesis [105, 106]. Likewise, exogenous FGF2 confers in vivo WAT formation via isolated human SVF cells [107]. FGF-10 is expressed primarily in WAT preadipocytes and facilitates increased proliferation, but does not affect their differentiation [108]. FGF-1 has been shown to enhance the adipogenesis of human preadipocytes [109].

The Origin of Adipocyte Progenitors

Adipocytes are generally thought to arise from mesodermal stem cells residing in the adipose tissue stroma; however, previous work has postulated that adipocyte precursors may exist in the adipose vasculature, embedded in the walls of blood vessels in WAT [58, 59]. Additional studies have also shown that preadipocytes may derive from mural cell origin, as adipocytes and pericytes may share a common origin [17, 55, 57, 110, 111]. Committed preadipocytes have been shown to express pericyte markers, notably SMA, NG2, and PDGFRB [58], which is characteristic of mural cells and required for their formation (reviewed in [112]. Hence, committed preadipocytes may constitute a subset of mural cells (i.e., pericytes) in WAT. These findings support earlier studies indicating that angiogenesis and adipogenesis are tightly correlated (reviewed in [113]) and [114, 115]. Consequently, other evidence suggests that proliferating progenitor cells are located in the stromal fraction of human adipose tissue [62]. Interestingly, recent analysis of intact human WAT revealed that ASCs were found scattered in the adipose tissue stroma, and these ASCs did not express pericytic markers *in situ,* as previously reported [62]. Though it has been widely accepted that adipocytes arise entirely from the mesoderm, evidence has also shown that neuroepithelial cells derived from mouse ESCs can undergo adipogenesis in vitro [116, 117]. Hence, the neuroectoderm could be a source of adipocytes. Though Billon et al. were able to show that adipocytes in vivo arise from the neural crest, only a subset of adipocytes in specific depots, notably the cephalic region, may be of neuroectoderm origin.

Interestingly, evidence suggests that nonadipose tissue-resident progenitors are able to migrate to adipose tissue, undergo adipogenesis, and contribute to the white adipocyte pool. Hong et al. demonstrated that circulating fibrocytes (peripheral blood mononuclear cells) can undergo adipogenesis in vitro as well as form adipocytes in vivo after implantation into SCID mice [118]. It was also reported by several studies in rodents that adipocytes may derive from circulating bone marrow cells [119–121]. However, an additional study found the opposite and suggests that bone marrow-derived cells do not differentiate into adipocytes or contribute to adipose tissue development [122]. Additional bone marrow reconstitution studies demonstrate that bone marrow progenitor-derived adipocytes and adipocyte progenitors do indeed derive from hematopoietic cells via the myeloid lineage [123]. Yet, the adipocytes developed from these progenitors were different from traditional white adipocytes, in that they had increased expression of inflammatory cytokines and decreased expression of leptin and other genes involved in mitochondrial biogenesis and lipid oxidation, supporting previous conclusions that contribution of bone marrow-derived progenitors to functional WAT may be negligible [120, 122]. Of consideration, these bone marrow progenitor-derived adipocytes accumulated more in VAT depots compared to SAT and were more plentiful in women compared to men; therefore, accumulation of adipocytes from bone marrow origin may contribute to adipose tissue depot heterogeneity.

Overall, evidence to support the origin of adipocytes from areas outside the mesoderm is controversial, and whether the adipocyte precursor population is resident within the adipose tissue and/or originates from the recruitment of circulating progenitor cells remains to be determined. Lack of specific cell surface markers to identify human adipocyte origins precludes the accurate isolation of human APCs and analysis of the adipogenic cascade. Though resident pools of APCs have been identified in rodents, these cells are not fully identified in humans; hence, the exact nature of human preadipocytes still remains unclear.

Effects of Obesity on ASC Pool

Due to the inability to analyze the varying degrees of cell turnover in humans, few data are available concerning human adipocyte precursor renewal within adipose tissue; although this process is essential to maintain a preadipocyte pool to be available during WAT expansion. The development, availability, and response of the adipocyte progenitor pool define an individual's capacity for adipose tissue expandability. Hence, characterizing factors that regulate the size and differentiation of adipocyte progenitor pools may denote novel therapeutic strategies to control the deposition of lipid due to excess energy surplus. Likewise, new lines of evidence are analyzing how obesity impacts ASC biology. Detrimental consequences of adipose tissue remodeling, resulting from adipocyte hypertrophy, hypoxia, and local inflammation [124], include enhanced proliferation of preadipocytes [125, 126], with concurrent inhibition of preadipocyte differentiation [127–130] and increased preadipocyte apoptosis [131]. Therefore, phases of adipocyte hyperplasia would be achieved with increased requirements for proliferation coupled with successive less efficient adipogenesis. Frequent cycling will thus promote replicative senescence of adipocyte progenitor cells with gradual impairment of adipocyte function and viability. Overall, obesity would promote accelerated exhaustion of the adipocyte progenitor pool, decreased capacity for preadipocyte self-renewal, and extensive adipose tissue remodeling, all leading to impaired expandability of subcutaneous adipose tissue, ectopic lipid accumulation, and obesity-related metabolic perturbations (insulin resistance). Isakson et al. demonstrated impaired differentiation of preadipocytes from the stromal fraction of subcutaneous abdominal adipose tissue from obese versus lean individuals [3]. Early studies using thymidine incorporation into fat cell DNA reported increased preadipocyte proliferation in high fat diet-fed rats [132]. More recent reports demonstrate that human subcutaneous abdominal adipose tissue has increased proliferation of adipocyte precursors in increasing obese conditions [126]. Yet, other studies indicate that preadipocyte numbers in the SVF were lower in obese women as compared to lean [4]. However, the aforementioned observations could be attributed to greater recruitment of preadipocytes to adipogenesis or greater preadipocyte apoptosis. Recent evidence suggests that adipocyte precursor/preadipocyte number may depend on the degree of obesity; as humans with morbid obesity, with corresponding excessive AT development, had decreased ASCs (heterogeneous fraction), compared to individuals with moderate obesity [62]. This decrease was accompanied by smaller mean adipocyte diameter and a marked increase in the expression of adipogenic markers, suggesting increased proliferation of preadipocytes and/or increased differentiation of new preadipocytes. Indeed, recent compelling data reported decreased replicative potential, premature cellular senescence, and loss of the multilineage differentiation potential of omental ASCs from patients with morbid obesity compared to lean individuals [133]. In addition, recent findings have also shown that chronic thiazolidinedione treatment decreases the adipogenic potential of ASCs, exhausting the pool of committed preadipocytes in WAT [134].

Depot Differences of ASC Pool

It is well documented that differences in regional fat distribution affect metabolic parameters in humans, presumably due to intrinsic differences in function of the adipose tissue [135–139]. The two types of WAT, visceral (VAT) and subcutaneous (SAT), are defined by location, and the mechanisms and developmental signals that account for each depot's unique characteristics are steadily emerging. Studies have revealed that subcutaneous upper body depots and visceral depots both correlate with an increased susceptibility for metabolic perturbations [140, 141], while lower-body fat is protective [138, 142–144] (reviewed in [145]). In addition, evidence suggests that VAT expands predominantly by adipocyte hypertrophy, while SAT by adipocyte hyperplasia with nutritional overload [146]. While numerous studies have investigated regional differences in adipose tissue metabolism [147–150],

few have examined depot-specific differences in adipocyte progenitor development. Subsequently, convincing evidence for distinct depot-dependent populations of ASC pools is emerging, as adipocyte progenitors may contribute to regional variation in WAT function and development. Early studies from the Kirkland laboratory revealed that abdominal subcutaneous preadipocytes derived from adipose stromal cells accumulated more lipids and had higher differentiation capacity and levels of adipocyte markers compared to visceral preadipocytes from obese subjects [151]. Studies performed in primary cultures also showed that the proliferation and differentiation capacity of ASCs from subcutaneous precursor cells was higher than in omental cells in obese individuals [148]. Flow cytometric analysis supported previous data by validating that the number of $CD34^{+}/CD31^{-}$ SVF cells from gluteal SAT positively correlated with increasing BMI of overweight individuals [152]. Additional lines of evidence indicate that SAT adipocyte precursors in rodents are more abundant and have increased proliferation as compared to VAT adipocyte progenitors in response to high-fat diet [146]. Notably, recent studies by Macotela et al. that highlight the intrinsic differences of VAT versus SAT preadipocyte pools in rodents reveal that visceral APCs display less differentiation capacity, and VAT has a decreased percentage of APCs following high-fat diet, with subsequent increase in other SVF cells (i.e., macrophages). They also demonstrate that visceral APCs highly express antiadipogenic factors, as opposed to subcutaneous APCs, which show higher expression of proadipogenic genes [153]. Overall, the reduced differentiation capacity of visceral preadipocytes may account for the increased hypertropy of existent adipocytes and the metabolic abnormalities associated with visceral adipose tissue. Hence, depot-specific differences in adipocyte progenitor abundance and proliferation influence whether a fat depot expands by hypertrophy or hyperplasia, and thus may have important implications on the development of metabolic disease.

Though the aforementioned data collectively indicate that subcutaneous depots contain a greater number of functional adipose progenitors as compared to visceral depots, these findings are controversial. Other investigations indicate that preadipocytes from upper body (abdominal) SAT of obese women differentiate less readily and are more susceptible to apoptosis as compared to the lower body (femoral) depot [4]. These results support previous reports in primary cultures showing that subcutaneous abdominal preadipocyte differentiation inversely correlates with increased obesity and central adiposity [154]. Thus, the SVF of subcutaneous abdominal fat tissue from centrally obese individuals might contain more preadipocytes with impaired differentiation potential than tissue from lean individuals. This provides evidence that abdominal VAT and abdominal SAT may share similar properties, as previously shown [74]. Overall, these studies are complicated due to the lack of distinct markers of ASCs and preadipocytes and the complexity in defining precisely where in the commitment and differentiation phase a given cell may be.

Transcriptional profiling has revealed limited yet valuable information about depot-specific differences in adipose tissue, as morphological and functional differences in developmental gene expression have been reported in rodents and humans [155–158]. Adipocytes from VAT express higher levels of *HoxA5*, *HoxA4*, *HoxC8*, *Glypican 4* (*Gpc4*), *Thbd*, and *Nr2f1* (*nuclear receptor subfamily 2 group F member 1*), whereas subcutaneous WAT has higher levels of *HoxA10*, *HoxC9*, *Tbx15*, *Shox2* (*Short stature homeobox 2*), *En1* (*Engrailed 1*), and *Sfpr2*, and most of these differences are observed in rodents and humans. Notably, depot-specific variations in gene expression were also observed in preadipocytes [147, 157]. In addition, select developmental genes, *Tbx15*, *Glyp4*, and *HoxA5*, demonstrate changes in expression that correlate with levels of obesity (body mass index) and fat distribution (waist-to-hip ratio) [157]. More extensive gene expression analyses reveal that additional genes that regulate early development, such as homeobox family members and pregnancy-associated factors, are distinct between fat cell progenitors of both rodent and human adipose tissue depots [147, 159, 160]. The observed differences in gene expression appear to be intrinsic and persist through in vitro culture and differentiation; hence, the microenvironment does not appear to be an influence. Furthermore, the results from the aforementioned experiments by Tchkonia et al. highlighting the differences in lipid

accumulation and differentiation capacity of SAT versus VAT preadipocytes [151] were associated with distinct patterns of gene expression and conserved over multiple cell generations [158]. Collectively, these data suggest that WAT depots originate from different precursor cells, whose function is presumably controlled by genes involved in development and pattern specification. Moreover, pre-B-cell leukemia transcription factor (PBX1), a family member of the homeodomain transcription factors, has been shown to be induced after commitment of mouse ESCs to the adipocyte lineage following treatment with RA [161]. A siRNA-mediated silencing of PBX1 expression in hMADs shows that PBX1 may play a role in human adipogenesis by maintaining the proliferation of ASCs and prevention of their commitment to adipocyte lineage [161]. Although the expression of PBX1 in different depots has not yet been explored, these data strongly suggest that the depot-specific differences in preadipocyte pools are established during development. Thus, the apparent differences in adipose tissue distribution in normal and obese individuals may be derived from distinct precursors in the different WAT depots.

Sex steroids are endogenous modulators of adipose tissue development, function, and distribution of SAT versus VAT depots [162], though little is known about the cellular and molecular mechanisms of this regulation. Men often have more adipose tissue distributed in the abdominal or visceral region ("android" or "apple" phenotype), which carries a much greater risk for metabolic disorders than does adipose tissue distributed subcutaneously (reviewed in [163]. In contrast, women, have more subcutaneous adipose tissue ("gynoid" or "pear" phenotype), and this distribution is predominantly sex hormone (estrogen)-dependent [164]. Likewise, in men and menopausal women, conditions in which estrogen levels are low, visceral adiposity increases. These distinct sex differences in patterns of fat distribution often develop during puberty; hence, sex steroids may potentially regulate fat distribution through epigenetic mechanisms involving adipose progenitors. Likewise, suboptimal maternal diet predisposes to visceral obesity and metabolic syndrome [165], further supporting the role of epigenetic mechanisms in the interaction between maternal nutrition and the regional fetal development of adipose tissue.

Complexity of Characterization and Analysis of ASCs

Though much progress has been made to elucidate the mechanisms that underlie the commitment of stem cells to the adipocyte lineage, many challenges remain in elucidating the function of ASCs in adipose tissue development. SVF subpopulations that contain committed preadipocytes can only confer WAT formation in vivo under certain inducible conditions that are conducive to alterations in adipose tissue expansion, such as HFD or lipodystrophy [57]. Other studies provide evidence that dietary stimulus can modulate the proliferation of adipogenic progenitors [146]. Hence, the ASC natural microenvironment is significant, but cannot be fully recapitulated in the realm of culture experiments. Consequently, to date, little is known about the capacity of these adipose "stem cells" to self-renew and produce new preadipocytes in humans or undergo adipogenesis. Of note, preadipocyte replication is often analyzed as an indicator of progenitor pool activity, yet this proliferation could be either a mechanism to replenish the local pool of immature progenitor cells of the expanding adipose tissue or also an index of adipocyte progenitor cell entry into adipogenesis. Frequently, the ASCs commonly utilized for experimentation are a heterogeneous cell population with the potential to commit to other lineages; so functional differences may exist between ASCs and committed preadipocytes in vivo. Though recent evidence suggests that ASCs are involved in the adipogenic process [62], additional studies are necessary to elucidate the contribution of ASCs to committed preadipocytes. More knowledge about the mechanisms that regulate ASCs is necessary in order to refine and standardize laboratory techniques to isolate, characterize, and manipulate ASCs.

Importantly, analyses to understand ASCs and adipocyte origins have clinical implications, as these findings may offer insight into diseases linked to adipose tissue. The identified novel transcriptional and auto/paracrine factors that regulate adipocyte development present potential therapeutic avenues to modulate the size and management of the ASC pools as well as the adipose cell turnover rate. Likewise, this would allow the manipulation of subcutaneous adipose tissue expandability, with subsequent prevention of metabolic abnormalities associated with ectopic fat deposition and improvement of insulin sensitivity in conditions of obesity as well as lipodystrophy. Hence, continued efforts to investigate the contribution of these pathways to the regulation of adipocyte progenitor pools in different depots may lead to the prevention of metabolically unfavorable fat distribution [166].

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Chapter 3
Metabolism of White Adipose Tissue

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Keywords Lipid storage • Lipolysis • Lipid droplet associated proteins • Adipokines

Key Points

- The main metabolic role of adipose tissue remains the storage of energy as triacyglycerols and its release when needed through lipolysis.
- Besides the classical hormone-sensitive lipase, other lipases and numerous lipid droplet associated proteins are now known to control this synthesis and release of triacylglycerols by adipose tissue.
- Adipocytes are also involved in cholesterol metabolism and through the production of adipokines, and perhaps of microvesicles, in the control of other metabolic pathways in other tissues

Introduction

The contribution of white adipose tissue (WAT) to whole body oxygen consumption and energy production is limited as it represents only about 5 % of whole body energy expenditure [1]. This is, on a per-kilogram basis, much less than organs such as the liver, kidney or brain. Moreover, WAT is heterogeneous at the cellular level and this estimate represents the activity of adipocytes but also other cells such as stroma and immune cells. This review deals only with the metabolism of white adipocytes. Despite this low metabolic activity, adipocytes are continuously synthesizing and breaking down triacylglycerols (TAG), around 50–60 g/day, with a half-life of TAG stores in humans of about 200–270 days [2], and have important metabolic functions that have a significant role in the regulation of lipids and also glucose metabolism. WAT is by far the largest site of TAG and therefore of energy storage. These TAG, stored within intracellular lipid droplets (LD) represent in a young healthy adult around 12–15 kg, i.e., 110,000–135,000 cal. This energy is stored during the postprandial periods, and most of TAG comes from ingested lipids. Some of the fatty acids used for the synthesis and storage of TAG may be synthesized form carbohydrates through the pathway of de novo lipogenesis, but this contribution is minor in humans except in situations of massive and prolonged carbohydrate overfeeding.

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This energy is released from WAT between meals and in situations of energy restriction and of exercise to meet the energy need of the body. This release, through the process of lipolysis, i.e., the hydrolysis of intracellular TAG, provides glycerol and fatty acids. Most of the glycerol will be used by gluconeogenic tissues to produce new molecules of glucose. Fatty acids appear in plasma as albumin-bound fatty acids (NEFA) that will be used mainly by muscles (mostly for oxidation), liver (oxidation, complete to CO_2 or incomplete to ketone bodies, and also TAG synthesis, storage, and secretion as very-low-density lipoprotein (VLDL-TAG)), and adipose tissue (reesterification). TAG storage and hydrolysis in WAT are highly controlled by metabolic, hormonal (mainly insulin and catecholamines), metabolic (glucose, NEFA), and nutritional (energy intake, contribution of carbohydrates and lipids to this intake) factors. Some cytokines participate also in this regulation. These regulations are essential to maintain body weight homeostasis; alterations in these processes, resulting in imbalance between TAG storage and mobilization in WAT, may result in obesity or depletion of WAT (lipoatrophy). Moreover, the way plasma lipids are cleared from circulation by adipose tissue and fatty acids released during lipolysis has an important role in the everyday regulation of plasma lipids concentrations, and alterations of WAT metabolism may result in increased plasma lipids levels and therefore increased risk of cardiovascular disease. In addition, fatty acids released by lipolysis and some of their derivatives can participate to cellular signaling, for example through activation of PPARs [3]. Last, adipocytes, through the secretion of hormones such as leptin and adiponectin, and also of some cytokines, and the way they store TAG and release fatty acids, control in part the amount of lipids stored in other tissues and the sensitivity to insulin of tissues such as muscles or liver. Therefore, modifications of WAT metabolism may be implicated in the excessive accumulation of lipid substrates in non-adipose tissues, their adverse effects (lipotoxicity [4]) and the development of insulin resistance and diabetes. Finally it must be kept in mind that WAT is heterogeneous, with differences in metabolism between small and large adipocytes [5, 6] and also between its various sites [7]. Alterations of metabolism limited to some sites (such as visceral or peri-arterial adipose tissue) may have profound effects on neighboring tissues [8, 9].

TAG Synthesis and Storage

TAG stored in adipocytes are synthesized within these cells from fatty acyl-CoAs and glycerol-3-phosphate (G3P). Most of the fatty acids used for this synthesis are provided by circulating plasma lipids, while G3P has two main possible origins, glycolysis and glyceroneogenesis. The exact intracellular site of TAG synthesis, the way new TAG molecules are directed toward the lipid droplet (LD) and the morphology of LD are still debated despite recent advances in this field.

Sources of Fatty Acids

Fatty Acids from Circulating Lipids

Fatty acids from circulating lipids are provided either by the albumin-bound NEFA pool or by the TAG incorporated in TAG-rich lipoproteins, mainly VLDL in the post-absorptive state and chylomicrons in the postprandial state. Lipoproteins-TAG must first be hydrolyzed by the enzyme lipoprotein-lipase (LPL) bound to the wall of capillaries in adipose tissue [10] in order to release their fatty acids. The expression and activity of LPL is increased in adipose tissue in the fed state, particularly during a high-carbohydrate diet, probably through the action of insulin, whereas both expression and activity are decreased in adipose tissue during fasting and a high-fat diet [11]. The decrease of LPL during

fasting involves the action of angiopoietin-like 4 (Angptl4) [12]. LPL is synthesized by adipocytes and must, to become active, undergo a maturation process and be transported to the capillary endothelium. These processes are dependent at least in part of the protein lipase maturation factor 1 (LMF1) expressed in most tissues [13, 14]. LMF1 deficiency leads to low LPL activity and hypertriglyceridemia [14]. Lipoproteins-TAG hydrolysis is also probably controlled in part through the VLDL receptor, a member of the LDL-receptor family, which is expressed in adipose tissue [15]. The VLDL receptor binds apoprotein E-rich lipoproteins, such as VLDL, chylomicrons and remnants, and probably brings them in close contact with LPL, facilitating its action. Mice deficient in VLDL receptor have a decreased fat mass and are resistant to diet-induced obesity; moreover, VLDL-receptor deficiency reduces the obesity of ob/ob mice [16]. The expression of VLDL receptor is stimulated by PPARγ agonists [17] and decreased by PCSK9 [18]. The exact role of this receptor in humans remains to be defined. Apolipoprotein AV is also a regulator of the degradation of lipoproteins-TAG, probably by enhancing the binding of these lipoproteins to the proteoglycans of the vascular wall [19, 20]. Lastly a glycosylphosphatidylinositol-anchored HDL-binding protein 1 (GPIHBP1), located also on the capillary endothelium, has an important role in the lipolysis of chylomicrons [21] by enhancing interactions between LPL and chylomicrons.

Whatever their origin, the uptake of long chain fatty acids by adipocytes requires specific processes in order to allow them to cross the plasma membranes [22, 23]. It is probable that both a transport by specific transporters and a passive diffusion co-exist, their respective roles being still highly debated [24]. Human white adipocytes express several fatty acid transporters that facilitate and control the transport of fatty acids: the protein CD36 (homologue to the murine fatty acid transporter FAT), the Fatty Acids Transport Proteins (FATP1 and 4) and the Fatty Acid Binding Protein plasma membrane (FABPpm, which is identical to the mitochondrial aspartate aminotransferase), with FAT appearing as responsible for most of fatty acids uptake [25]. This transport is dependent of the presence of lipid rafts in the membrane [26]. Insulin promotes this transport by stimulating the expression of transporters and their trafficking, particularly of FATP1, to plasma membranes [27]. Endurance training and activation of AMP-activated kinase (AMPk) also increase expression of these transporters [24]. Caveolin-1, an essential structural protein of caveolae [28], could also been involved in the uptake of fatty acids and in their intracellular transport [29]. It binds fatty acids [30] and moves from plasma membrane to the LD in response to exposure of cells to fatty acids [31]. Caveolin-1 deficiency leads to a lipoatrophic phenotype in mice [29], and Cav-1 mutations in humans are a cause of Berardinelli–Seip congenital lipodystrophy [32]. However, it is unclear whether Cav-1 acts in fatty acids uptake directly or indirectly by controlling the formation of caveolae and the correct localization on plasma membranes and function of FAT [33].

Fatty acids are not soluble in the cytosol and may exert toxic effects on membranes, although adipocytes appear remarkably resistant to this toxicity. Fatty acids inside the cells are therefore tightly bound by cytosolic Lipid-Binding-Proteins also called Fatty Acid Binding Proteins (FABP). These proteins carry them from membrane to membrane or to the site of action of the enzyme Acyl-CoA synthase [34, 35]. Human white adipocytes express two FABPs: Adipocyte Lipid Binding Protein (ALBP or AFABP or aP2), expressed only in adipocytes, and Keratinocytes Lipid Binding Protein (KLPB) that is expressed also in macrophages. AFABP is much more abundant than KLPB in human (and rodent) adipocytes; however, this ratio varies between different sites of adipose tissue and this may impact on the metabolism of different fat depots [36]. The first step in the metabolism of fatty acids taken up from circulation is their activation in long chain fatty acyl-CoA (LCFA-CoA). This can be achieved by Acyl-CoA synthase-1, the isoform of ACS expressed in adipocytes, but also by FATP-1 and -4, that possess ACS activity [37], contributing to the close link between fatty acid uptake and activation [38]. LCFA-CoAs can then be directed toward oxidation or to the synthesis of more complex lipids such as TAG. Oxidation requires, as in other tissues, the entry of LCFA-CoA inside mitochondria through the action of the enzyme Carnitine-Palmitoyl Transferase I (CPT I). In other tissues this step is an important site of the regulation of fatty acids metabolism through the inhibition of

CPT-I by malonyl-CoA, the product of Acetyl-CoA Carboxylase that catalyzes the first step in the lipogenic pathway [39]. Whether this step is also highly regulated in adipose tissue is unclear despite some evidence [40]; however, the main metabolic fate of fatty acids taken up by adipocytes appears to be reesterification into TAG.

De Novo Lipogenesis (DNL)

DNL is the synthesis of new fatty acid molecules from non-lipid substrates, mainly carbohydrates in mammals. The expression and activity of the glycolytic and lipogenic pathways are therefore linked together in lipogenic tissues. The two main sites of DNL are liver and adipose tissue; the quantitative importance of this pathway and the respective contribution of liver and adipose tissue vary between species [41]. Overall, DNL is less active in humans than in rodents and contributes much less than TAG dietary intake to adipose tissue lipids stores [42]. Indeed studies of hepatic DNL in healthy humans concluded that this pathway is a minor contributor to the fatty acids used for liver TAG synthesis and secretion and represents only about 1–2 g/day [43–46]. Liver lipogenesis is stimulated by insulin and glucose and can be largely increased (two to fourfold) by high-carbohydrate (CHO) diet [43, 47–49]; it is increased in ad libitum fed obese subjects [50], hypertriglyceridemic type 2 diabetic subjects [51] and in subjects with nonalcoholic fatty liver disease [52], but still remains minor compared to oral TAG ingestion (usually more than 100 g/day). The new fatty acid molecules provided by hepatic DNL can be exported as TAG-VLDL for uptake and storage by adipocytes, but are a minor contributor to these stores in humans [43]. The key enzymes for lipogenesis are also expressed in adipocytes [53], but the expression and activity of these enzymes are lower in human than in rat adipocytes [49]. DNL in humans is less active in adipocytes than in liver when expressed per gram of tissue but, on a whole body basis, the contributions of liver (1.5 kg) and adipose tissue (12–15 kg) appear comparable (1–2 g/day for each tissue) [43].

The regulation of DNL by hormonal (mainly insulin and glucagon), metabolic (glucose, polyunsaturated fatty acids (PUFA)) and nutritional (total energy intake, dietary CHO over fat ratio) factors is less well defined in humans, either in liver or adipose tissue, than in rodents, and less well known in adipocytes than in liver. Overall it is clear that hepatic lipogenesis is highly responsive to modifications of hormonal and nutritional conditions. Insulin and glucose stimulate it, while glucagon and PUFA inhibit it [54]. The regulation by insulin and PUFA is mediated by the transcription factor sterol response element binding protein 1c (SREBP-1c) [55] and also in part by LXRα (insulin and PUFA) [56] and carbohydrate response element binding protein (ChREBP) (PUFA) [57], whereas the inhibitory action of glucagon and the stimulatory one of glucose are mediated by ChREBP [57]. Insulin stimulates the transcription of SREBP-1c, directly and indirectly through stimulation of the expression of LXRα. Insulin also stimulates cleavage of the precursor form of the protein SREBP-1c and the release of its mature form [58]. LXRα stimulates the expression of lipogenic genes directly and through an increase in the expression of SREBP-1c [56]. A full stimulation of liver lipogenesis requires the simultaneous and synergistic action of insulin and glucose [54]. Glucose acts by dephosphorylation of ChREBP, allowing its entry into the nucleus and its binding to a specific response element in the promoter of glycolytic (L-PK) and lipogenic (FAS, ACC) genes [59–61]. Glucagon and PUFA, on the contrary, phosphorylate ChREBP respectively through the protein kinase A (PKA) and the AMP-dependent kinase (AMPK), inhibiting its action [59, 62].

The regulation of DNL in adipocytes, particularly in humans, is less well defined. It is clear that insulin increases FAS expression and activity in human and rodents adipocytes [63, 64]. This action involves probably both SREBP-1c [65] and LXRα. Whether insulin stimulates SREBP-1c maturation in adipocytes is unclear. Glucose also stimulates lipogenesis in adipocytes [66], and as in liver, a full stimulation requires the simultaneous presence of insulin and glucose. The action of glucose could be transmitted by ChREBP, since this transcription factor is expressed in adipocytes [49, 61, 67, 68].

A stimulation of adipocyte ChREBP expression by glucose and insulin was reported but only in the presence of high, unphysiological glucose levels [67]. In vivo, ChREBP expression is poorly responsive to metabolic and nutritional factors in liver and adipose tissue, and is clearly increased only in the situation of high CHO refeeding after starvation [67–69]. The roles of glucose and ChREBP in adipose tissue are strongly supported by the recent demonstration that glucose induces, through the activation of the classical ChREBP isoform (ChREBP-α), the expression of a novel isoform, ChREBP-β [70]. This new, shorter isoform is a potent stimulator of the lipogenic pathway. Thyroid hormones stimulate the expression of ChREBP in liver and adipocytes [71, 72] through the receptor TRβ. Lastly, PUFA have an inhibitory action on lipogenesis in adipose tissue but this effect is less marked than in liver [73]. Endoplasmic reticulum stress stimulates lipogenesis in liver and can contribute to hepatic steatosis [74]; this effect remains to be established in adipose tissue. Overall, the expression and activity of lipogenesis appears less responsive to metabolic and nutritional factors in adipose tissue than in liver, in rodents and in humans, and is still less responsive in humans than in rodents [43, 49, 68], although some stimulation has been observed during prolonged carbohydrate overfeeding [75]. It is noteworthy that the expression of ChREBP, SREBP-1c, FAS, and ACC is decreased in adipose tissue of human obese subjects and of experimental models of obesity with long-standing obesity, while the expression and activity of liver lipogenesis are increased [49, 50, 76].

Lastly, the renin–angiotensin system (RAS) is involved in the control of lipogenesis and TAG storage [77]. WAT expresses the components of a functional RAS [78, 79] and mice with overexpression of angiotensinogen in adipose tissue have an increased fat mass with adipocyte hypertrophy [80]. Angiotensin II stimulates lipogenesis in 3T3-L1 and human adipocytes [81]. This effect involves SREBP-1c and is mediated by the angiotensin type 2 receptor (AT2R) [82]. Deletion of this receptor results in adipocyte hypotrophy and resistance to diet-induced obesity [83]. These mice have reduced adipocyte expression of SREBP-1c and FAS, but also of LPL, FAT and aP2, suggesting that angiotensin II stimulates several pathways of TAG storage. In addition, angiotensin II is antilipolytic through AT1R [77]. Angiotensinogen is overexpressed in the adipose tissue of obese subjects [84], particularly in visceral adipose tissue and could therefore have a role in the development of obesity.

Sources of Glycerol-3-Phosphate

TAG synthesis requires glycerol-3-phosphate (G3P) for the initial step of fatty acids esterification. Glycerokinase activity is very low in adipocytes. Its expression could be increased by the PPARγ agonists thiazolidinediones [85], but this remains debated in humans [86]. G3P is therefore produced either from glucose through the first steps of glycolysis or from gluconeogenic precursors through glyceroneogenesis [87]. Glucose enters adipocytes through the glucose transporters 1 and 4 (Glut-1 and Glut-4) responsible respectively of basal glucose and insulin-stimulated glucose uptake. Insulin acutely stimulates glucose uptake by promoting the translocation of Glut-4 from an intracellular pool to the membrane, an effect mediated through the PI-3 kinase Akt pathway [88]. Glucose uptake is also stimulated by the Acylation Stimulating Protein (ASP) [89]. The other source of G3P is glyceroneogenesis, an abbreviated version of gluconeogenesis that provides G3P from gluconeogenic substrates such as lactate and pyruvate [87]. The regulatory step of this pathway is controlled by the cytosolic form of PEPCK. PEPCK-C expression and activity is increased by PUFA and thiazolidinediones and inhibited by glucocorticoids (see reference [90]). Glyceroneogenesis is decreased by dyslipidemia-inducing HIV-protease inhibitors through the induction of adipose tissue inflammation [91]. The relative contribution of glycolysis and glyceroneogenesis to G3P production thus varies with nutritional and pharmacological factors. The overall availability of G3P controls the esterification rate of fatty acids provided by DNL and circulating lipids but also the partial reesterification of fatty acids released by the lipolysis of stored TAG.

TAG Synthesis and Formation of Lipid Droplets

TAG biosynthesis needs the successive esterification of the alcoholic groups of G3P by different enzymes: glycerol-3-phosphate acyltransferases (GPATs), 1-acylglycerol-3-phosphate acyltransferases (AGPATs), and diacylglycerol acyltransferases (DGATs) [92–95]. All these enzymes exist in different isoforms and are encoded by different genes. The isoforms GPAT1, GPAT2, AGPAT2, DGAT1 and 2 are present in adipose tissue [96], but the tissue repartition and substrate specificity of these different isoforms are not yet fully clarified. The expressions of DGAT1 and 2 are stimulated in adipose tissue by glucose and insulin [97] and both insulin and glucose increase TAG synthesis. ASP also stimulates adipocyte TAG synthesis [89]. The important role of these enzymes in controlling adipose TAG stores is demonstrated by studies of mice lacking DGAT and of subjects with congenital lipodystrophy 1 (BSCL-1, caused by mutations of AGPAT2) [96, 98, 99]. A point of dicussion is the intracellular site of TAG synthesis and how new TAG molecules are directed to LD for storage. Classically, TAG synthesis occurs in the endoplasmic reticulum. However, there is recent evidence that this synthesis takes place also in a subclass of caveolae in the plasma membrane [100]. Since caveolae are important for the correct localization and function of fatty acid transporters [33], fatty acid uptake and TAG synthesis would be closely linked. These caveolae also contain perilipin 1 [100, 101], a protein coating LD, and this protein could, in addition to its regulatory role in lipolysis (cf next section: lipolysis and release of fatty acids), be involved in the incorporation of newly synthesized TAG into LD. The way the size and number of LD per cell are controlled are only partially understood. The constitution of LD needs the synthesis of phospholipids for the monolayer wrapping them. Seipin, whose mutations cause BSCL-2, seems, in addition to its role in adipogenesis, implicated in the synthesis of this phospholipids monolayer and the morphology of LD (see [102]). LD-associated proteins are also implicated: deficiency in fat specific protein of 27 kDa (FSP27) results in the formation of small multilocular LD instead of a large unilocular one [103, 104] (see paragraph: perilipin and other LD-associated proteins).

Lipolysis and Release of Fatty Acids

During intracellular lipolysis TAG are hydrolyzed successively into diacylglycerols (DAG) and monoacylglycerols (MAG) to finally release three molecules of fatty acids and one molecule of glycerol per molecule of TAG. The first steps are controlled by adipose tissue triglyceride lipase (ATGL) (hydrolysis of TAG into DAG) and hormone-sensitive lipase (HSL) (hydrolysis of DAG) Monoacylglycerols are hydrolyzed by a different enzyme, a monoacylglycerol lipase, which releases glycerol and the last fatty acid and that has no known regulatory role. This hydrolysis is usually complete although some DAG and MAG can accumulate. Since adipose tissue has very low glycerol kinase activity, the end-product glycerol is released in the circulation for use by other tissues. Release of glycerol depends in part on adipose tissue aquaporin 7 (AQP7), a channel-forming integral protein of the cell membrane. AQP7 is a member of a family of at least 13 proteins that function as water channels [105] and, for some members, as glycerol channels. Its expression is increased during fasting and reduced by refeeding and insulin [106], while thiazolidinediones stimulate it [107]. In addition, there is an acute regulation by catecholamines through β-adrenoceptors, with phosphorylation of AQP7 and its translocation to the cell membrane [105]. Deletion of AQP7 in mice induces lack of plasma glycerol increase in response to beta-adrenergic stimulation and during fasting, with fasting hypoglycemia [105], and results in obesity [108]. Missense mutations resulting in the loss of transport activity have been described in humans [109]. One subject homozygous for such a mutation had a normal body weight and normal basal plasma glycerol concentration but lack of increase during

exercise [109], suggesting that AQP7 has a role in glycerol efflux in humans but is not the only mechanism. Fatty acids released by the hydrolysis of TAG can, on the contrary, be either released or reesterified into TAG without appearing in the circulation. This intracellular recycling of fatty acids depends of the availability of G3P and of the expression and activity of esterification enzymes. In the basal, post-absorptive state, this recycling appears limited [110], but high reesterification rates can occur during exercise [111] or in pathological situations such as hyperthyroidism [110] and stress [112]. Fatty acids released by lipolysis could theoretically also be oxidized, but this fate appears negligible in normal adipocytes (<1 %) [113]. The mechanisms responsible for the transport of fatty acids released by lipolysis to the plasma membrane are debated. aP2 is probably involved, since it forms a complex with HSL [114] and aP2$^{-/-}$ mice have decreased release of fatty acids from adipose tissue [115]. The efflux of fatty acids, as their uptake, probably involves both diffusion and transport by specific plasma membranes proteins.

Lipolysis is mainly controlled by the enzyme HSL, whose activity is regulated principally by catecholamines and insulin through the cAMP-PKA pathway. However, it is now clear that HSL is also controlled by other mechanisms and that other lipases are involved in adipocytes TAG hydrolysis. Lastly, the role of lipid droplet-associated proteins, described first for perilipin1 [116], has been extended to other proteins and appears increasingly important in the control of lipolytic enzymes activity.

HSL

HSL was first characterized in rats as a 84 kDa protein with 768 amino acids. In human adipose tissue HSL is a 88 kDa immunoreactive protein of 775 amino acids encoded by nine exons and whose gene is on chromosome 19. It is expressed also in brown adipose tissue, steroidogenic cells, skeletal muscle, heart, insulin-secreting beta-cells, mammary glands, and, at least in rodents, macrophages [117]. HSL is a serine protease that can hydrolyze TAG, DAG, cholesterol, and retinyl esters. In adipose tissue it hydrolyzes TAG and DAG with a higher activity for DAG and, when acting on TAG, a preference for the *sn*1-ester and 3-ester bond (see in [117]).

Analysis of the structure of HSL has shown several functional domains. The N-terminal part of approximately 300 amino acids is involved in the dimerization [118] and therefore in the activity of HSL, since there is evidence that its functional form is a homodimer [119]. Residues 192–200 are critical for the interaction with aP2 (see in [117]), an interaction that probably has a role in the efflux of fatty acids released by HSL and in preventing the inhibition of HSL activity by these fatty acids. The C terminal part contains the catalytic and regulatory domains. The active serine of the catalytic triad is at position 423 in rat and 424 in humans, located in a Gly-Xaa-Ser-Xaa-Gly motif found in lipases and esterases [120]. This serine is encoded by exon 6. A truncated, short form of HSL of 80k DA generated by alternative splicing of exon 6 during the processing of HSL mRNA has been described in human but not rodent tissue. This short from lacks serine 424 and is devoid of activity [121]. The presence of this variant in some obese subjects is associated with decreased in vitro HSL activity and reduced maximal lipolytic response to catecholamines [122]. The other amino acids of the catalytic triad are Asp 703 and His 733 in rats (Asp 693 and His 723 in humans) [123]. The regulatory domain is encoded principally by exon 7 and most of exon 8. It runs from residue 521 to 669 in rats [117] and contains the serines (serine 563, 565, 659, and 660 in rats) whose phosphorylation status controls the activity of HSL.

HSL activity is stimulated by catecholamines through the classical adenylate cyclase-cAMP-PKA pathway. The action of catecholamines is potentiated by Angptl4 [124]. Actually, catecholamines stimulate lipolysis through their β-receptors and inhibit it through α-receptors, the net result depending on the balance between the two actions and usually resulting in humans in physiological situations in stimulation of lipolysis [7]. Regional differences between different adipose tissue sites in the

proportion of alpha and beta receptors result in differences in the response to catecholamines [125] and regional differences in the regulation of adipose tissue metabolism (see ref [126]). Stimulation of lipolysis results from the phosphorylation of serine 563 that is the regulatory site [127]. Serine 565 (basal site) is phosphorylated in basal conditions. The two sites are mutually exclusive and the basal site can block the phosphorylation of serine 563 and thus exerts an anti-lipolytic action [128]. Serine 565 can be phosphorylated by several kinases, particularly AMPK [128]. Compounds activating AMPK, such as metformin, may thus exert an anti-lipolytic action [129]. Lastly, evidence has been provided that serines 659 and 660 are also phosphorylated by cAMP-dependent protein kinase in vitro in rat adipocytes and that this phosphorylation could also stimulate lipolysis [130].

Other pathways of phosphorylation have been described. Increased cAMP concentrations can activate the mitogen-activated protein kinase/extracellular regulated kinase (MAPK/ERK) pathway [131, 132]. Activated ERK phosphorylates HSL at serine 600 and increases its activity [131]. Lastly. The natriuretic peptides Atrial Natriuretic Peptide (ANP) and Brain Natriuretic Peptide (BNP) have been shown to phosphorylate HSL and stimulate lipolysis [133]. This effect is present only in primates. ANP and BNP activate guanylate cyclase and stimulate cGMP-dependent protein kinases. They probably play a role in the stimulation of lipolysis during exercise [133] and could explain the maintenance of lipolysis during treatment with β-adrenergic receptors antagonists [133].

Dephosphorylation of the regulatory site(s) inhibits HSL. Insulin, the main anti-lipolytic hormone, acts by phosphorylating and stimulating the activity of phosphodiesterase 3B (PDE3B), that breaks down cAMP and thus reduces the phosphorylation of HSL [134]. This action is mediated by the PI3kinase-PKB pathway [135]. Insulin also activates ERK in adipocytes, but the relation of this action with the regulation of lipolysis is unclear. Ser-563 can also be dephosphorylated by the protein phosphatases 2A and 2C [136] and insulin could stimulate these phosphatases. Other mechanisms are possible: internalization of β adreno-receptors [137] or disruption by insulin of β adrenergic signaling [138]. There is also evidence that hypothalamic action of insulin could inhibit HSL and lipolysis through suppression of sympathetic nervous system outflow to WAT [139]; adipocyte DNL could be simultaneously stimulated. Adenosine, NPY and PGE2 also inhibit lipolysis through Gi coupled receptors (see [24, 140]). Lastly, homocysteine was recently shown to inhibit lipolysis through the AMPK pathway [141].

Other Lipases

HSL was long considered as the only, and therefore regulatory, enzyme hydrolyzing adipose tissue TAG. This view was challenged by the finding that mice lacking HSL do not develop obesity and have a reduced fat mass [142, 143]. They always have a marked basal lipolysis and a response of lipolysis to beta-adrenergic stimuli [142–144], suggesting than another lipase was present and active in the absence of HSL. The finding that DAG accumulate in adipocytes of these mice [145] suggested that another lipase preferentially hydrolyzed TAG, and was rate-limiting for the first step of lipolysis, while HSL was limiting for the hydrolysis of DAG. This lipase, named adipose tissue lipase (ATGL) or PNPLA2, has been identified [146]. The enzyme is identical to the protein desnutrin [147] and to the calcium independent phospholipase A2ζ [148] described nearly simultaneously.

ATGL in mice and humans is expressed predominantly in white and brown adipose tissue, localized to the adipocyte LD. It is also present, to a lesser extent, in heart, skeletal muscle, and testis [146, 147]. It specifically hydrolyzes TAG, has low activity against DAG and little or no activity against cholesterol esters. Its expression is increased by fasting, PPAR agonists and glucocorticoids, and reduced by refeeding and insulin [3, 147]. ATGL expression increases during the differentiation of human preadipocytes to adipocytes, simultaneously with HSL expression, and the expression of these two lipases appears co-regulated in human adipose tissue [149]. Expressionof ATGL is also reduced

in the adipose tissue of ob/ob and db/db mice [147]. ATGL-overexpressing mice have increased lipolysis and attenuation of diet-induced obesity [150], while ATGL KO mice have increased adipose tissue mass, low basal and isoproterenol stimulated lipolysis [151] and also accumulation of TAG in non-adipose tissue. However, human subjects with mutations of ATGL accumulate TAG in multiple tissues but are not obese (see [151]). Studies of ATGL expression in human obesity have shown reduced protein, but not mRNA, levels in adipose tissue [152, 153]. ATGL contains in its N terminal part a patatin domain and thus belongs to a large family of proteins with patatin domain which have acyl-hydrolase activity (see reference [154] for more details on this family of protein). The N terminal part also contains a consensus sequence Gly-Xaa-Ser-Xaa-Gly for serine lipase, with the possible active serine at position 47. Another domain between residues 309 and 391 contains large amounts of hydrophobic residues, suggesting it could be a lipid/membrane binding site; this domain could be responsible for the constitutive presence of ATGL on the LD. ATGL activity is mainly controlled by posttranslational regulation. Its activation requires the protein CGI-58 (or ABHD-5). Mutations in the human CGI-58 gene induce the Chananin-Dorfman syndrome, characterized by TAG accumulation in multiple tissues [155]. In the basal state CGI-58 is bound to perilipin 1 and cannot access ATGL. PKA activation phosphorylates perilipin 1 (the phosphorylation site probably responsible is serine 517 [156]) and releases CGI-58, which can then bind and activate ATGL [146, 155]. It has been recently shown that the A-kinase anchoring protein (AKAD) implicated in this action of PKA on perilipin 1 is Optic atrophy 1 (OPA-1) [157]. ATGL is not a target for PKA, but AMPK phosphorylates serine 406 and activates ATGL [158].

Other potential lipases have been described in adipocytes. Carboxyl esterase 3 (known also as hepatic triglyceride hydrolase) is present in adipocytes and could contribute to basal lipolysis [159–161]. Adiponutrin (PNPLA3) is highly expressed in adipose tissue, has high sequence homology with ATGL with a patatin domain, the consensus sequence for serine hydrolase and possible lipid/membrane- binding domains [162]. The regulation of its expression is however quite different, since it is repressed during fasting and increased in fa/fa rats [162, 163]. Divergent results on a possible TAG hydrolase activity of adiponutrin have been reported [146, 148] and its role in adipose tissue TAG metabolism remains uncertain. Lastly, two other members of the adiponutrin family (GS2, GS2-Like) could also be involved in lipolysis [164].

Perilipin 1 and LD-Associated Proteins

Phosphorylation of purified HSL induces only a modest two to threefold increase in activity, whereas the stimulation of lipolysis in intact adipocytes by beta-adrenergic agents induces a much larger increase of lipolytic rate. A first explanation to this discrepancy appeared when it was demonstrated that phosphorylation of HSL induced, in addition to a stimulation of its activity, its translocation from the cytosol to the surface of LD, where it can hydrolyze TAG [165]. This requires the phosphorylation of serines 659 and 660 [166]. A second explanation emerged when it appeared that PKA phosphorylated not only HSL but also perilipin 1, a protein surrounding LD which acts as a gatekeeper for the access of HSL to TAG. Perilipin 1 is one of the numerous proteins surrounding LD and belongs, together with adipophilin (or ADRP, adipocyte related differentiation protein, now perilipin 2), TIP-47 (now perilipin 3), S3-12 (perilipin 4), and LSDP5 (perilipin 5), to the perilipin family of proteins [167] (for a review of PAT proteins see ref [168]). ADRP is expressed in all cells storing lipids [169]. In adipocytes, it is highly expressed during differentiation and constitution of LD and its expression decreases in mature adipocytes. Its role is still unclear, but it could be involved in the transport of lipids to droplets [170]. Perilipin 1 is expressed in adipocytes, steroidogenic cells [171] and foam cells of atheroma plaques [172]. Perilipin 1 expression appears during the differentiation of adipocytes and is high in mature adipocytes. Expression requires the presence, and intracellular metabolism, of fatty

acids [173] and is also stimulated by PPARγ agonists [174]. There are at least three forms of perilipin 1, A, B, and C, resulting from different splicing of a common premessenger RNA, and sharing a common N protein part [171]. Perilipin A and B are expressed in adipocytes, A being the predominant form. Perilipins are phosphorylated on multiples serine sites by PKA (three serines on the N terminal part common to perilipin A and B and three other on the C terminal part specific of perilipin A). In the basal, unphosphorylated state, perilipin 1 opposes the hydrolysis of TAG by HSL [175]. The phosphorylation of perilipin 1 allows phosphorylated HSL to bind it and to access lipids of LD to hydrolyze them [176]. Thus perilipin 1 regulates the action of both HSL and ATGL. This role of perilipin is demonstrated by studies in perilipin 1 null mice. These mice have a reduced fat mass and are resistant to genetic and diet-induced obesity [177, 178]. Their lipolysis is increased in the basal state, but the response to beta-adrenergic stimulation is reduced [177, 178]. Surprisingly, perilipin 1-overexpressing mice are also resistant to obesity [179]; this could be related to a decrease in FSP27 expression (see next paragraph). Perilipin 1 is expressed in human adipose tissue and evidence for a role in the regulation of lipolysis in humans was provided [180–183]. These studies showed that a low total perilipin 1 content was associated with high basal lipolytic rate of isolated adipocytes and high concentrations of glycerol and NEFA in vivo, thus supporting a role for perilipin in the regulation of lipolysis in humans [181]. The possible role of perilipin 1 in human obesity remains unclear; both decreased [181–183] and increased expression [180] in obese subjects has been reported.

The role of perilipin 5 has recently been clarified. However, perilipin 5 expression is very low, or absent, in WAT. Perilipin 5 is highly expressed in oxidative tissues. It recruits mitochondria to LD and in basal conditions opposes lipolysis and shifts fatty acids towards TAG synthesis and storage in LD. This is due to interactions with ATGL. Under β adrenergic stimulation this inhibition is relieved, with perilipin 5 favoring lipolysis and channelling of fatty acids toward beta-oxidation (see [113, 184–186] for details). The very low expression of perilipin 5 in white adipocytes could contribute to their low oxidative rate of fatty acids.

FSP27 (Cidec in humans) belongs with Cidea and Cideb to the CIDE family of proteins (Cell death-inducing DFF45-like effector). FSP27 is expressed in WAT and BAT, Cideb in liver and Cidea in BAT. All three proteins have, in addition to a role in cell death, roles in lipids and energy metabolism, since their deletion results in lean mice with decreased fat mass, resistance to diet-induced obesity, increased insulin sensitivity, energy expenditure, lipolysis and fatty acid oxidation, although the precise mechanisms involved appear different [187]. FSP27 expression is highly induced during adipocyte differentiation. It is localized to the surface of LD [103, 104]. Its overexpression increases lipids accumulation in large unilocular LD, while its ablation results in the formation of small multilocular LD [103, 104]. This protein clearly promotes the clustering of LD and the formation of large unilocular LD [188]. White adipocytes from FSP27 KO mice have increased lipolysis and fatty acid oxidation. The increased lipolysis seems related to the fragmentation of LD, increasing the total surface accessible for lipolytic enzymes. Enhanced lipid oxidation is explained by an increase in the number and activity of mitochondria in adipocytes [103, 189]. The expression of WAT-selective genes is reduced while that of some BAT-selective genes is up-regulated [190] and the WAT of FSP27-deficient mice acquires BAT-like properties [189]. Therefore, FSP27 is important to promote efficient lipid storage in WAT. Its expression is stimulated by PPARγ and decreased by TNFα [191] and this contributes to the lipolytic action of this cytokine. FSP27 expression is decreased by perilipin 1 overexpression in mice and this probably explains the unexpected resistance to obesity of these mice [179, 192].

Cholesterol Metabolism

Adipocytes store TAG but also relatively large amounts of cholesterol (1–5 mg/g of total lipids) [193], making adipose tissue the body's largest cholesterol pool. Contrary to what is observed in steroidogenic cells and foam cells, most (about 95 %) of this cholesterol is in the free, nonesterified form.

In adipocytes, cholesterol is present in two major pools, the plasma membrane and the phospholipid monolayer surrounding LD. This last pool may represent up to one third of the total free cholesterol pool and the percent of free cholesterol present in plasma membrane is thus much lower in adipocytes than in other cells [194]. Since the cholesterol synthetic rate is very low in adipocytes [195], most of the adipocyte cholesterol comes from plasma lipoproteins. The LDL-receptor (LDL-R), the oxidized LDL receptor 1, LRP, and the scavenger receptor BI (SR-BI) are expressed by adipocytes, but their respective quantitative importance in the uptake of cholesterol has not been defined. Interestingly, the expression of SR-BI is stimulated during differentiation of adipocytes [196] and most of the cholesterol taken up through this receptor is targeted in mature adipocytes toward LD [197]. Caveolins could be implicated in this intracellar cholesterol transport [198]. In addition, insulin and angiotensin induce the translocation of SR-BI from intracellular pools to the plasma membrane and stimulate the uptake of cholesterol from HDL; these actions are mediated by the PI3-kinase pathway [196]. There is a strong correlation between adipocyte cell size and cholesterol content. This content increases thus during replenishment of LD and increases further in hypertrophic adipocytes in the obese state [199]. Thus, adipose tissue can store large amounts of cholesterol, particularly during obesity. Adipocytes express the transporter ABCA1 [200] and can also release cholesterol. However, a significant increase in this efflux is observed in vitro only during prolonged stimulation of lipolysis by lipolytic agents [200]. Efflux is independent of caveolins. Whether influx is increased during reduction of total body fat mass and how it is regulated in this situation remains to be investigated. Overall, these data suggest that adipose tissue could play a significant role in whole body cholesterol metabolism and have a buffering role of not only plasma TAG but also plasma cholesterol.

Increased adipocyte size also results also in modification of the intracellular repartition of cholesterol: more of the cholesterol is present on the surface of LD. Despite increase in the cell total cholesterol content, the membrane of hypertrophied adipocytes contains less cholesterol [193]. This depletion in membrane cholesterol results in a stimulation of expression of SREBP-2 and its target genes HMG-CoA reductase and synthase and LRL-R, whereas expression of ABCA1 is repressed [199], modifications aimed at restoring the membrane pool of cholesterol. FAS expression is also stimulated; since SREBP-1c is not modified. Increase of FAS expression is perhaps mediated by LXRα. In addition, relative cholesterol depletion in the membrane of adipocyte decreases the expression of Glut-4, with reduced glucose uptake and metabolism, and increases those of TNFα, Interleukin 6 and angiotensinogen [199]; all these modifications favor development of insulin resistance. These data suggest the interesting possibility that cholesterol might be a sensor for the amount of fat stored in adipocytes and serves as a link between the increase in fat stores and some of the modifications of metabolism observed in obesity [193, 199].

Microvesicles

Adipocytes, as most cells, receive and can release microvesicles [201–205]. Microvesicles are an heterogenous population of subcellular membrane-enclosed vesicles with a diameter between 50 and 1,000 nm, usually separated in microparticles (>100 nm) and exosomes (<100 nm). These microvesicles contain in their lumen components of cytosol such as soluble proteins, phospholipids, and also mRNAs and microRNAs [203, 205]. They also contain transmembrane and GPI-anchored proteins. Materials released by one cell can thus be transferred to another cell receiving the microvesicles, allowing communication between cells in a paracrine and possibly endocrine manner [205]. Adipocytes release microvesicles in the basal state and this release can be stimulated by exogenous palmitate, H_2O_2 or pharmacological compounds such as glimepiride [203]. Adipocyte-derived microvesicles (ADMs) contain mRNA for adipocyte-specific proteins, such as perilipin, and FSP27. There is evidence that microvesicles released by large adipocytes transfer to small adipocytes signals

decreasing lipolysis and stimulating lipogenesis and development of LD, thus transferring the burden of lipid storage from adipocytes with already large LD to small adipocytes [202–204]. ADMs can also transfer also material, and thus specific information, to other cells such as macrophages [205]. The composition and the material transferred by ADMs may be modified in pathological situations such as obesity [206]. Therefore, the information received by adipocytes from microvesicles originating from other cells and sent by adipocytes through ADMs to other cells could contribute to the development of inflammation and cardiovascular abnormalities during obesity and insulin resistance.

Conclusion

The recent years have brought important and exciting insights on adipocytes metabolism and changed our view of how processes such as lipolysis and TAG storage are controlled. Lipid droplets have emerged as important and dynamic organelles. An increasing number of proteins surrounding these droplets are described [207], and increasingly important roles for these proteins have been discovered. Microvesicles could appear in the next years as an important tool for the exchange of information both between adipocytes themselves and between adipocytes and other cells. All these new data should help to better understand the physiopathology of obesity and of its complications.

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Chapter 4
Bad Fat or Just More Fat? Murine Models of Metabolically Healthy Obesity

Grace Bennett, Andrew S. Greenberg, and Martin S. Obin

Keywords Obesity • Mouse • Adipose tissue • Inflammation • Insulin resistance • MHO • Fat transplantation

Key Points

- Many (15–25 %) obese individuals are "metabolically healthy" (MHO), remaining insulin sensitive with cardiometabolic, glycemic, and inflammatory profiles comparable to lean individuals.
- MHO individuals store proportionally more fat in subcutaneous as opposed to visceral adipose depots, coincident with reduced adipocyte size and inflammation in the latter.
- Fat topography and adipocyte/adipose function are potentially more critical than obesity per se in promoting the inflammatory and metabolic complications of obesity.
- Fat transplantation experiments in mice and rats demonstrate that increasing subcutaneous fat can enhance the metabolic and inflammatory status of recipients, in particular when subcutaneous fat is transplanted intra-abdominally.
- Genetic mouse models of MHO are characterized by adipose tissue expansion with minimal increases in adipocyte stress and/or adipose tissue inflammation. In a number of these models, the MHO phenotype is maintained despite *greater* adipocyte size (hypertrophy) and intra-abdominal fat mass than in obese wild-type mice.
- These observations support the concept that metabolic protection results not only from good fat but also in the case of MHO individuals from excess of it.

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Introduction

Worldwide obesity has more than doubled since 1980, with more than 500 million individuals currently obese (BMI > 30) [1]. The potential public health, economic and social impacts of this "epidemic" are daunting, as obesity is an independent risk factor for debilitating comorbidities, including type 2 diabetes mellitus (T2DM), cardiovascular disease (CVD), stroke, nonalcoholic steatohepatitis (NASH), certain cancers, and overall mortality [2, 3]. These comorbidities reflect in large part the metabolic dysregulation that typifies the chronically obese state. Intriguingly, however, a significant proportion (25 %) of obese individuals remains relatively protected from metabolic complications typically associated with obesity [4]. This group, referred to as the metabolically healthy obese (MHO) remain insulin sensitive with favorable hormonal and liver enzyme profiles in the relative absence of hypertension, dyslipidemia, and/or inflammation [2, 4–13]. Criteria for defining "metabolic health" in MHO individuals vary in the literature. As a rule, however, the criteria are based on the absence or "below cutoff" values for cardiometabolic risk factors, metabolic syndrome hallmarks or insulin resistance in individuals with BMI > 30 [14–16]. Independent of the criteria used to assess MHO, the metabolic profiles of these individuals approximate those of young lean individuals [17] and have been longitudinally associated with reduced incidences of T2DM and cardiovascular disease. Accordingly, elucidating the factors that underlie the MHO phenotype is an important undertaking.

An Adipocentric View of MHO: Fat Topography and the Maintenance of Functional Adipose Tissue

Hallmark features of the MHO phenotype in humans are the preferential deposition of fat in subcutaneous as opposed to visceral depots [18, 19] and a greater frequency of smaller visceral adipocytes [4, 19–21]. It is widely accepted that visceral fat is more metabolically pathogenic than subcutaneous fat. Indeed the beneficial effects of thiazolidinedione (TZD) therapy on insulin resistance are associated with preferential accumulation of subcutaneous rather than visceral adipose tissue (AT) [22, 23]. The metabolic pathogenicity of visceral fat in part reflects relative insensitivity to the anti-lipolytic actions of insulin and thus relatively high rates of lipolysis [24]. High rates of fatty acid release promote fatty acid uptake, lipid deposition, and ultimately "lipotoxicity" and/or "glucolipotoxicity" within key insulin sensitive tissues (liver, muscle, pancreas), thereby promoting the development of insulin resistance (IR) and T2DM [25–31]. In addition to constitutively higher rates of lipolysis, visceral fat has more immune cells and, in obese individuals, significantly greater production of inflammatory mediators (e.g., cytokines, chemokines) than subcutaneous fat [32–37]. These inflammatory mediators further promote fatty acid release via activation of inflammatory (Toll-like receptor [TLR]4/inhibitor of nuclear factor kappa-B kinase subunit β [IKKβ]) and stress kinase (mitogen-activated protein kinase [MAPK]) pathways that impair adipocyte insulin signaling, thereby compromising the metabolically "safe" storage of triacylglycerol in adipocytes [30, 38, 39]. Notably, MHO individuals have less ectopic (hepatic and skeletal muscle) fat than comparably obese metabolically unhealthy individuals [20]. An additional and potentially critical feature of visceral fat is its direct drainage into the liver via the portal venous circulation. This drainage directly exposes insulin-sensitive hepatic cells and resident inflammatory (Kupffer) cells to the deleterious actions of released fatty acids, adipokines, and other inflammatory mediators. Portal drainage of mesenteric and omental fat is considered an important mechanism by which increased visceral adiposity predisposes to metabolic pathology in obese individuals [40]. Together, these observations support the hypothesis that the greater metabolic pathogenicity of visceral fat reflects both the inherent properties of visceral adipose

tissue as well as its location (i.e., portal drainage). In section "Fat Transplantation and Metabolic Protection: Good Fat, Bad Fat, or Just More Fat?," we review fat transplantation studies in mice which identify both depot-autonomous and location-dependent impacts of transplanted AT on metabolic health.

Adipocyte size is another important factor that determines the metabolic impact of obesity. Visceral AT is reported to have less capacity for preadipocyte differentiation and contain a greater percentage of large adipocytes as compared with subcutaneous AT [37]. Larger (more hypertrophic) adipocytes are considered more diabetogenic, in part due to their greater rate of lipolysis and fatty acid release [41–43]. In addition, hypertrophy subjects adipocytes to chronic stressors (e.g., endoplasmic reticulum (ER) stress, shear stress, hypoxia, fibrosis) that disrupt normal adipocyte triacylglycerol storage, fat oxidation, and adipokine secretion [43–45]. For example, production of inflammatory mediators such as tumor necrosis factor (TNF)-α and interleukin (IL)-6 increases with adipocyte size, whereas secretion of the insulin-sensitizing adipokine adiponectin decreases with adipocyte size [43–48]. High levels of circulating adiponectin are proposed as an additional defining feature of the MHO phenotype [4].

In section "Mouse Models of Metabolically Healthy Obesity" we review mouse models of MHO. Many of these MHO models result from genetic lesioning of inflammatory or stress kinase signaling pathways or from deletion of select immune cell populations. Of particular interest are genetic models in which metabolically healthy obese mice are significantly *more* obese than their metabolically impaired wild-type counterparts. These "hyper-obese" models of MHO underscore the metabolic protection afforded by lipid storage in healthy adipose tissue and identify factors that constrain metabolically healthy AT expansion in obesity.

Fat Transplantation and Metabolic Protection: Good Fat, Bad Fat, or Just More Fat?

Fat transplantation is a powerful experimental tool for assessing the impact of different fat depots on metabolic health. Key questions in such studies are as follows: (1) Does the metabolic benefit of fat transplantation reflect increased fat mass (i.e., lipid storage capacity) per se, or functional differences in the implanted fat (e.g., reduced inflammation, increased insulin sensitizing, or weight-attenuating adipokine secretion)? (2) Do functional differences in implanted fat reflect depot-intrinsic qualities of the transplant or alternatively, modulating effects of the site to which it is implanted? An important caveat is that most studies in mice involve transplantation of the gonadal adipose depot (epididymal fat pad in males). The gonadal depot is not a true visceral (i.e., portally draining) depot. Thus, its direct relevance to the metabolic impacts of visceral (omental, mesenteric, umbilical) fat in humans remains unclear.

Overall, fat transplantation studies indicate that fat depots have cell-autonomous characteristics and metabolic effects. Subcutaneous fat consistently confers metabolic benefits when transplanted, particularly when it is transplanted intra-abdominally [49–52]. Transposition of subcutaneous fat is associated with subsequent reductions in visceral adipose depots, visceral AT inflammation, and portal lipids, and these are proposed as mechanisms by which subcutaneous fat confers metabolic protection [49–52]. These observations suggest that, as in MHO individuals, the balance between subcutaneous fat and visceral fat is an important determinant of systemic insulin sensitivity. The metabolic impacts of intra-abdominal fat are less clear-cut. Removal of epididymal fat can enhance insulin sensitivity in nonobese chow fed rodents [53, 54] and mice transplanted with epididymal fat can develop glucose intolerance and hepatic IR [55]. However, transplantation of epididymal fat can paradoxically improve glucose-insulin homeostasis in both lipoatrophic and nonobese recipients [31, 49, 53, 56]. Metabolic improvements associated with transplantation of epididymal fat included

decreased portal lipids and improved liver metabolism [53, 56]. Thus, evidence suggests that both removal and addition of intra-abdominal fat can promote metabolic benefits, presumably by different mechanisms [55]. In other studies, however, epididymal fat transplanted into the subcutaneous or intra-abdominal/visceral cavity of either lean or obese mice was unable to improve insulin sensitivity or significantly attenuate glucose intolerance [50, 51].

The transplantation site also shapes transplant responses and metabolic outcomes. Intriguingly, these location-dependent effects vary with the physiological model used. For example, in lipoatrophic and partially lipectomized mice, fat transposed to subcutaneous depots is metabolically beneficial independent of depot of origin [31, 52, 56], presumably reflecting the salutary effects of increased fat storage capacity per se. However, in mice made obese by 7 weeks of HFD feeding, neither inguinal nor epididymal fat transplantation into the subcutaneous space improved metabolic outcomes [50]. In contrast, transplantation of inguinal fat to the visceral side of the peritoneum (where it would drain into the portal circulation) significantly attenuated glucose intolerance coincident with reductions in endogenous inguinal, epididymal, and retroperitoneal fat [50]. Similarly, in nonobese mice, inguinal (but not epididymal) fat transplants reduced endogenous adiposity and improved glucose-insulin homeostasis when transplanted into the visceral cavity (portal drainage), but had significantly less benefit when transplanted into the subcutaneous space.

Portal drainage is also reported in some [55] but not all [49] studies to be required for the negative metabolic impacts of transplanted intra-abdominal (epididymal) fat. Nonobese mice receiving epididymal fat transplants to the mesenterium (portal venous drainage) but not those receiving transplants to the parietal peritoneum (caval/systemic venous drainage) developed impaired glucose tolerance and hepatic insulin resistance [55]. Moreover, portal vein IL-6 was elevated only in mice receiving a portal fat transplant, and such transplants from IL-6 KO mice failed to induce glucose intolerance, implicating portally drained, adipose-derived IL-6 in impaired glucose tolerance [55].

In summary, fat transplantation studies demonstrate that both depot-intrinsic and location-dependent factors interact to either confer metabolic benefit or promote metabolic dysregulation. These studies support the view that subcutaneous fat has intrinsic salutary properties and that a predominantly subcutaneous fat distribution may be metabolically beneficial in obesity. In addition, portal drainage appears to be important for the metabolic benefits of transposed subcutaneous fat and the metabolic derangements of transposed epididymal fat, respectively.

Mouse Models of Metabolically Healthy Obesity

Our review of murine models of MHO focuses on genetic models in which obesity is associated with a salutary profile of glucose-insulin homeostasis and adipocyte/AT function. The genetic models discussed below and summarized in Table 4.1 are grouped into five (overlapping) categories of proximate physiological effect: (1) altered expression of adipokines or inflammatory mediators, (2) disrupted inflammatory signal transduction, (3) reduction or enhancement of AT immune cell populations, (4) attenuated adipocyte stress, and (5) enhanced adipogenesis and/or adipocyte lipogenesis. Intriguingly, in a number of these models, MHO mice are *more* obese than their obese but metabolically unhealthy wild-type counterparts. In light of the metabolically beneficial effects of weight loss per se, our discussion does not include the myriad genetic models in which metabolic protection is associated with reduced adiposity or body weight, including groundbreaking studies of immunity in which metabolic improvements in obese mice were obtained following ablation of cluster of differentiation (CD)8^+ T lymphocytes [57] or mast cells [58], or upon supplementation of T lymphocyte-depleted mice with $CD4^+$ T cells [59].

Table 4.1 Mouse genetic models of metabolically healthy obesity

Model	BW	Fat mass[a]	GAT mass	ScAT mass	Adipocyte size	AT inflammation stress signaling	Metabolic protection	Ectopic fat	Citations
Adiponectin Tg (*ob*/*ob*)[b]	↑	↑	↓	↑	↓	↓	↑Glucose Tolerance ↓Fasting Glucose, Insulin		[63]
TBP-2 KO HcB-19 (HFD, *ob*/*ob*)	↑	↑	↑	↑	-	-	↑Glucose Tolerance ↑Insulin Signaling[c]		[64–67]
COL6 KO (*ob*/*ob*)	↔	↔	-	-	↑	↔, ↓[d]	↑Glucose Tolerance		[68, 70]
TWEAK KO (HFD)	↔	↔	↑	↔	↑	↓	↑Glucose/Insulin Tolerance	↓	G. Bennett, M. Obin, (in prep.)
aP2 KO (*ob*/*ob*, HFD)	↑	↑	↑	-	-	↓	↑Glucose/Insulin Tolerance		[78–83]
IL-1R1 KO (HFD)	↔	↑	↑	↑-	-	↓	↑Glucose/Insulin Tolerance		[87]
AIM KO (HFD)	↑	↑	↑	↑	↑	↓	↑Glucose/Insulin Tolerance ↑Insulin Signaling[c]		[88–90]
ERK-1 KO (HFD)	↔	↔	↓	↑	-	↓-	↓Fasting Glucose ↑GD	↓-	[93]
Tpl2 KO (HFD)	↔	-	↔	↔	↔	↓	↑GlR; ↓HGO	↓	[95]
iNOS/NOS2 KO (HFD)	↔	↔	↔	↔	-	-	↑Glucose/Insulin Tolerance ↑Insulin Signaling[c]		[97–99]
JNK1 KO BM (HFD)	↔	↔	-	-	-	↓	↑GlR; ↑GD ↓HGO ↑Glucose/Insulin Tolerance	↓	[104]
TLR4 KO C3H/HeJ (HFD)	↑	↑	-	-	-	↓	↓Fasting Glucose, Insulin ↑Insulin Tolerance ↓Lipid-Induced IR[e]	↓	[105–108]
CD11c-DTR BM (HFD)	↔	↔	↔	-	-	↓	↑GlR; ↓HGO ↑Glucose/Insulin Tolerance	↓	[115, 116]
aP2-DGAT1 Tg aP2DGAT-TgBM (HFD)	↑	↑	-	-	-	↓	↓Fasting Glucose, Insulin ↑Glucose/Insulin Tolerance	↔	[117, 118]

Increased (↑), decreased (↓), or no significant difference (↔) in obesity complications relative to comparably or less obese wild-type mice. Cells with hyphens reflect data not reported. See section "Mouse Models of Metabolically Healthy Obesity" for details of gene nomenclature and function

BW body weight, *GAT* gonadal adipose tissue, *ScAT* subcutaneous adipose tissue, *KO* knockout mouse, *HFD* high fat diet, *GIR* glucose infusion rate during the euglycemic/hyperinsulinemic clamp, *HGO* hepatic glucose output during the euglycemic/hyperinsulinemic clamp, *GD* glucose disposal rate during the euglycemic/hyperinsulinemic clamp, *BM* bone marrow transplanted into wild-type mouse, *Tg* transgenic mouse

[a]Total fat mass (by magnetic resonance imaging or computed tomography) or aggregate mass of multiple fat pads

[b]Obesity phenotype expressed on *ob*/*ob* mouse background

[c]Enhanced insulin-dependent phosphorylation of Akt and/or IRS-1 and/or GSK3β in insulin-sensitive tissue(s)

[d]No difference in adipose tissue macrophages, but reduced MAPK activation (phosphorylation of JNK and ERK)

[e]Improved GIR, glucose turnover, and glucose uptake in skeletal muscle and adipose tissue during the euglycemic/hyperinsulinemic clamp conducted 5 h post lipid infusion

Adiponectin Transgenic Mice

Adiponectin is an anti-inflammatory, insulin-sensitizing adipokine expressed exclusively by adipocytes that exerts salutary effects on lipid and glucose homeostasis via multiple mechanisms [60–62]. Elevated circulating adiponectin is a hallmark and predictor of the MHO phenotype in humans [4]. Adiponectin transgenic mouse on an *ob*/*ob* background (AdTg) had two to threefold higher levels of circulating adiponectin than *ob*/*ob* controls [63]. On a normal diet, AdTg mice became extraordinarily obese, weighing on average 40 g more than ob/ob mice at 21 weeks of age. This extreme obesity largely reflected preferential hyperplastic expansion of subcutaneous AT depots. Visceral depots and adipocytes were reduced in size to wild-type (ob/+) levels. Hyper-obesity AdTg mice remained insulin sensitive and glucose tolerant, had fewer AT macrophages and inflammatory markers, less liver steatosis and improved circulating insulin, glucose and triacylglycerol. Thus, adiponectin overexpression results in a hyper-obese mouse with predominantly subcutaneous fat, smaller adipocytes, attenuated inflammation and enhanced metabolic protection that recapitulates human MHO.

Thioredoxin Binding Protein (TBP)-2 KO Mice

Thioredoxin binding protein (TBP)-2 (also referred to as thioredoxin interacting protein (Txnip) or vitamin D_3 upregulated protein [VDUP]-1) is a member of the α-arrestin protein family with demonstrated roles in the regulation of cell fate, immune responses, and energy metabolism. TBP-2 is a negative regulator of adipogenesis, in part through its inhibitory actions on peroxisome proliferator-activated receptor (PPAR)γ expression and activity [64–67]. Consistent with these observations, TBP-2 KO mice fed either a normal or high fat diet (HFD) or TBP-2 KO mice on an *ob*/*ob* background gained significantly more weight (up to 100 %) and adiposity (up to 50 %) than similarly fed wild-type (WT) or *ob*/*ob* mice [64, 66]. Greater adiposity reflected increased energy intake and adipocyte hyperplasia in multiple AT depots [66]. Despite greater adiposity, both models of obesity in TBP-2 KO mice were more glucose tolerant and insulin sensitive than obese controls, reflecting augmented glucose transport in AT and skeletal muscle and improved glucose-stimulated insulin secretion (GSIS) [69]. Thus, as in adiponectin-overexpressing mice [63] adipose accretion by adipocyte hyperplasia is associated with an MHO phenotype in obese TBP-2 KO mice. Similarly, the HcB-19 mouse strain with a naturally occurring TBP-2 nonsense mutation (truncation) becomes more obese than WT mice at an early age but retains glucose tolerance and insulin responsiveness [67]. HcB-19 mice crossed with the *ob*/*ob* mice become even more obese than *ob*/*ob* mice, but are protected against peripheral insulin resistance, β-cell apoptosis and subsequent T2DM [67].

Collagen VI (COL6) KO Mice

Adipocyte hypertrophy and AT expansion require a coordinated tissue remodeling program in which extracellular matrix (ECM) turnover plays a critical role ([44], and reviewed in [68]). Recent studies suggest that excessive deposition of collagen(s) or other ECM components (fibrosis) resulting from chronic AT remodeling promotes inflammatory and metabolic pathology associated with hypertrophic obesity. Excessive AT collagen constrains adipocyte expansion, thereby activating inflammatory stress kinase pathways and impairing adipocyte function [69, 70]. Consistent with these observations, collagen VI (COL6) KO mice on the *ob*/*ob* background developed extra-hypertrophic adipocytes and greater AT mass in both the gonadal and mesenteric depots, but had lower fasting glucose, enhanced

glucose tolerance and reduced circulating triacylglycerol following lipid challenge as compared with control *ob*/*ob* mice [70]. This metabolic protection was associated with reductions in markers of endoplasmic reticulum (ER) stress, attenuated c-Jun N-terminal kinase (JNK) and extracellular signal-regulated kinase (ERK) activation, reduced frequency of adipocyte death, and lower circulating IL-6 and TNF-α following lipopolysaccharide (LPS) challenge. Thus, reducing AT collagen facilitates the development of metabolically "benign" adipocyte hypertrophy and an MHO phenotype.

Tumor Necrosis Factor-Related Weak Inducer of Apoptosis (TWEAK) KO Mice

Additional evidence implicating AT fibrosis in obesity complications comes from analysis of mice deficient for tumor necrosis factor (TNF)-related weak inducer of apoptosis (TWEAK/TNFSF12), a cytokine of the TNF superfamily [71–73]. Through engagement with its signaling receptor, fibroblast growth factor-inducible (Fn)-14, TWEAK activates mitogen activated protein kinase (MAPK), and nuclear factor (NF)κB pathways coordinating tissue remodeling and repair, including the expression of matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) [71, 74, 75]. Both TWEAK and Fn14 are upregulated in AT of morbidly obese humans [76, 77] and in gonadal AT of mice fed HFD for >8 weeks (G. Bennett and M.S. Obin, in preparation). Notably, TWEAK KO mice fed HFD for >8 weeks develop (30 %) larger adipocytes and gonadal AT mass than HFD-fed WT mice. This greater hypertrophy is associated with an altered profile of MMPs and TIMPs favoring collagenolyis, reduced levels of mature (cross-linked) collagen-I, decreased JNK activation, and reduced expression of interferon (IFN) γ and interferon-induced inflammatory mediators (G. Bennett and M.S. Obin, in preparation). Consistent with larger adipocytes and attenuated AT inflammation, TWEAK KO mice also have reduced circulating free fatty acids and ectopic fat, and they develop improved glucose tolerance and enhanced peripheral and whole-body insulin resistance between weeks 12 and 16 of HFD. The MHO phenotype of obese TWEAK KO mice suggests that TWEAK's fibro-inflammatory actions in gonadal AT constrain healthy adipocyte expansion, thereby promoting AT inflammation and metabolic complications of obesity.

Adipocyte-Fatty Acid Binding Protein (AFABP/aP2) KO Mice

Adipocyte-fatty acid binding protein (AFABP/aP2) is an intracellular lipid chaperone that coordinates metabolic and inflammatory responses in adipocytes and macrophages. In response to a 12-week HFD-challenge initiated at 5–6 months of age, aP2 KO mice gained more weight and epididymal AT mass than WT mice, yet exhibited lower fasting glucose and insulin, improved performance in the intraperitoneal glucose tolerance test (GTT) and insulin tolerance test (ITT), lower circulating triacylglycerol and attenuated TNF-α expression in the epididymal depot [78]. These findings were recapitulated in obese aP2 KO mice on the *ob*/*ob* background [79]. Reduced basal and β-adrenergic-stimulated lipolysis, suggested that "lipolytic inefficiency" in aP2 KO mice promoted greater fat storage in adipocytes, enhanced peripheral insulin sensitivity and altered the plasma fatty acid profiles sufficiently to attenuate β-adrenergic-stimulated insulin secretion from islets [80, 81]. Intriguingly, younger (8 week-old) and thus lighter KO mice fed HFD for 12 weeks did not become either more obese or more insulin sensitive than WT mice [82], suggesting that the MHO phenotype of aP2 KO mice arises in the context of severe obesity. Obese WT mice transplanted with aP2-deficient bone marrow had improved fasting glucose and insulin levels and were more glucose tolerant and insulin sensitive than obese mice that received WT bone marrow [83]. Thus, both adipocyte and macrophage aP2 contribute to the metabolic complications of obesity.

Interleukin-1 Receptor 1 (IL-1R1) KO Mice

IL-1β is a hallmark of obesity-associated AT inflammation, glucose intolerance and IR [84–86]. IL-1β signals through the IL-1 receptor (IL-1R) to activate NFκB and JNK pathways controlling cytokine production and, via activation of the Janus kinase (JAK)2/signal transducers and activators of transcription (STAT)3 pathway, regulates suppressor of cytokine signaling molecule (SOCS)-3 [87]. When fed a HFD, IL-1RKO mice developed greater gonadal, subcutaneous, and whole-body fat mass than WT mice [87]. Despite greater adiposity, obese IL-1R KO mice remained insulin sensitive and glucose tolerant (assessed by ITT and GTT). In addition, pooled AT macrophages and whole adipose explants of IL-1R KO mice secreted less IL-6 and TNF-α protein, indicating that metabolic protection in the obese KO mice reflected reduced inflammation. However, HFD-fed KO mice had higher fasting and glucose-stimulated insulin levels than WT counterparts, suggesting that the MHO phenotype of IL-1R1 KO mice reflects at least in part alterations in β cell physiology.

Apoptosis Inhibitor of Macrophage (AIM) KO Mice

AIM (also known as Spa, Api6, and CD5L) is a macrophage-secreted member of the scavenger receptor cysteine-rich superfamily (SRCR-SF). AIM expression and serum levels are upregulated in murine obesity (reviewed in [88]). Macrophage-derived AIM is taken up by adipocytes via CD36-mediated endocytosis. AIM inhibits fatty acid synthase, thereby stimulating lipolysis and the release of fatty acids [89], which activate adipocyte chemokine production via Toll-like receptor (TLR) 4 [90]. The pathophysiologic impact of AIM on inflammation and obesity complications was assessed in AIM-deficient (AIM KO) mice [90]. AIM KO mice fed a HFD for 12 weeks became more obese than WT mice. However, greater adiposity was associated with fewer inflammatory (M1-polarized) macrophages, reflecting reductions in AT chemokine expression. Systemic protection was also evident, including reduced circulating TNF-α, IL-6, and IL-1β, enhanced insulin-dependent phosphorylation of Akt (also known as protein kinase B), and glycogen synthase kinase (GSK)3β in peripheral tissues and improved GTT and ITT performance [90]. Thus, despite greater adiposity, impaired recruitment of M1 macrophages into AT protects AIM KO mice from the inflammatory and metabolic complications of obesity.

ob/ob-Extracellular Regulated Kinase (Erk)-1 KO Mice

Extracellular regulated kinase (ERK) activity promotes adipogenesis, is increased in adipocytes from obese patients [91], and is induced in murine AT by high fat diets [92]. ERK1 knockout mice on an *ob*/*ob* background become as obese as *ob*/*ob* mice, but have reduced circulating glucose and improved glucose tolerance [93]. Improvements in glucose metabolism reflected improved glucose disposal in skeletal muscle and AT. Intriguingly, metabolic protection in *ob*/*ob-Erk-1*$^{-/-}$ mice was associated with a trend for increased subcutaneous and decreased epididymal AT, and this was coincident with robust downregulation of chemokines (CCL2), cytokines (IL-1β, TNF-α) and inflammatory kinases, as well as attenuated T-lymphocyte (CD3^{+}, CD4^{+}) infiltration and downregulated T helper (Th)1 gene activation (i.e., IFNγ and T-box 21 [Tbx21]) in AT. Thus, as in MHO humans, both a proportional increase in subcutaneous fat and reduced intra-abdominal AT inflammation promote the MHO phenotype of *ob*/*ob-Erk-1*$^{-/-}$ mice.

Tumor-Progression Locus 2 (TPL2) KO Mice

TPL2 (also known as MAP3K8) is a serine/threonine kinase that is activated by TNF-α, TLR4 signaling and inflammatory mediators that activate the NFκB and MAPK pathways [94]. Thus, TPL2 is uniquely situated to integrate inflammatory signaling pathways that play critical roles in obesity-associated inflammation and insulin resistance. Perfield et al. [95] placed TPL2 KO mice on a HFD for 16 weeks and reported increases in body weight, adipose depot weights and gonadal adipocyte size comparable to those observed in HFD-fed WT mice. Yet, HFD-fed KO mice had decreased fasting glucose and insulin and improved glucose infusion rate and hepatic glucose output during the euglycemic/hyperinsulinemic clamp. This protected metabolic phenotype was associated with reduced MAPK (ERK, JNK) activation in peripheral tissues, as well as reduced frequency of adipocyte death (i.e., crown like structures [CLS]), CLS-associated macrophages and inflammatory gene expression in epididymal AT. Thus, abrogation of TPL2-mediated inflammatory signaling confers an MHO phenotype of reduced AT inflammation and enhanced insulin sensitivity despite hypertrophic obesity.

Inducible Nitric Oxide Synthase (Nos2) KO Mice

Inducible Nitric Oxide Synthase (iNOS) is the product of the *Nos2* gene, which is upregulated by inflammatory cytokines in skeletal muscle and fat of obese mice and is considered a hallmark of pro-inflammatory (M1) macrophages in AT [96]. *Nos2* induction in obese wild-type mice is associated with skeletal muscle impairments in insulin-stimulated phosphatidylinositol 3-kinase (PI3K) and Akt activation. Notably, Nos2 KO mice become as obese as wild type mice on a high fat diet but are protected from insulin resistance [97]. Obese KO mice exhibit improved glucose tolerance and remain fully insulin sensitive, reflecting unimpaired insulin-stimulated PI3K and Akt activation and normal insulin-stimulated glucose uptake in muscle [97]. With regard to mechanism, studies with *Nos2*$^{-/-}$ mice implicate the iNOS/NO pathway in S-nitrosylation (and thus inactivation) of insulin receptor (IR)β/IR substrate-1 (IRS-1) and Akt and demonstrate that the pathway antagonizes beneficial effects of PPARγ activation, including the MHO hallmarks, adiponectin production and AT remodeling with smaller adipocytes [98, 99].

Inhibitor of κB Kinase-β (IKK-β) KO Mice

The IKK-β protein kinase phosphorylates IκBs in response to proinflammatory stimuli, thereby releasing IκB-dependent inhibition of NFκB–dependent gene transcription. Activation of IKK-β plays critical roles in adipose, hepatic, myeloid, and hypothalamic inflammation in the obese state [27, 100, 101]. When fed a HFD or crossed onto the *ob*/*ob* background [101], *Ikbkb*$^{+/-}$ mice become comparably obese as control mice (homozygous deletion of *Ikbkb* results in embryonic lethality). Heterozygous mice had lower fasting glucose and insulin levels, reduced circulating free fatty acids and increased insulin signaling in peripheral tissues when compared to *Ikbkb*$^{+/+}$ littermates [101]. Thus, abrogation of upstream NFκB activation results in an MHO phenotype. When mice deficient for IKK-β in hepatocytes (*Ikbkb*Δ^{hep}) or myeloid cells (*Ikbkb*Δ^{mye}) [102, 103] were made obese by HFD or by crossing to *ob*/*ob* mice [100], *Ikbkb*Δ^{hep} mice retained insulin responsiveness in liver, but developed insulin resistance in muscle and fat. In contrast, *Ikbkb*Δ^{mye} mice remained insulin responsive in all tissues and were more glucose tolerant and insulin sensitive in euglycemic/hyperinsulinemic clamp studies as compared with WT (floxed) controls. A caveat is that *Ikbkb*Δ^{mye} mice weighed less than WT controls,

Thus, although the MHO phenotype associated with myeloid IKK-β deletion is consistent with the role of proinflammatory macrophages in obesity-associated AT inflammation and IR, we cannot rule out differences in adipose mass as additionally contributing to the protected phenotype.

Chimeric Mice Deficient for Myeloid Jun Kinase (JNK)-1

JNK-1 activation attenuates insulin action via inhibitory phosphorylation of serine/threonine residues at receptor and post-receptor levels. Radiation chimeras receiving bone marrow from JNK-1 KO mice become as obese on HFD as WT mice transplanted with WT bone marrow [104]. However, these chimeric KO mice are metabolically protected, based on GTT, ITT and euglycemic/hyperinsulinemic clamp studies [104]. Metabolic protection was coincident with a favorable inflammatory profile in AT characterized by fewer CLS and attenuated expression of macrophage markers (F4/80) and inflammatory genes (TNF-α, IL-6, IL-12, and macrophage migration inhibitory factor [MIF]). In addition, myeloid deficiency of JNK-1 resulted in elevated circulating adiponectin (as in human MHO) and reduced systemic free fatty acids in response to HFD [104]. Complementary in vitro studies demonstrated that JNK-1-deficient macrophages were less responsive to pro-inflammatory stimulation by palmitate [104]. Together, these results identified myeloid JNK-1 as a critical mediator of AT inflammation and metabolic dysfunction in obesity.

Toll-Like Receptor (TLR) 4 KO Mice

Saturated fatty acids (e.g., palmitate) exert pro-inflammatory effects through the activation of TLR-4, a pattern recognition receptor that is ubiquitously expressed in insulin sensitive tissues and macrophages. TLR signaling activates transcription factors (activator protein [AP]-1, NFκB, and interferon regulatory factors [IRFs]) controlling the expression of cytokines and chemokines that direct the adaptive immune response [105]. Initial reports indicated that TLR4 ablation protected mice from fatty acid-induced insulin resistance in muscle and mitigated HFD-induced insulin resistance in female mice despite greater adiposity [106]. Moreover, C3H/HeJ mice that naturally express a loss-of-function TLR4 mutant allele developed comparable [107] or greater [108] adiposity and adipocyte hypertrophy on HFD but were protected from fasting hyperglycemia and hyperinsulinemia, impaired glucose transport, and hepatic steatosis, and expressed more adiponectin and less TNF-α in epididymal AT [107, 108]. In addition, chimeric mice deficient for TLR4 selectively in hematopoietic (bone marrow-derived) cells became obese but were protected from fasting hyperglycemia and IR and AT inflammation. These observations implicate macrophage TLR4 signaling in obesity-associated inflammation and complications [109]. However, other studies report that C3H/HeJ and 10ScN mice (in which the TLR4 locus is deleted) are in fact resistant to HFD-induced obesity (in particular diets rich in saturated fat) [110, 111]. Mechanisms by which disruption of TLR4 signaling confers obesity-resistance on the one hand and an MHO phenotype on the other remain unclear.

Chimeric Mice Deficient for Myeloid CD11c

The integrin CD11c is a cell surface hallmark of pro-inflammatory "M1" macrophages that infiltrate AT of obese mice and humans, aggregate in CLS around dead adipocytes, prime $CD8^+$ cytotoxic T lymphocytes, and promote insulin resistance [44, 112–114]. To assess the role of $CD11c^+$ macrophages in obesity-associated metabolic dysregulation, irradiated WT mice were transplanted with

bone marrow from mice expressing the simian diphtheria toxin receptor (DTR) driven by the CD11c promoter (CD11c-DTR mice) [115, 116]. Chimeric CD11c-DTR mice were then made obese by 16 weeks of HFD. $CD11c^+$ cells were subsequently selectively ablated by injection of diphtheria toxin (DT) and both HFD and DT injection were maintained for an additional 17 days [116]. At the termination of the experiment, weights of epididymal and inguinal AT depots were identical in obese mice that had received either WT or CD11c-DTR bone marrow. However, CD11c-DTR chimeric mice exhibited robust improvement in obesity-associated inflammation and metabolic dysregulation. First, ablation of $CD11c^+$ cells by DT injection resulted in complete abrogation of HFD-induced $CD11c^+$ macrophage infiltration into epididymal AT in conjunction with a 70 % reduction in CLS, abrogation of IL-6 and MCP-1 induction and a threefold increase in epididymal IL-10 gene expression. These anti-inflammatory effects in AT were associated with attenuated skeletal muscle and systemic inflammation (MCP-1, TNF-α, IL-6, IL-12, IFN-γ), improved liver steatosis and normalization of ITT, GTT, glucose infusion rate, hepatic glucose production and free fatty acid release during the euglycemic/hyperinsulinemic clamp [116]. These results strongly support the notion that $CD11c^+$ AT macrophages contribute to the unhealthy metabolic phenotype of obese individuals.

Diacylglycerol Acyl Transferase (DGAT)-1 Transgenic Mice

Diacylglycerol acyl transferase (DGAT)-1 is the rate-limiting enzyme in triacylglycerol synthesis. Transgene-driven DGAT-1 overexpression in adipocytes (under the control of the aP2 promoter) leads to an increase in adipocyte triacylglycerol storage and thus, increased adipose mass [117]. DGAT-Tg mice fed either a chow or HFD diet develop more AT mass than WT mice due to greater adipocyte size. However, this increased adipose mass is not associated with a worse metabolic profile, based on comparable levels of ectopic fat in the two genotypes and protection from inflammatory macrophage activation, macrophage accumulation in WAT, systemic inflammation, and insulin resistance [118]. To assess the contribution of macrophage DGAT1 expression to this phenotype, irradiated WT mice were transplanted with bone marrow from either WT (control) or DGAT-Tg mice [118], thereby limiting the expression of the transgene to myeloid cells. When fed a HFD, DGAT-Tg BM mice became as obese as mice receiving WT bone marrow, but they stored more triglyceride in AT macrophages. This enhanced storage of triglyceride by macrophages attenuated FA-induced macrophage inflammatory activation and both whole-body insulin resistance and glucose intolerance. Thus, overexpression of DGAT1 in macrophages is sufficient recapitulate both the obesity and associated metabolic protection observed in DGAT-Tg mice.

Summary and Conclusion

MHO humans and mice (this review) demonstrate that differences in fat topography and adipocyte/AT function are potentially more critical than obesity per se in promoting the inflammatory, glycemic and cardiometabolic complications of obesity. Hallmark features of the MHO phenotype in humans are the preferential deposition of fat in subcutaneous as opposed to visceral depots [18, 19] and a greater frequency of smaller visceral adipocytes [4, 19–21]. Transplantation studies in rodents described above demonstrate that subcutaneous fat confers metabolic benefits, in particular when it is transplanted intra-abdominally. In non-lipoatrophic mice these benefits are often associated with reductions in the mass of other adipose depots (and total adiposity). The preferential sequestration of triglycerides in transposed subcutaneous fat may be one mechanism by which transplanted subcutaneous fat enhances the inflammatory and metabolic phenotype of recipients.

Consistent with the association of human MHO with reduced AT inflammation, the preponderance of murine MHO models discussed in this review result from genetic manipulations that attenuate AT inflammation. AT expansion with smaller adipocytes (hyperplastic obesity) is typically less inflammatory and provides one way to achieve MHO in humans and mice (e.g., adiponectin transgenic and TBP-2 KO mice). Surprisingly, *greater* adipocyte size (hypertrophy) and *greater* hypertrophic intra-abdominal fat mass can also be metabolically protective (e.g., mice deficient for IL-1R1, COL-6, aP2, AIM, TLR4, and TWEAK). Together, these observations in mouse models of MHO support the concept that adipose tissue expansion in the relative absence of inflammation or stress-activated signaling can protect from metabolic derangements of obesity. Thus, metabolic health results not only from good fat but also in obese individuals from excess of it.

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Chapter 5
The Aging Adipose Organ: Lipid Redistribution, Inflammation, and Cellular Senescence

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Keywords Aging • Cellular senescence • Inflammation • White adipose tissue

Key Points

- Adipose tissue regulates systemic metabolism and inflammation.
- Adipose tissue plasticity decreases with advancing age.
- Adipose tissue inflammation and senescent cell accumulation increase with advancing age.

Introduction

The traditional dogma that white adipose tissue (WAT) is an inert, energy-storing organ has been disproven over the past 30 years through intensive scientific investigation. Indeed, this work has elucidated the immense complexity of the organ, thereby illuminating its integral role in metabolic homeostasis and systemic inflammation [1]. Most intriguingly, ongoing work has demonstrated that WAT from separate anatomical locations manifests differing metabolic, expression, and secretory phenotypes [2]. A striking divergence is observed between subcutaneous and visceral WAT. Visceral WAT is related to pathological risk as evidenced by its strong association with insulin resistance, nonalcoholic fatty liver disease (NAFLD), type 2 diabetes mellitus (T2DM), and cardiovascular disease (CVD) [3–5]. Subcutaneous WAT displays protective attributes by sequestering and storing circulating lipid thereby mitigating the aforementioned comorbidities [4]. Interestingly, the comorbidities listed above are much more prevalent in older human populations [6, 7], which is coincidently aligned with the time frame when humans begin to demonstrate age-associated reductions in subcutaneous WAT mass [8–12]. These observations suggest that WAT could be a pivotal regulator of health-span and longevity; therefore, WAT dysfunction is potentially causal for disease onset and progression.

Throughout the lifecycle, WAT morphs in a way that is conducive to redistribution of lipid from subcutaneous to visceral depots with advancing age. This phenomenon occurs in conjunction with the accumulation of lipid in nonadipose tissues including liver, skeletal muscle, and bone marrow

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[13–15]. Loss of subcutaneous WAT, expansion of visceral WAT, and increased ectopic lipid accumulation are commonly observed in aging populations and are strong predictors of age-related disease progression [16, 17]. Clinical studies suggest that a reduction in visceral WAT through caloric restriction [18], physical activity [19], and/or bariatric surgery [20] may mitigate age-associated pathologies in humans. Dietary or pharmacological interventions known to increase mean and maximal lifespan in animals have profound effects on WAT function [21–23]. Genetic manipulations that lead to reduced visceral WAT accumulation are associated with extended lifespan in a variety of animal models [24–28]. Collectively, the body of evidence suggests that WAT distribution and function are associated with longevity.

The redistribution of lipid with advancing age implies a decline in subcutaneous WAT functional capacity over time. This is supported by the observation that older adults esterify more dietary lipid in visceral WAT as compared to young controls [29]. The causes of age-related lipid redistribution are likely multifactorial. Reduced preadipocyte proliferation and differentiation are paramount to this process [30]. Inflammation likely plays a role by promoting replicative exhaustion of the preadipocyte pool and preadipocyte senescence. Accumulation of senescent cells has emerged as a frequent feature in aging WAT. Interestingly, the removal of senescent cells improves health-span in a progeroid mouse model, coupled with delayed age-related loss of subcutaneous WAT [31]. The reduction of WAT functional capacity with aging and the role that inflammation and cellular senescence may play in this process will be explored throughout this review. Potential therapeutic interventions for dysfunctional WAT will also be discussed.

White Adipose Tissue Function

The primary function of WAT is to sequester and esterify calorically dense, cytotoxic fatty acids until times of energy scarcity. As demonstrated in the non-age-related forms of lipodystrophy, normal WAT function is important in mitigating ectopic lipid accumulation and its associated pathologies [32]. Most lipid in white adipocytes is stored as neutral triglycerides in unilocular lipid droplets [33]. The accumulation of triglyceride occurs through numerous mechanisms including the uptake of circulating nonesterified fatty acids, the hydrolysis and uptake of chylomicrons and cholesterol, and de novo lipogenesis. White adipose is a very dynamic tissue that has the ability to adapt to vast swings in energy availability. Considerable changes in WAT mass are commonly observed in times of caloric scarcity or excess. The rapidity and magnitude of the response of WAT to changes in energy availability is depot-specific and dependent upon local microenvironments.

As previously stated, subcutaneous and visceral WAT display differing phenotypes and play differing roles in the storage of lipid. Subcutaneous WAT may have evolved to store excess energy as a long-term reserve. Subcutaneous WAT resides under the skin and therefore can expand unabated due to few anatomical restrictions. The primary means by which subcutaneous WAT expands is through the proliferation and differentiation of resident preadipocytes [34]. Since subcutaneous WAT serves as a long-term energy reservoir, it is the primary defense against ectopic lipid accumulation and expansion of visceral WAT [4]. The ability of subcutaneous WAT to expand through proliferation and differentiation is generally blunted during the aging process, possibly contributing to the pathogenesis of age-related diseases [8, 35, 36]. In contrast to its subcutaneous counterpart, visceral WAT evolved for rapid release of fatty acids and has been shown to have frequent turnover of lipid [37]. Visceral WAT is located inside the abdominal cavity, thus has limited area for expansion. An increase in visceral WAT mass is typically the result of adipocyte hypertrophy due to limited progenitor replication [38, 39]. The excessive expansion of visceral WAT, especially in conjunction with the loss of subcutaneous WAT, is associated with dramatic deleterious effects on metabolic homeostasis. This

phenomenon has clinically been referred to as "adiposopathy" [38, 40] or "acquired lipodystrophy" [41, 42]. Although often occurring in association with obesity, adiposopathy or acquired lipodystrophy is also common with the aging process.

In addition to its role in storing lipid, WAT possesses mechanical, immune, and endocrine functionality. Adipose tissue located on the hands, feet, and face provides structural support while also providing protection from potential mechanical stressors [1]. Both subcutaneous and visceral WAT protect against infection by inhibiting the entry of pathogens into the circulation. For instance, subcutaneous WAT aids the skin in preventing the entry of external environmental pathogens [30], whereas visceral WAT prevents the spread of infection by walling off inflamed or ruptured enteral organs [43, 44]. The ability of WAT to mitigate infection is multifactorial and related to its microenvironment. WAT is not conducive to pathogen survival because local free fatty acid levels are very high [30]. Additionally, WAT has robust innate and adaptive immune response capabilities due to its pool of resident immune cells [45]. Furthermore, the expression and secretion of cytokines and chemokines by various cells within WAT activate resident immune cells while also inducing leukocyte infiltration [45].

In recent years, WAT endocrine functionality has garnered considerable attention due to its association with metabolic homeostasis and systemic inflammation. These functions are mediated through the expression and secretion of numerous proteins. These proteins, referred to as adipokines, can act locally or enter the circulation and signal throughout the body in a hormonal fashion [46]. The number of individual adipokines and their respective functions are immense and outside the scope of this review. The type of adipokine produced is highly depot-specific and can generally be characterized as anti- or proinflammatory in nature. Subcutaneous and visceral WAT displays unique adipokine expression profiles [47, 48]. The secretome of healthy subcutaneous WAT is mostly anti-inflammatory, whereas visceral WAT is more proinflammatory [48–50]. These general characteristics hold true in young and healthy populations but become confounded during aging. Although adipocytes are involved in WAT endocrine activity, they are not solely responsible. In fact, WAT is made up of numerous other cell types including macrophages, lymphocytes, fibroblasts, endothelial cells, and the aforementioned preadipocytes [46]. The abundance of these cell types is depot-specific, affecting their contribution to WAT expression and secretory profiles in different depots. Interestingly, the endocrine profile of WAT becomes generally more proinflammatory with aging, as evidenced by increased tumor necrosis factor-α (TNFα) and interleukin-6 (IL6) expression [51, 52]. Although there is evidence to suggest that WAT macrophage infiltration can increase with age, macrophages do not appear to be central to age-related proinflammatory cytokine release [53]. These findings indicate that preadipocytes and/or adipocytes themselves may contribute substantially to age-associated functional declines in subcutaneous WAT.

Preadipocytes

Preadipocytes appear to be one of the most abundant types of progenitor in the body. They account for anywhere between 15 and 50 % of the total number of cells in WAT [30]. This range may be related to depot-specific variation in proliferation, differentiation, and susceptibility to apoptosis. These properties contribute to differences in the propensities of each depot to expand through proliferation and differentiation as seen in subcutaneous WAT [34], or primarily through adipocyte hypertrophy as observed in visceral WAT [38, 39]. It is believed that the majority of preadipocytes arise from multipotent mesenchymal stem cells that reside along vascular tissue in WAT [54, 55], although circulating progenitors also contribute [56, 57]. While still debated, it appears that distinct subtypes of progenitors give rise to the depot-specific characteristics of white adipocytes.

The primary role of preadipocytes is to differentiate into mature, lipid-storing adipocytes. Preadipocyte differentiation is a tightly controlled process that involves an array of transcription factors that act in a stepwise manner. Although subcutaneous and visceral adipocytes likely differentiate from progenitors with distinct characteristics [36, 58–62], they share similar transcriptional programs. Peroxisome proliferator-activated receptor gamma (PPARγ) is the key regulator of differentiation [63]. PPARγ is a ligand-dependent transcription factor that induces gene expression by binding to PPARγ responsive elements (PPRE) following dimerization with retinoid X receptors (RXR) [64, 65]. Two isoforms of PPARγ exist, termed PPARγ1 and PPARγ2 [66]. PPARγ1 is ubiquitously expressed, whereas PPARγ2 is almost exclusively expressed in adipocytes. Both isoforms are strongly induced during preadipocyte differentiation [67], although their functional differences remain elusive. PPARγ directly controls the expression of genes involved in adipocyte lipid uptake, transport and metabolism, insulin signaling, and adipokine production [68]. PPARγ expression is regulated by members of the CCAAT/enhancer-binding protein (C/EBP) transcription factor family. Early in the differentiation process, C/EBPβ and C/EBPδ expression and transcriptional activity are induced, which leads to expression of PPARγ and C/EBPα [69, 70]. PPARγ and C/EBPα expression is then sustained through a positive feedback loop in which each transcription factor maintains the expression of the other [69, 71, 72], thereby promoting lipid accumulation and terminal differentiation.

Preadipocyte metabolic and secretory phenotypes differ from those of adipocytes [61, 73]. Interestingly, preadipocyte gene expression profiles are much more aligned with macrophages than mature adipocytes [74]. Preadipocytes express toll-like receptors and play an active role in WAT immune response capabilities [75–77]. Furthermore, preadipocyte exposure to TNFα induces the expression and secretion of proinflammatory mediators [78, 79], which are known to increase macrophage recruitment and infiltration [80]. These "activated" preadipocytes can also acquire morphology that resembles that of macrophages [78, 79, 81]. Although preadipocyte immune activity is important to host-defense, it also predisposes WAT to chronic inflammation during the aging process and obesity. Inflammation in WAT reduces preadipocyte proliferation [82, 83] and differentiation [78, 79, 84] capacity in addition to perpetuating ancillary inflammatory responses [85, 86]. These characteristics provoke a vicious cycle that plays a vital role in the age-associated loss of subcutaneous WAT functional capacity.

Aging White Adipose Tissue—Functional Changes

Lipid redistribution during the aging process is commonly observed. White adipose mass gradually increases through middle age and begins to decline during late-middle and old age [12, 87]. This change occurs due to reductions in subcutaneous WAT and is accompanied by an expansion of visceral WAT [9, 10, 12, 88–90]. These findings are consistent with the observation that older men and women store less dietary lipid in subcutaneous WAT [29]. Additionally, in adult women, abdominal circumferences increase by 4 cm every 9 years [15], which is indicative of increased visceral WAT [91]. The removal of visceral WAT in rats improves metabolic parameters and extends lifespan [92–94]; however findings in humans are inconsistent and remain very controversial [95–97]. Ectopic lipid accumulation is also increased throughout this time frame during the aging process [16, 17]. As addressed previously, this so-called acquired lipodystrophy is associated with profound changes in metabolic function in elderly humans and is linked to functional declines and all-cause mortality [6, 7, 98]. Decrements in subcutaneous WAT likely contribute to the systemic lipotoxicity and metabolic dysfunction frequently observed in the elderly. This deterioration is related to curtailed functional capacity of preadipocytes in subcutaneous WAT [38, 41, 42].

Over the lifecycle, extensive functional changes occur in preadipocyte populations [35, 36, 82–85, 99–103]. Collectively, these changes appear depot-specific. However, some individual preadipocytes

within the same depot can morph at different rates and sometimes resemble the properties of preadipocytes from much older or younger individuals [36, 83, 85, 100]. Preadipocyte replication [36, 82, 83, 100] and adipogenic potential [35, 36, 84] are decreased, while proinflammatory and chemotactic secretomes are commonly increased with aging [85, 102]. The reduction in preadipocyte adipogenesis appears to be related to decreased C/EBPα and PPARγ expression and activity. Expression of C/EBPα and PPARγ is decreased in primary preadipocytes isolated from aged rats and humans as well as in intact WAT from various species [35, 83, 104]. These changes are inducible by serially passaging preadipocytes obtained from young humans [59]. The aging process does not appear to blunt the expression of C/EBPβ or C/EBPδ [35]; therefore, inhibition of C/EBPβ activity or C/EBPα and PPARγ expression or activity may be responsible. Several mechanisms leading to impaired adipogenesis have been observed, among them are pathways involving C/EBPβ liver-inhibitory protein (C/EBPβ-LIP) and C/EBP homologous protein (CHOP). Both C/EBPβ-LIP and CHOP impede terminal differentiation by forming heterodimers with various adipogenic transcription factors, thereby inhibiting promoter interaction [84, 102, 105]. Interestingly, both C/EBPβ-LIP and CHOP expression are increased in aged preadipocytes, adipocytes, and/or intact WAT [84, 106]. Cellular stress responses to DNA damage, metabolic dysfunction, and inflammatory insults induce the expression of C/EBPβ-LIP and CHOP. A regulator of C/EBPβ-LIP translation is CUG triplet repeat RNA binding protein (CUGBP). CUGBP binds to the 5′ end of C/EBPβ mRNA, which results in translation of the truncated isoform of C/EBPβ, known as C/EBPβ-LIP [107]. C/EBPβ-LIP does not contain the transactivating domain required for C/EBPα and PPARγ induction. CUGBP is induced by TNFα [30], which is known to be increased in aged WAT [102]. Changes in cap-dependent translation due to activation of mammalian target of rapamycin (mTOR) also favor C/EBPβ-LIP translation [108]. Similarly, CHOP induction is driven by inflammatory responses brought about by endoplasmic reticulum and mitochondrial stress [109, 110].

As discussed throughout this review, aging-related changes of transcriptional, metabolic, and secretory profiles are frequently depot-specific. The age-related diminution of preadipocyte proliferation and differentiation is particularly evident in subcutaneous WAT [30]. This is potentially detrimental since subcutaneous WAT is crucial for long-term storage of lipid and protection against ectopic lipid accumulation. These inherent properties of aging WAT may lead to a stepwise degradation of metabolic homeostasis and eventual age-related morbidity and mortality. The central theme to the onset and perpetuation of subcutaneous WAT functional decline is inflammation.

Inflammation in White Adipose Tissue

The aging process is associated with increased WAT proinflammatory cytokine and chemokine secretion. Two proinflammatory mediators that are increased in aging WAT are TNFα and IL6 [51, 52]. As addressed above, these cytokines directly inhibit adipogenesis [78, 79, 84], while also promoting resident preadipocytes to develop a secretory profile similar to that of an activated macrophage [80]. Although macrophage recruitment into WAT is still observed with aging, the macrophages themselves do not appear to be responsible for most of the proinflammatory cytokine production [53]. Furthermore, WAT macrophage infiltration with aging is less robust than observed in the setting of obesity and is completely absent in some cases [53, 111]. These findings suggest that preadipocytes or differentiated adipocytes are primarily responsible for the increase in WAT proinflammatory secretion with aging. This notion is supported by the finding that co-cultures of human preadipocytes and adipocytes express TNFα, IL6, and monocyte chemoattractant protein-1 (MCP1) in response to lipopolysaccharide exposure [75]. Interestingly, the magnitude of expression was suppressed with differentiation, indicating that preadipocytes account for the majority of proinflammatory expression. Similar

findings in rat preadipocytes indicate that the older the animal, the greater the expression of TNFα and IL6, with some demonstrating expression profiles similar to activated macrophages [61, 85].

As was the case with previously addressed aging-associated phenomena in whole adipose tissue, the most intense preadipocyte inflammation and stress responses are observed in subcutaneous WAT. For instance, markers of inflammation and tissue remodeling are greater in subcutaneous than visceral preadipocytes from aged rats [85]. Furthermore, the inflammatory response to lipopolysaccharide exposure is approximately sixfold greater in subcutaneous WAT relative to visceral WAT in aged mice [52]. The disproportionate inflammatory response between depots with aging likely plays a role in the loss of subcutaneous WAT, the expansion of visceral WAT, and ectopic lipid accumulation. Although strong evidence suggests that dysfunctional preadipocytes play a role in age-associated inflammation, the possibility exists that other cell populations also contribute. In recent years, the state of cellular senescence has garnered considerable attention due to the potential role these cells may play in driving aging phenotypes. In fact, markers of senescence are increased in models of accelerated aging [13, 112] and the global removal of senescent cells improves health-span in aged mice [31]. Collectively, these findings suggest that senescent cells may play a role in WAT dysfunction with chronological aging.

Cellular Senescence in White Adipose Tissue

Cellular senescence is characterized by an irreversible cell-cycle arrest in cellular populations that would otherwise continue to replicate [113–116]. This phenomenon is induced by numerous mechanisms including telomere shortening, chromatin and/or DNA damage, oncogene activation, chronic mitogen exposure, and metabolic and inflammatory stressors [117–120]. The senescent state may have evolved as a means to prevent the hyperproliferation of dysfunctional cells, thereby mitigating cancer and other hyperplastic morbidities [117, 121–123]. Senescent cells appear large and flattened, possess enlarged nucleoli, commonly overexpress lysosomal beta-galactosidase (β-gal) [30, 124], and usually have increased p16 expression [115]. Senescent cells accumulate in various organs with advancing age [125]. The onset of cellular senescence can lead to a secretory phenotype that is proinflammatory in nature, the senescence-associated secretory phenotype or SASP [123, 126–128]. The secretion of cytokines, chemokines, and extracellular-matrix-modifying (ECM) proteases by senescent cells adversely affects the local microenvironment by inducing tissue remodeling and apoptosis [123].

Cellular senescence in WAT has emerged as a potential contributor to the reduction in preadipocytes with advancing age. This is especially true in subcutaneous WAT. Work in our laboratory has demonstrated a strong association between aged WAT and the emergence of cellular senescence. The prevalence of senescent cells, as measured by β-gal staining, is nearly threefold higher in subcutaneous WAT from old rats (30 month) as compared to young rats (3 month) (unpublished observations, Kirkland JL). Old rats also have significantly higher expression levels of p16 mRNA and protein in subcutaneous WAT. Interestingly, primary subcutaneous preadipocytes isolated from old rats also demonstrate a nearly threefold higher presence of senescent cells. Additionally, the bulk of IL6 expression in the stromal vascular fraction of mouse subcutaneous WAT appears to occur in senescent cells. We demonstrated this by FACS-sorting GFP-positive senescent cells from WAT of older mice with an accelerated aging syndrome, in which a p16 promoter drives a GFP reporter [31]. Preliminary experimentation in human subcutaneous WAT appears to mirror the findings in rats. Collectively, our data suggest that senescent cells and the associated secretory phenotype emerge in subcutaneous WAT with chronological aging. We speculate that not only is senescent cell accumulation in subcutaneous WAT a hallmark of aging, but that it also plays a mechanistic role in age-associated morbidities.

Model of Aging-Associated White Adipose Tissue Dysfunction

It is well accepted that the aging process results in diminished subcutaneous WAT and an increase in visceral WAT. The reduction in subcutaneous WAT arises, in part, through the decline of resident preadipocyte function. The cause of this is multifactorial, with contributions from at least two mechanisms: (1) preadipocytes take on a macrophage-like secretory phenotype and (2) senescent cells emerge from stressed preadipocytes. These detrimental characteristics then spread throughout the depot in a feed-forward manner due to the perpetuation of cytokine, chemokine, and ECM protease expression. This process further degrades the WAT microenvironment by reducing preadipocyte proliferation and differentiation, spreading inflammatory activation to nearby preadipocytes, and increasing tissue-remodeling and apoptosis (Fig. 5.1). These circumstances impede long-term lipid storage in subcutaneous WAT. We speculate that this contributes to increases in systemic inflammation, visceral WAT, and ectopic lipid accumulation with lipotoxicity. The expansion of visceral WAT and ectopic lipid storage, coupled with the spread of inflammation systemically, instigates aging-associated morbidities including insulin resistance, NAFLD, T2DM, and CVD. This stepwise process, if true, would suggest that functional declines in subcutaneous preadipocytes play a crucial role in the aging phenotype. Preadipocytes within subcutaneous WAT might be a target for pharmacological intervention.

Conclusions and Future Directions

White adipose is an extremely dynamic tissue that likely plays a pivotal role in regulating metabolic homeostasis and systemic inflammation. Consequently, WAT appears to have profound effects on health-span and longevity. The aging process is associated with a redistribution of lipid from

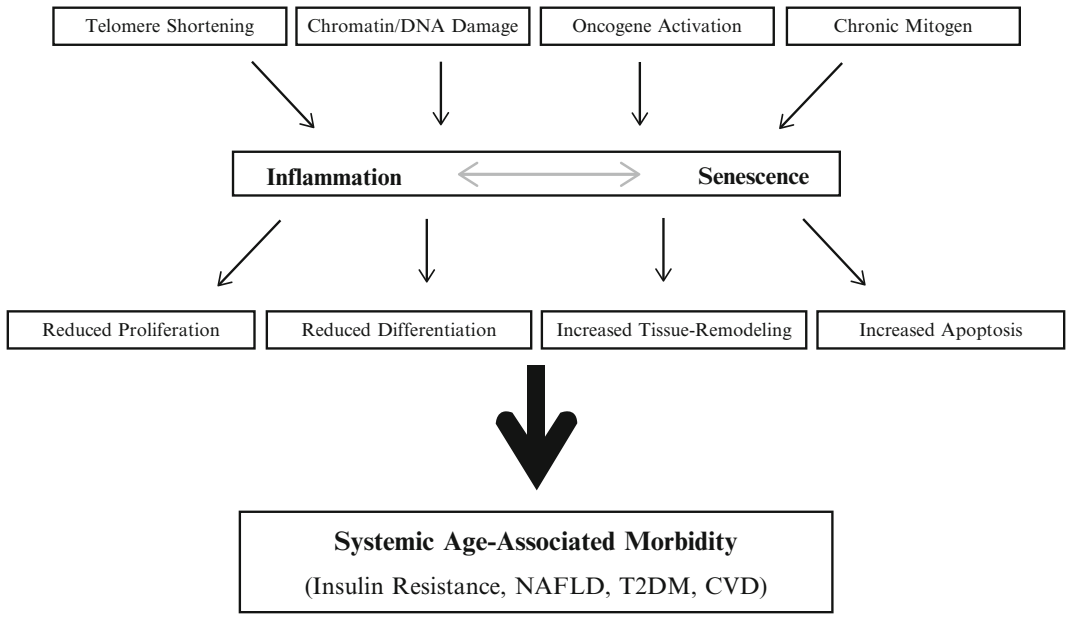

Fig. 5.1 Speculative mechanistic model for the age-associated decline in subcutaneous WAT function and the subsequent downstream effects on morbidity

subcutaneous to visceral WAT with a concomitant expansion of ectopic lipid storage. These phenomena are referred to as adiposopathy or acquired lipodystrophy and might contribute to numerous metabolic disorders including insulin resistance, NAFLD, T2DM, and CVD. The onset or progression of these disorders in the elderly population is linked with the loss of independence, frailty, and all-cause mortality. We speculate that this process begins with the decline of subcutaneous preadipocyte functional capacity due to increased inflammation and the emergence of senescent cells. Over time, these stressors inhibit the proliferation and differentiation of preadipocytes, thereby diminishing lipid storage capacity. As this process progresses, the local microenvironment is changed through the chronic expression of cytokines, chemokines, and ECM proteases.

It is yet to be elucidated if inflammation induces the emergence of cellular senescence in WAT or if senescent cells initiate the inflammatory response. Regardless of the sequence, the removal of senescent cells appears to have beneficial effects on health outcomes, at least in mice with an accelerated aging-like syndrome [30]. This observation could have important ramifications in the clinical setting if borne out in chronologically aged humans: removal of senescent cell populations could potentially mitigate age-related metabolic disease. If true, this methodology could be extended to obese and diabetic populations in which senescent cell numbers are increased [30, 112]. Numerous potential therapeutic options exist including: (1) direct pharmacological targeting of senescent cells for removal, (2) utilizing existing drug therapies that increase subcutaneous preadipocyte differentiation, thereby minimizing preadipocyte inflammation and senescence onset, and (3) diminishing the emergence of senescent cells through caloric restriction and/or aerobic exercise. Regardless of the approach to alleviating senescent cell burden, it appears that their removal would benefit WAT microenvironments and diminish age-related morbidity. Much more investigation is needed to develop definitive therapeutic interventions.

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Chapter 6
Adipokines: Leptin and Adiponectin in the Regulation of Inflammatory and Immune Responses

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Keywords Obesity • Adipokines • Inflammation

Key Points

- Adipokines are critical mediators that convey information about adipose tissue status to the body.
- Leptin is an anorexigenic adipokine. Obese subjects produce high levels of leptin but are resistant to its activity.
- Leptin is a survival and activating factor for T lymphocytes and promotes proinflammatory responses.
- Adiponectin levels are reduced in obesity and negatively correlated to markers of inflammation in metabolic disease.
- Adiponectin promotes insulin sensitivity and has anti-inflammatory effects.
- Levels of adiponectin are paradoxically elevated and positively associated with markers of inflammation in chronic inflammatory diseases.

Introduction

Adipokines are peptides mostly secreted by adipocytes and capable of acting at both the local (autocrine/paracrine) and systemic (endocrine) levels, providing a critical link between obesity, insulin resistance (IR), and inflammatory disorders [1–3]. Although several adipokines have been described, the two that best fit the description and that are best understood are leptin and adiponectin (APN), the first two adipokines to be discovered. Other mediators, including cytokines, chemokines, and others, are also secreted by adipocytes; however, these are not strictly classified as adipokines because adipocytes are not the main source of these mediators [3, 4]. Production of adipokines by adipocytes is one of the most important ways for white adipose tissue (WAT) to influence physiological and pathological processes throughout the body, including appetite, glucose and fat metabolism, vascular health, bone strength, endocrine function, and so on. This chapter discusses leptin and APN, with emphasis on their role in regulating inflammatory and immune responses, a critical mechanism linking nutritional status and adiposity to several pathologies, such as infectious and autoimmune diseases, as well as chronic conditions including diabetes, cardiovascular (CVD) disease, and cancer.

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Leptin

A genetic defect causing a severely obese phenotype due to overeating and decreased energy expenditure was identified in mice at the Jackson Laboratories in the 1950s and these mice were called *ob/ob* [5]. Parabiotic experiments indicated that *ob/ob* mice lacked a satiety factor, but were able to respond to that, putatively soluble, factor from a parabiotic mouse [6]. Similar experiments performed with obese diabetic *db/db* mice, also identified at the Jackson Laboratories, led to the hypothesis that the *db* gene encoded for the ob receptor [7]. It took nearly three decades for leptin to be discovered, in 1994, by Friedman and colleagues at the Rockefeller University [8]. This adipokine encoded by the *ob* gene was called leptin from the Greek word *leptos*, meaning thin.

Leptin is almost exclusively produced by adipocytes, but can also be synthesized in small amounts by cells in the stomach, skeletal muscle, liver, lymphocytes, and placenta [9–14]. Nonadipocyte sources of leptin are unlikely to contribute to systemic leptin levels, though they may exert important effects in the microenvironment. Circulating levels and mRNA expression of leptin in adipocytes are strongly associated with fat mass and measures of adiposity such as body mass index. Leptin expression is higher in subcutaneous than in visceral adipose tissue, leading to circulating levels of leptin two to three times higher in women—who have a higher percentage of subcutaneous WAT—than in men [1, 4, 11, 15–18].

Leptin exerts its biological activities by binding to its receptor, called LEPR. Six alternatively spliced isoforms of LEPR have been identified and are known as LEPR a to f, each isoform forming homodimers [10, 11, 19, 20]. However, only the long form of LEPR, LEPR-b, contains a cytoplasmic region capable of downstream signaling upon leptin binding. This receptor belongs to the class 1 family of cytokine receptors that require activation of Janus tyrosine kinase 2 (JAK2) for propagation of signaling. Binding of leptin to LEPR-b induces autophosphorylation of JAK2, leading to phosphorylation of the three intracellular tyrosine residues of LEPR-b, specifically those in positions 985, 1,077, and 1,138 [21–24]. When activated, each of these tyrosine residues recruits specific downstream signaling proteins, such as SH2-domain containing phosphatase-2, signal transducer and activator of transcription (STAT)-5, and STAT-3, which mediate leptin's signaling [21, 22, 25, 26].

Binding to LEPR-b present in neurons in specific areas of the brain mediates the anorexigenic activity of leptin, the most important function of this adipokine [19, 27]. By controlling appetite, leptin helps to maintain long-term control of adiposity and regulates metabolic changes in response to nutritional inputs. Leptin can also regulate short-term energy intake by modulating meal size according to changes in energy balance [28, 29]. In agreement with these functions, elevated expression of LEPR-b is present in the feeding centers of the hypothalamus, including the arcuate, dorsomedial, ventromedial, and premammillary nuclei [10, 11, 30–32]. Within the arcuate nucleus, LEPR-b is found in two distinct populations of neurons [28, 33], those that produce the orexigenic mediators neuropeptide Y (NPY) and agouti-related peptide (AgRP), and those that synthesize the anorexigenic molecule proopiomelanocortin (POMC) [28, 30, 33, 34]. Leptin decreases appetite and increases energy expenditure by activating POMC neurons while it inhibits NPY/AgRP neurons [24, 31, 33, 34]. In addition to its pivotal role in the hypothalamic control of food intake, leptin also acts in the cortex and limbic areas, where it regulates cognitive and hedonic responses to feeding [34, 35]. Obesity is associated with development of leptin resistance in the brain, resulting in loss of the anorexigenic effects of this adipokine even in the presence of significantly elevated circulating levels derived from the expanded adipose mass. Proposed mechanisms mediating leptin resistance in the setting of obesity includes changes in circulating levels of leptin-binding proteins, reduced transport of leptin across the blood-brain barrier, and reduced signaling through cellular LEPR-B signaling as a result of inflammation, endoplasmic reticulum stress or feedback inhibition [36].

In addition to being obese, experimental rodents and humans with leptin or leptin-receptor deficiency have other endocrine abnormalities, which include reduced fertility, alterations in bone metabolism, and dysfunction of the immune system. Alterations secondary to lack of leptin or its receptor resemble the adaptive response to starvation, in which leptin levels fall dramatically, out of proportion with fat mass [19, 37–39].

Early observations of thymus atrophy in *db/db* mice suggested a role for leptin in regulation of T lymphocyte survival and function [40]. Following the discovery of leptin, direct evidence was provided that leptin increases activation and proliferation of $CD4^+$ T lymphocytes, with a major impact on proliferation of naïve T lymphocytes compared to memory cells [41]. Moreover, leptin was shown to enhance proliferation of effector $CD4^+$ T cells while inhibiting responsiveness of T regulatory (Treg) cells, thus tipping the balance toward an activated, proinflammatory lymphocyte environment [42]. In vivo data in *ob/ob* and *db/db* mice supported the in vitro findings, with these mice having enhanced Treg proliferation compared to WT mice [42]. In addition, *ob/ob* and *db/db* mice have decreased size and cellularity of the thymus and spleen due to high cellular apoptosis, which is normalized by peripheral administration of leptin or by transplantation of WT adipose tissue in *ob/ob* mice [43–45]. Leptin protects lymphocytes from apoptosis through an IRS-1/ PI3-kinase signaling cascade, independent of JAK activation [46] and by suppressing Fas-mediated apoptosis [47].

In addition to supporting T cell survival and proliferation, in vitro leptin also exerts a variety of activities on both lymphocytes and other leukocytes, including enhancement of T lymphocyte activation and promotion of T helper (TH)-1 cytokine production, activation of monocytes and macrophages and secretion of proinflammatory mediators, promotion of neutrophil chemotaxis and production of reactive oxygen species by these cells as well as differentiation, proliferation, and activation of natural killer (NK) cells [19, 48, 49]. A particularly strong proinflammatory effect of leptin is mediated by its induction of TH17 cells through induction of RORγt expression [50]. Thus, in vitro evidence indicates an important role for leptin in modulating several aspects of immune and inflammatory responses, with a general tendency toward proinflammatory effects.

In vivo studies demonstrated that *ob/ob* and *db/db* mice are either resistant or less susceptible in models of innate and adaptive immune-mediated inflammatory diseases, possibly secondary to reduced secretion of proinflammatory cytokines coupled with increased production of anti-inflammatory cytokines and increased functionality of Treg cells [19, 49, 51–53]. For example, *ob/ob* mice are protected from disease in models of multiple sclerosis, intestinal inflammation, rheumatoid arthritis, and other inflammatory conditions [19, 49, 51–53], although their protection is highly dependent on the experimental model used, particularly when evaluating colitis [54–56]. Context-dependent effects of leptin have also been reported in other disease models. Thus, for example, o*b/ob* mice develop less severe arthritis compared to WT mice in antigen-induced arthritis, an immune-mediated model of joint inflammation [57, 58], while they have a more prolonged inflammatory response to zymosan-induced arthritis, that is independent of the adaptive immune system [59]. This could be partly explained by the observation that obesity increases and prolongs inflammatory responses independently of leptin, as demonstrated by the similar phenotype of *ob/ob* mice and mice with high fat diet-induced obesity in models of inflammatory diseases that do not involve adaptive immunity [60–63].

Leptin has been involved in the pathogenesis of several pathologies with an inflammatory component in humans, including multiple sclerosis, systemic lupus erythematosus, chronic obstructive pulmonary disease (COPD), allergies, asthma, infections, and several others [64–69].

In summary, leptin is an important factor in modulating immune and inflammatory reactions, both in humans and experimental animals. However, we still do not have a good understanding of the mechanisms by which leptin interacts with other mediators in the complex network of immune and inflammatory responses and how obesity may modify these interactions.

Adiponectin

Adiponectin (APN) is secreted predominantly by adipocytes and is present at a concentration of 5–30 μg/ml in blood of healthy humans, making it the highest circulating adipokine [70–74]. Although extra-adipocyte sources of APN may be important modulators of the local microenvironment, they are unlikely to significantly contribute to the circulating pool of APN under physiological conditions. APN was identified in 1995 and 1996 by four independent groups [75]. It is a 247-amino acid protein consisting of an amino-terminal signal sequence, a variable region, a collagenous domain, and a carboxy-terminal globular domain which form low molecular weight (LMW) trimers, which then further associate to form middle molecular weight (MMW) hexamers and high molecular weight (HMW) oligomers [76]. These HMW oligomers form bouquet-like structures through disulphide bonds located within the collagenous domains of each monomer [76]. All three forms of APN are present in serum, but whether the different forms of APN exhibit differential biological actions remains unclear. A globular form of APN may also exist. Leukocyte elastase, secreted by activated monocytes and neutrophils, cleaves APN and generates the globular domain that is capable of forming a trimer [77]. Activation of the transcription factors PPARα and γ and FOXO1 is critical in regulating production of APN in adipocytes [78].

The biological activity of APN is mediated by binding through its receptors, AdipoR1, AdipoR2, and T cadherin [79, 80]. AdipoR1 is ubiquitously expressed but predominantly found in skeletal muscle, whereas AdipoR2 is mostly expressed in the liver. AdipoR1 and AdipoR2 contain 7-transmembrane domains that are structurally and functionally different to other G-protein receptors, having the N terminus located in the cytoplasm and the C terminus located externally [79]. In vitro studies have shown AdipoR1 to be a high affinity receptor for globular APN and to have a low affinity for full-length APN, while AdipoR2 is an intermediate affinity receptor for both globular and full-length APN. Whereas AdipoR1 and AdipoR2 mediate APN signaling (see below), T cadherin likely functions by sequestering APN in tissues, thus creating an APN reservoir that is released into the circulation when T-cadherin is absent [81]. Genetic linkage studies associate mutations in the human *Cdh13* gene, which encodes for T-cadherin, with circulating APN levels [82], suggesting that modulation of this pathway is likely to be important in the physiological regulation of APN. Epigenetic modulation leads to loss of T-cadherin expression in cancer, indicating active regulation of this protein in humans [83–85], but data on modulation of T-cadherin in health and disease are lacking.

APN binding to its receptors activates signaling molecules such as peroxisome proliferator-activated receptor (PPAR)-α, AMP-activated protein kinase (AMPK), and mitogen-activated protein kinase (p38 MAPK) [79]. The activation of PPAR-α is important in APN-stimulated fatty acid (FA) oxidation, but not for glucose uptake, whereas AMPK and MAPK activation is likely involved in both FA oxidation and glucose uptake. An additional signaling mechanism involves activation of ceramidase that reduces intracellular levels of proinflammatory ceramides while increasing the concentration of sphingosine-1-phosphate, a molecule with important immunoregulatory and anti-inflammatory effects [86]. Finally, APN binds calreticulin and opsonizes apoptotic cells to facilitate their clearance by macrophages [87].

Unlike leptin, APN levels decrease with the increase in fat mass that is observed in obesity. Chronic inflammation associated with obesity inhibits production of APN, leading to the subsequent perpetuation of inflammation given the potent anti-inflammatory effects of this adipokine [88, 89]. An inverse correlation between APN and IR has been established both in animal and human studies [90]. Studies in obese and insulin-resistant individuals demonstrated a negative correlation between APN and markers of inflammation, again pointing to the strong association between APN and inflammatory responses [88, 89]. APN improves insulin sensitivity through various mechanisms. In the liver, APN activates AMPK, which increases FA oxidation, downregulates gluconeogenic enzymes, and increases glucose-6-phosphate biosynthesis [90]. Activation of PPAR-α by APN further increases insulin

sensitivity by increasing FA oxidation, and thereby decreasing FA synthesis in the liver [79]. In muscle, APN stimulates phosphorylation of acetyl-CoA carboxylase, FA oxidation, glucose utilization, and lactate production [79]. Treatment with thiazolidinediones (TZD), drugs that activate PPAR-γ and are used to treat patients with type 2 diabetes, increases APN levels and improves glucose tolerance and insulin sensitivity [79]. Reduced levels of APN in obesity also contribute to the endothelial dysfunction observed in subjects with CVD by reversal of the deleterious effects of inflammation on the endothelium [91, 92]. In addition, APN inhibits foam cell formation and smooth muscle cell migration, both of which play an important role in the development of atherosclerosis [93]. Moreover, APN protects the heart from ischemia-reperfusion injury by inhibiting oxidative stress [94, 95]. In vitro studies demonstrated that APN decreases production of proinflammatory cytokines and upregulates production of anti-inflammatory ones [88, 96]. Thus, extensive evidence indicates that APN exerts beneficial effects and that APN production is reduced in metabolic disease, possibly as a result of chronic inflammation. On the other hand, a less extensive body of evidence points to the paradoxical upregulation of APN levels in several types of inflammatory and immune-mediated conditions [88, 97–103]. If inflammation is indeed the most important mechanism regulating production of APN, one would expect to observe reduced levels of this adipokine in diseases characterized by elevated inflammation. However, data indicate a complex association between inflammation and APN outside the realm of metabolic disease.

Elevated circulating levels of APN have been reported in patients with autoimmune diseases such as systemic lupus erythematosus, rheumatoid arthritis, active autoimmune idiopathic recurrent pericarditis, and type 1 diabetes, though not all reports are in agreement [88, 104–108]. Upregulation of APN is also present locally in the inflamed joints of rheumatoid arthritis patients [105, 106], with data indicating a direct proinflammatory role of APN in chondrocytes [109, 110]. The presence of elevated levels of APN is particularly intriguing in type 1 diabetic patients, given that opposite findings are reported for type 2 diabetes [70, 90, 111, 112]. Unexpectedly, serum APN levels predict progression of renal damage and are positively, rather than negatively, associated with CVD in type 1 diabetes [113, 114]. Deregulated production of APN is also observed in experimental models of type 1 diabetes [115].

Although APN levels are low in CVD and this adipokine exerts potent vascular and cardioprotective effects [70], circulating levels of APN are high and associated with adverse outcome in patients with chronic heart failure, who also develop APN resistance [102]. APN is positively correlated with cardiac inflammatory infiltrate and systemic markers of inflammation in patients with dilated inflammatory cardiomyopathy [71]. Furthermore, induction of autoimmune myocarditis in mice leads to elevated circulating and cardiac levels of APN; however, APN gene transfer reduces inflammation and cytokine production in vivo and in vitro in cardiomyocytes [71]. Since several studies indicate an anti-inflammatory effect of APN in the heart, upregulation of APN in chronic heart failure may represent a failed attempt at controlling disease [102].

A complex association between APN and disease is also present in chronic kidney failure, with circulating levels of APN being high in patients with chronic kidney failure, particularly in end-stage renal disease. Elevated levels of APN in this population are positively correlated with markers of systemic inflammation and associated with higher risk of death [116]. Although clearance of APN is mostly mediated by the liver [117], reduced renal excretion—rather than suppressed production by adipocytes—has been proposed as the mechanism for the elevated circulating levels of APN in chronic kidney disease [100, 118].

Unorthodox levels of APN have been reported in several types of lung disease, with the strongest evidence available for COPD, where high APN is associated with worse lung function and greater disease severity [97, 101]. Furthermore, systemic levels of APN increase during acute exacerbations of COPD and are positively correlated with markers of inflammation [119]. Gene variants of APN have been associated with risk of COPD, thus pointing to a possible pathophysiological role for APN in this condition [120].

The exact mechanisms leading to increase in APN levels in chronic inflammatory diseases and the specific role of APN in the pathophysiology of these conditions remain to be elucidated. Carefully controlling for a variety of factors that may contribute to regulation of APN production, release, and clearance is necessary to dissect the mechanisms behind this apparently paradoxical association.

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Part II
Adipose Tissue Inflammation and Adipocyte Dysfunction in Obesity

Chapter 7
Adipose Tissue Inflammation

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Keywords Adaptive immunity • Cytokines • Inflammation • Innate immunity • Insulin resistance

Key Points

- Chronic inflammation is a hallmark of obesity and related disorders.
- Proinflammatory cytokines are the driving forces of inflammatory conditions throughout the body.
- Adipose tissue (AT) contains increased amounts of various proinflammatory cytokines such as interleukin-1 (IL-1) tumor necrosis factor-alpha (TNFα) and IL-6.
- Overwhelming production of proinflammatory cytokines in the adipose tissue (visceral and subcutaneous adipose tissue) leads to systemic inflammation and related clinical symptoms.
- Weight loss reduces production of proinflammatory cytokines in adipose tissue and therefore is a potent "anti-inflammatory" strategy.
- Neutralization of certain proinflammatory cytokines such as IL-1 might become an effective therapy in the future for obesity-related inflammatory disorders such as metabolic syndrome, type 2 diabetes and atherosclerosis.
- A complex cellular infiltrate contributes to adipose tissue inflammation.

Abbreviations

AT	Adipose tissue
CCL	Chemokine (C-C motif) ligand
CRP	C-reactive protein
DIO	Diet-induced obese/obesity
ERK	Extracellular signal-regulated kinases
HFD	High fat diet
IFN	Interferon
IL	Interleukin
IL-1Ra	Interleukin-1 receptor antagonist
iNKT	Invariant natural killer T cells

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IRS	Insulin receptor substrate
KO	Knockout
NAFLD	Nonalcoholic fatty liver disease
NK	Natural killer
PPAR	Peroxisome proliferator-activated receptor
RANTES	Regulated and normal T cell expressed and secreted
T2D	Type 2 diabetes
TH	T helper cells
TNF	Tumor necrosis factor
Treg	Regulatory T cells

Introduction

The incidence of obesity is rising dramatically all over the world leading to a further and enormous increase in obesity-related disorders such as atherosclerosis, type 2 diabetes (T2D), nonalcoholic fatty liver disease (NAFLD), and certain cancers. Obesity, in particular but not only visceral obesity, which is the accumulation of adipose tissue inside the abdominal cavity, is commonly associated with insulin resistance finally resulting in the development of T2D. Obesity and related insulin resistance are frequently correlated with a state of low-grade inflammation and therefore it is assumed that inflammation contributes to its development [25, 53]. Furthermore, although evidence is limited, obesity might be associated with some immune-mediated disorders such as asthma, psoriasis, and certain cancers.

Inflammatory events in our body are in general controlled and directed by soluble mediators, i.e., cytokines, which are released by many cell types but especially monocytes/macrophages. In addition to adipocytes, adipose tissue contains preadipocytes, endothelial cells, fibroblasts, and various leukocytes including macrophages, T lymphocytes, and neutrophils. Certain chemokines such as Chemokine (C-C motif) ligand (CCL2) are considered of critical importance to attract inflammatory leukocytes to the adipose tissue [16, 31]. Adipose-tissue infiltrating leukocytes are probably the key source of cytokines in case of obesity-related inflammation. The adipose tissue contains rather substantial amounts of various proinflammatory cytokines such as tumor necrosis factor-alpha (TNFα), interleukin-1 (IL-1), and IL-6. Although, for example, macrophages in adipose tissue seem to be the major source of TNFα, adipocytes contribute almost one third of circulating IL-6 in patients who are obese. Therefore, obesity is associated with a chronic inflammatory response characterized by abnormal cytokine production, increased synthesis of acute-phase reactants, such as C-reactive protein (CRP), and activation of inflammatory signaling pathways. In this chapter, we provide an overview of recent advances in the understanding of the role of proinflammatory cytokines in adipose tissue and in obesity-related disorders.

Adipose-Tissue Infiltrating Immune Cells: Major Cytokine Sources

Monocytes/Macrophages

Macrophage recruitment to the adipose tissue in obesity contributes to enhanced tissue inflammatory activity, and therefore may play an important role in obesity-associated metabolic dysfunction [67]. Macrophages may differentiate from preadipocytes and mesenchymal stem cells in the adipose tissue or may enter the adipose tissue attracted by certain chemokines. Adipocytes release many different chemoattractants thereby attracting monocytes. One such chemokine is CCL2 which is highly expressed in adipose tissue [31]. The absence of CCL2 in mice, however, does not limit obesity-associated infiltration of macrophages into adipose tissue suggesting that many other chemokines might be operative [27].

Macrophages have been recently characterized as exhibiting either a more proinflammatory M1 or a more anti-inflammatory M2 phenotype. Different types of macrophages accumulate in the adipose tissue [42]. Resident macrophages present in adipose tissue of lean mice display mainly the alternatively activated phenotype (M2 or "alternatively activated" macrophages characterized by activated genes for IL-10 and others) [42]. In contrast, in case of obesity, proinflammatory classically activated macrophages are dominating in the adipose tissue (M1 or "classically activated" macrophages producing enhanced levels of proinflammatory cytokines). In the process of becoming obese, adipose tissue macrophages change from an M2-polarized state in lean animals that protects adipocytes from inflammation to an M1 proinflammatory state leading to insulin resistance and related inflammation. All these studies are convincing examples of how adipocytes and macrophages might interact in the adipose tissue.

Lymphocytes: T and B Cells

After the discovery that adipose tissue macrophages are a major source of proinflammatory cytokines in obesity, several studies initially suggested that also T cells infiltrate the adipose tissue and modulate inflammation [71]. Wu et al. observed that infiltration of adipose tissue from diet-induced obese (DIO) insulin-resistant mice by T cells was accompanied by an increased expression of the T cell chemoattractant RANTES (regulated and normal T cell expressed and secreted) and furthermore, adiponectin$^{-/-}$ mice showed higher RANTES expression compared to wild-type mice [71]. Kintscher and colleagues showed that proinflammatory T cells are also present in visceral adipose tissue of patients with T2D. In this study, they observed that in a mouse model of obesity-mediated insulin resistance a marked T lymphocyte infiltration preceded recruitment of macrophages suggesting that T lymphocytes might contribute to obesity-associated inflammation even at a rather early stage [33].

In the following years, several studies revealed that various T cell subsets might be involved in adipose tissue inflammation: In lean mice, regulatory T cells (Treg) and T helper (T_H) 2 cells predominate over proinflammatory T_H1 cells and effector CD8+ T cells in adipose tissue and were recently implicated in controlling the inflammatory state of adipose tissue and, thereby, insulin sensitivity [18, 69]. With expanding adiposity anti-inflammatory T_H2 cells and Tregs fail to control "metainflammatory" signals and get overwhelmed by proinflammatory T_H1 and effector T cells [6, 18]. While T_H2 and Tregs promote alternative polarization of macrophages via anti-inflammatory signals, such as IL-4 and IL-10, T_H1 cells produce proinflammatory cytokines, such as IFNγ thereby enhancing classically activated macrophages [45]. To demonstrate the "antidiabetic" effect of CD4+ T_H2 cells in metabolic inflammation, Winer and colleagues transferred CD4+ T_H2 cells into lymphocyte-free (Rag1-null) DIO mice and reversed insulin resistance. Also the treatment with a CD3 specific antibody which reduced the predominance of T_H1 cells reversed insulin resistance in obese mice [69].

As mentioned above, Feuerer et al. showed that Tregs play an important role controlling inflammation in adipose tissue [18]. As the key driving force of visceral adipose tissue Treg cell accumulation, phenotype, and function, peroxisome proliferator-activated receptor (PPAR)-γ, the "master regulator" of adipocyte differentiation, was identified recently [5]. In this study, the PPAR-γ activating drug pioglitazone restored Tregs in DIO mice, but could not recover Tregs in PPAR-γ abrogated mice further suggesting the necessity of PPAR-γ signaling and the application of thiazolidinediones in obesity and insulin resistance.

Other T cell subsets which are involved in the "rivalry of T cell populations" in adipose tissue are proinflammatory CD8+ effector T cells and invariant natural killer T cells (iNKT): Nishimura and colleagues showed that effector T cells are significantly elevated in adipose tissue after few weeks of high fat diet (HFD) in mice and precede macrophage accumulation. To demonstrate the involvement of this T cell population in insulin resistance, they depleted CD8+ cells in mice and observed ameliorated systemic insulin resistance. Mechanistically, the authors showed that adipose tissue from obese

mice activates T cells in vitro and in this proinflammatory milieu monocytes differentiated into macrophages [51]. In contrast to this "diabetes-driving" T cell population, Lynch and colleagues recently identified iNKT cells, which are able to rapidly release high amounts of T_H1 and T_H2 cytokines, to control adipose tissue inflammation. They observed that serum iNKT cell numbers were increased in obese patients after weight loss and found the highest amounts of iNKT cells in lean humans. In this study, depletion of iNKT cells in fat and liver of obese mice decreased glucose tolerance and in concordance, in vivo activation of iNKT cells via their lipid ligand, alpha-galactocalceramide, improved metabolic parameters [43]. Another study by Schipper et al. corroborates these data and shows that especially under low-fat diet conditions, adipose tissue-resident iNKT cells maintain healthy adipose tissue through direct interplay with adipocytes and thereby prevent insulin resistance [59].

Collectively, recruitment of various proinflammatory T cell populations into adipose tissue and depletion of other anti-inflammatory subpopulations may have a crucial effect on the recruitment of macrophages or their M1/M2 phenotype polarization and, thereby, adipose tissue inflammation and insulin resistance.

Along with macrophages and T cells, also B cells and their pathogenic IgG antibody products are elevated in adipose tissue of obese mice in an early state of disease. They are believed to activate macrophages and proinflammatory T cells in a MHC-dependent manner. In concordance, mice which do not produce mature B cells (B^{null} mice) are less insulin resistant and show less infiltration of M1 macrophages and IFNγ producing CD8+ T cells. In addition, transferred IgG from obese wild-type mice into obese B^{null} mice impairs insulin sensitivity by acting as autoantibodies and targeting various self proteins. Supporting this observation, antigens which are associated with insulin resistance were found up to 70 % in patients with T2D [68].

Neutrophils

Usually, neutrophils are one the earliest immune cells infiltrating inflammatory processes ("acute infiltration") and thereby initiating a "chronic inflammation" [60]. To observe if neutrophils also occur initially in adipose tissue inflammation, Elgazar-Carmon et al. specifically detected neutrophils by measuring the neutrophil-specific protein MPO and staining for NIMP-R14 in visceral adipose tissue of C57BL/6J mice in the course of DIO. They demonstrated an increase in neutrophilic infiltration in the intra-abdominal adipose tissue early (3–7 days) after starting a HFD and as expected, this infiltration was followed by macrophage infiltration. In this study, the authors further demonstrated that neutrophils adhere to adipocytes, which was dependent on the expression and complex formation of the neutrophil integrin CD11c (Mac1) with the adipocyte ICAM-1 that is upregulated during adipogenesis [13]. Recently, a study by Talukdar et al. identified neutrophil elastase as one possible guilty enzyme of mediating insulin resistance: In mice, this protease was elevated from day 3 of HFD (along with elevated neutrophils) and remained high for several weeks on HFD. Additionally, treatment of primary mouse and human hepatocytes with neutrophil elastase causes cellular insulin resistance by IRS-1 degradation, lower insulin signaling and higher glucose production. In a mouse model of DIO, genetic deletion of neutrophil elastase (B6.129X1-*Elane*^{tm1Sd}/J) or treatment with the neutrophil elastase inhibitor GW311616A resulted in substantially improved glucose tolerance as well as diminished adipose tissue inflammation. In neutrophil elastase deficient mice, a shift from M1 polarized macrophages toward an alternative phenotype was observed further indicating that neutrophils might reflect a relevant leukocyte population in the area of adipose tissue inflammation and neutrophil elastase may worsen metabolic control by directly causing cellular insulin resistance and stimulation of proinflammatory pathways via toll like receptor 4 [63].

Mast Cells and Eosinophils

Mast cells are widely known to exist predominantly in the submucosa of the intestine and the airways and in pathologic conditions, they contribute to type I allergic reactions via the interaction with IgE antibodies. A recent study by Liu et al. has shown that in white adipose tissue of obese humans and mice mast cells are quantitatively increased compared with lean controls. In a murine model of DIO, they observed that either mast cell deficient mice ($Kit^{W\text{-}sh/W\text{-}sh}$) or wild-type mice treated with the mast cell stabilizer disodium cromoglycate (DSCG) gained significantly less body weight, had significantly less fat mass, and showed a better glucose tolerance and insulin sensitivity than wild-type or untreated controls. Mast cells stimulate protease activity via Interferon (IFN) γ and IL-6 in adipose tissue. As a result, they induce angiogenesis, apoptosis, and leukocyte infiltration and thereby may contribute to adipose tissue inflammation and insulin resistance [41].

Along with mast cells, eosinophils are well established in the pathogenesis of allergic and asthmatic reactions and moreover limit helminth infections. Recently, it was shown that eosinophils are the predominant IL-4 producing cells in adipose tissue [70]. IL-4 is necessary for the induction of M2 polarized macrophages via induction of PPARγ and arginase-1. Wu et al. showed that the number of eosinophils in adipose tissue correlates inversely with mouse weight and HFD diminishes eosinophils in adipose tissue. In concordance, hypereosinophilic mice (IL-5 tg) exhibited a better glucose tolerance compared to wild-type controls and eosinophil-deficient mice had a significantly impaired glucose tolerance. Also mice fed a HFD and simultaneously infected with a helminth (therefore exhibiting higher levels of eosinophils) showed improved insulin sensitivity early postinfection suggesting the protective role of eosinophils in insulin resistance [70].

Therefore, there is increasing evidence that many different immune cells not only accumulate in adipose tissue but might also direct the "immune-inflammation" concert observed in obesity in a pro- or anti-inflammatory manner.

Proinflammatory Cytokines: Key Players in Systemic Inflammation in Obesity

TNF-Alpha (TNFα)

In 1993 it had been reported for the first time that a proinflammatory cytokine, namely TNFα, is highly expressed in adipose tissue and interferes with insulin action [24]. This had been a real shift in "cytokine paradigms" as TNFα at that time has been considered to play a role in cachexia and not in obesity. These findings led to the concept of inflammation in obesity and demonstrated that adipocytes are a potential source of TNFα. Expression of this cytokine in obese animals (*fa/fa* rat and *ob/ob* mouse) was increased and shown to regulate insulin action [24]. Further evidence into this direction suggesting a key role for TNFα in insulin sensitivity came from studies published by Uysal et al. where they observed that mice lacking TNFα or TNF receptors had improved insulin sensitivity in both dietary and genetic (*ob/ob)* models of obesity [64]. Importantly obese humans also demonstrated increased TNFα expression in their adipose tissue with reduced cytokine expression following weight loss [32]. First mechanistic insights suggested that TNFα-stimulated inhibitory phosphorylation of serine residues of insulin receptor substrate-1 (IRS-1) could contribute to insulin resistance [56]. In summary, TNFα had been the first and "the" classical proinflammatory cytokine which provided a link between inflammation, obesity, and insulin resistance.

Interleukin-1

IL-1α and IL-1β are among the first identified cytokines and exert strong proinflammatory functions [10]. The potent proinflammatory properties of IL-1 are tightly regulated by expression, processing, secretion, and antagonism by natural inhibitors such as IL-1 receptor antagonist (IL-1Ra) or other members of the IL-1 family such as IL-37 [7]. Concentrations of IL-1β are elevated in the circulation of patients with severe obesity and also in pancreatic cells during the progression from obesity to T2D [12]. IL-1α$^{-/-}$ mice have lower fasting glucose and insulin levels and improved insulin sensitivity as determined by insulin tolerance testing, compared with wild-type controls [46]. Both IL-1α and IL-1β knockout (KO) mice are almost entirely protected from inflammation after diet-induced steatosis [30]. IL-1β is able to reduce IRS-1 expression at a transcriptional level through a mechanism that is extracellular signal-regulated kinases (ERK) dependent and at a posttranscriptional level independent of ERK activation [28]. Neutralization of endogenous IL-1 by a specific antibody improves glycemic control in a mouse model of diet-induced obesity [52]. These effects were paralleled by a decrease in the levels of serum amyloid A.

Several IL-1F members have anti-inflammatory functions. IL-1Ra, which binds to IL-1 receptors thereby preventing IL-1 signal transduction, is markedly upregulated in the serum of obese patients, correlates with body mass index and insulin resistance, and is overexpressed in the white adipose tissue of obese humans [29, 62]. IL-37 is a unique anti-inflammatory cytokine with similar functions as IL-10. IL-37 potently suppresses the production of proinflammatory cytokines by macrophages and IL-37 transgenic mice are protected from lipopolysaccharide-induced septic shock [7]. IL-1F members have been associated with the development of insulin resistance and T2D, and neutralization of IL-1β improves metabolic parameters both in preclinical and clinical trials of T2D [38, 54]. We recently investigated adipose tissue and liver expression of various IL-1F members in severe obese patients undergoing bariatric surgery. Subcutaneous adipose tissue and liver biopsies were performed before surgery and within 6 months after substantial weight loss. Visceral adipose tissue samples were also available presurgery. Importantly we demonstrated that (1) in severe human obesity especially visceral adipose tissue but also subcutaneous adipose tissue are a much more prominent source of IL-1F members in comparison to liver tissue; (2) weight loss results in a decrease especially of IL-1β accompanied by improved insulin sensitivity, and (3) weight loss leads to an increase in the adipose expression of certain anti-inflammatory IL-1F members such as IL-37 [49]. Therefore, it is evident that in case of severe obesity not only the visceral adipose tissue but also the subcutaneous adipose tissue is a major source of proinflammatory IL-1 members and even more interestingly weight loss results in an increase in the expression of anti-inflammatory IL-1F members such as IL-37. This proves that weight loss is indeed an anti-inflammatory strategy and again that the adipose tissue in obesity is also a major source of inflammatory cytokines. Improvements in systemic inflammatory parameters after weight loss may therefore be explained partly by here described changes in cytokine expression profiles.

Interleukin-6

The IL-6 cytokine family (or gp130 cytokines) consists of IL-6, ciliary neurotrophic factor, IL-11, leukemia inhibitory factor, oncostatin M, and cardiotrophin 1. IL-6 signals via induction of a gp130 homodimer after binding to the IL-6 receptor [34]. IL-6 was one of the first cytokines considered as a predictor of insulin resistance and cardiovascular disease. Serum levels of IL-6 decrease in parallel with weight loss and improvement of insulin resistance in patients undergoing bariatric surgery [35]. Visceral fat has been shown to be a major site for IL-6 production in humans [20]. Fried et al. demonstrated that visceral adipose tissue releases around three times more IL-6 into

the circulation than subcutaneous adipose tissue [21]. IL-6 synthesis in abdominal adipose tissue is several times higher compared with subcutaneous adipose tissue thereby potentially contributing to hepatic insulin resistance. Whether this is indeed the case remains unclear as we have shown that subcutaneous and visceral adipose tissue in severe obese patients show similar IL-6 expression [48]. In this study, we observed that adipose tissue including subcutaneous adipose tissue is a major source of IL-6 and TNFα compared to hepatic tissue in human obesity, and excessive weight loss results in a dramatic decrease especially of IL-6 and TNFα expression.

Adipose tissue is composed of many different cell types including adipocytes, preadipocytes, monocytes/macrophages, stromovascular cells, and others. Fain and colleagues found that adipocytes are a minor IL-6 source, and cells retained in the tissue matrix after collagenase digestion are the major adipocytokine and IL-6 source [14]. Another important aspect is the fact that the release of IL-6, TNFα, and various other mediators such as IL-1Ra by adipocytes is approximately 10–12 % of that by nonfat cells such as macrophages present in human adipose tissue [15]. Whereas the visceral adipose tissue is a major IL-6 source, Bastard et al. showed that subcutaneous adipose tissue-derived IL-6 is biologically relevant and regulates systemic insulin sensitivity [2]. These studies altogether clearly indicate that both subcutaneous and visceral adipose tissues are major sources of IL-6 and TNFα in human obesity.

Interleukin-18

IL-18 is another proinflammatory cytokine which plays a role in septic shock, joint inflammation, and inflammatory bowel diseases [9]. IL-18 is another proinflammatory IL-1F member [10]. Similar to IL-1β, IL-18 is first synthesized as an active precursor (pro-IL-18) and requires caspase-1 for processing and activation. Its bioactivity on the other side is under tight control of its physiologic antagonist, the IL-18 binding protein. As IL-18 concentrations are increased in patients with T2D, this might reflect a role in the regulation of insulin resistance [73]. Indeed, as demonstrated, IL-18$^{-/-}$ mice and IL-18R$^{-/-}$ mice have increased body weight accompanied by insulin resistance, hyperglycemia, lipid abnormalities, and atherosclerosis compared to wild-type mice [50]. Intracerebral administration of recombinant IL-18 inhibited food intake and reversed hyperglycemia in these mice by activation of STAT3 phosphorylation. IL-18 is also upregulated in adipose tissue of obese mice and men [44, 47]. In our study we also observed increased expression of IL-18 in the subcutaneous adipose tissue in severe obesity; however, excessive weight loss did not result in a significant reduction [49]. Therefore, overall the role of IL-18 as an inflammatory marker in obesity remains unclear.

Anti-inflammatory Strategies to Counteract Adipose Tissue Inflammation

Anti-TNF Approaches

In animal models of DIO it has been shown that TNF blockade not only improves inflammation but also insulin resistance and steatosis [1, 40]. TNFα-neutralizing antibodies, such as infliximab or adalimumab, are widely used for the treatment of chronic inflammatory diseases, such as rheumatoid arthritis and inflammatory bowel diseases [61]. Initial studies suggested improved insulin resistance in humans by anti-TNF strategies. Gonzalez-Gay et al. demonstrated that nondiabetic patients with rheumatoid arthritis treated with infliximab showed improved insulin sensitivity after a single infusion [23]. Other studies did not observe such an effect neither induced by a single treatment in obese patients [55] nor after a treatment period of 4 weeks in nondiabetic [3] and diabetic subjects [11].

Although infliximab treatment came along with a significant reduction of CRP levels and systemic inflammation, insulin resistance was not improved in patients with rheumatoid arthritis or inflammatory bowel disease [37, 58]. Furthermore, cholesterol levels increased after neutralizing TNF for weeks [37] and also adipocytokine levels such as adiponectin and leptin did not change after 1 year of anti-TNF treatment [17]. Wascher et al. performed an anti-TNF study in insulin-resistant men without any additional diseases. These volunteer men with metabolic syndrome were given three infusions of infliximab (at weeks 0, 2, and 6) and insulin resistance was compared baseline to 70 days after start of treatment. Interestingly, anti-TNF treatment in this cohort significantly reduced inflammatory parameters such as CRP and fibrinogen but did not improve insulin resistance [66]. Taken these small human studies together, one may conclude that neutralizing TNFα (despite improving inflammation) isn't a very effective treatment for insulin resistance in humans and that other cytokines might play a more important role in the progression from low-grade inflammation in obesity to insulin resistance.

IL-1 Blockade

As mentioned above, IL-1α and IL-1β KO mice are protected from inflammation in DIO [30] and in line with this, treatment of DIO mice with a specific anti-IL-1 antibody improved glycemic control [52]. Anakinra acts as an Il-1Ra and is used for the treatment of rheumatoid arthritis, gout, and Still's disease [8]. In a long-term human study Larsen and colleagues have shown that daily injections of anakinra to patients with T2D not only reduced inflammatory markers such as IL-6 and CRP but also significantly improved hyperglycemia and insulin production [38, 39], and another study using the anti-IL-1β neutralizing antibody Gevokizumab (XOMA 052) yielded similar results [4]. A study by van Asseldonk et al. showed that anakinra treatment (150 mg anakinra daily for 4 weeks) in subjects with metabolic syndrome but without diabetes improved inflammation (reduced CRP levels) and β-cell function (increased disposition index) but did not increase insulin sensitivity [65].

In summary, IL-1 blockade seems to be more promising than neutralizing TNFα for improving glycemic control and insulin resistance. Based on these promising results of IL1β blockade various ongoing studies such as the CANTOS (Canakinumab Anti-inflammatory Thrombosis Outcomes Study) (Canakinumab among stable patients with coronary artery disease) will prove whether IL-1 is an attractive therapeutic target not only for metabolic inflammation and IR but also for stroke, myocardial infarction, and cardiovascular death [57].

Reversing Inflammation by Nonacetylated Salicylates

In the past, it became more and more evident that obesity and insulin resistance are linked with subtle low-grade inflammation. Therefore several studies aimed at determining the effect of nonacetylated salicylates as potent anti-inflammatory drugs. Interestingly, aspirin (a nonacetylated salicylate) and sodium salicylate treatment was shown to improve insulin resistance via inhibition of the serine kinase IKKbeta in vitro and in vivo [72]. In 2002, Hundal and colleagues demonstrated that high-dose aspirin treatment over 2 weeks improves glucose tolerance, CRP levels, and triglyceride and cholesterol levels among T2D subjects [26]. Subsequently, studies among obese or T2D subjects using Salsalate (a prodrug of salicylate with similar effects but less side effects compared to Aspirin) were also shown to reduce CRP and HbA1c levels, and further to improve insulin sensitivity and to increase adiponectin serum levels [19, 22, 36]. These studies highlight the close interplay of inflammatory signaling and adipose tissue inflammation/insulin resistance. Nevertheless, it remains unclear, whether long-term applications improve hard end point criteria of human diseases and whether this compensates the side effects of a long-term treatment.

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Chapter 8
Endoplasmic Reticulum Stress and Adipose Tissue Function

Michael Pagliassotti, Gretchen Moran, Andrea Estrada, and Michelle T. Foster

Keywords Obesity • Unfolded protein response • Inflammation • Hypoxia • Insulin resistance

Key Points

- Impairments in energy storage, energy mobilization, and secretory function in white adipose tissue are a characteristic feature of obesity.
- The endoplasmic reticulum is a cellular organelle involved in the synthesis and processing of secretory and membrane proteins.
- Disruption of endoplasmic reticulum (ER) homeostasis or ER stress has been observed in adipose tissue of obese, human subjects.
- Several perturbations associated with obesity such as endotoxemia, insulin resistance with hyperglycemia, elevated free fatty acids, overnutrition, and local tissue hypoxia may also induce ER stress.
- The synthesis and processing of several adipose tissue secreted proteins may be influenced by or contribute to ER stress.
- Adiponectin secretion is in part controlled by ER chaperone proteins.
- The unfolded protein response (UPR) is a signaling pathway that is engaged in response to ER stress.
- The UPR is linked to inflammatory and insulin signaling pathways.

Abbreviations

ATF4	Activating transcription factor-4
ATF6	Activating transcription factor-6
C/EBP	CCAAT/enhancer-binding protein
DsbA-L	Disulfide-bond A oxidoreductase-like protein
EDEM	ER degradation-enhancing α-like protein

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eIF2α	Eukaryotic initiation factor 2α
ER	Endoplasmic reticulum
Ero-1α	ER oxidoreductin-1
ERp44	ER resident protein-44
GADD34	Growth arrest and DNA damage-inducible protein-34
GCN2	General control non-derepressible 2 kinase
GRP78	Glucose-regulated protein 78/immunoglobulin-heavy-chain-binding protein
GRP94	Glucose-regulated protein-94
HMW	High molecular weight multimer
HSP	Heat shock protein
IRE1α	Inositol-requiring 1α
NFKβ	Nuclear factor kappa-β
Nrf2	Nuclear erythroid 2 p45-related factor-2
PERK	Double-stranded RNA-dependent protein kinase-like ER kinase
PKR	Double-stranded RNA-activated protein kinase
UPR	Unfolded protein response
XBP1	X-box binding protein-1

Introduction

White adipose tissue consists of adipocytes embedded in a connective tissue matrix and includes a well-organized vasculature and nerve supply. Though a principal feature of adipose tissue is to store and release energy, it also regulates cellular function in tissues/organs such as skeletal muscle, liver, and brain. This regulation occurs, at least in part, through endocrine-mediated mechanisms that involve the synthesis, processing, and secretion of biologically active proteins or adipokines. Impairments in energy storage, energy mobilization, and secretory function in white adipose tissue are a characteristic feature of obesity. Although the mechanisms leading to these impairments are complex, an increasing body of literature points to the involvement of the endoplasmic reticulum (ER), a subcellular organelle involved in lipid synthesis and protein processing. In this chapter the potential role of ER stress and the unfolded protein response (UPR) will be discussed in the context of adipose tissue biology and obesity.

ER Stress and UPR Fundamentals

Essential functions of the ER include the synthesis and processing of secretory and membrane proteins. Nascent proteins entering the ER lumen interact with folding machinery (e.g., protein chaperones), which promote disulfide bond formation, glycosylation, and protein assembly. Accumulation of unfolded proteins in the ER lumen leads to disruption of ER homeostasis or ER stress. ER stress, in turn, activates the UPR. In mammalian cells, three ER-localized proteins initiate the canonical UPR: inositol-requiring 1α (IRE1α), double-stranded RNA-dependent protein kinase-like ER kinase (PERK), and activating transcription factor-6 (ATF6). It is currently thought that in unstressed cells all three proteins are maintained in an inactive state via their association with the ER protein chaperone glucose-regulated protein 78/immunoglobulin-heavy-chain-binding protein (GRP78) (Fig. 8.1). In the presence of ER stress, GRP78 is released and sequestered on unfolded proteins, thereby activating PERK, IRE1α, and ATF6 [1, 2]. PERK activation leads to phosphorylation of the α-subunit of the

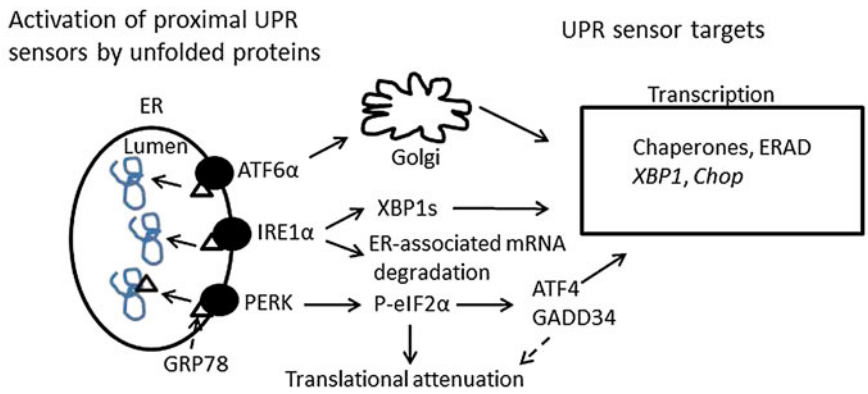

Fig. 8.1 Overview of the mammalian unfolded protein response. Accumulation of unfolded proteins results in the release of GRP78 and activation of ATF6α, IRE1α, and PERK (*left side*). Activation of UPR sensors results in the attenuation of protein translation, ER-associated mRNA degradation, and activation of gene transcription (*right side*). *ER* endoplasmic reticulum, *ATF6α* activating transcription factor-6α, *IRE1α* inositol-requiring 1α, *PERK* protein kinase-like ER kinase, *GRP78* glucose-regulated protein-78, *XBP1s* X-box protein-1 splicing, *P-eIF2α* phosphorylation of eukaryotic initiation factor-2α, *ATF4* activating transcription factor-4, *GADD34* growth arrest and DNA damage-inducible protein-34, *ERAD* endoplasmic reticulum-associated degradation, *Chop* CCAATT/enhancer-binding homologous protein. See text for additional details

translation initiation factor eIF2 (p-eIF2α) and subsequent attenuation of translation initiation. Paradoxically, p-eIF2α leads to selective translation of mRNAs containing open reading frames, such as activating transcription factor-4 (ATF4) [3, 4]. Increased expression of GADD34, a member of the growth arrest and DNA damage family of proteins, is involved in dephosphorylation of eIF2α and reversal of translational attenuation [5]. Activation of IRE1α promotes the splicing of X-box-binding protein-1 (XBP1s) mRNA and subsequent transcription of molecular chaperones (e.g., GRP78) and genes involved in ER-associated degradation (e.g., EDEM) [4]. IRE1α-mediated degradation of select ER-associated mRNAs provides an additional mechanism to reduce the load presented to the ER lumen [6]. Activation of ATF6 leads to its release from the ER membrane, processing in the Golgi, and entry into the nucleus. Transcriptional targets of ATF6 include protein chaperones and XBP1 [7]. Overall, the UPR attempts to reestablish ER homeostasis via transient attenuation of global protein synthesis, reduction of mRNAs whose protein products would be processed in the ER lumen, and increased capacity to fold and degrade proteins.

Adipose Tissue ER Stress in Obesity

At least three studies have observed markers of ER stress in adipose tissue of obese, human subjects [8–10]. In one of these studies, subcutaneous fat biopsies were obtained from the upper thigh in lean (body mass index [BMI] of 24 ± 1.2 kg/m^2, $n = 6$) and obese (BMI 33.5 ± 1.6 kg/m^2, $n = 6$) healthy subjects [8]. Adipose tissue from obese subjects was characterized by increased protein levels for several ER chaperones (calnexin, calreticulin, and protein disulfide isomerase) and XBP1 mRNA splicing. In another study, adipose tissue was obtained from 78 healthy, nondiabetic subjects over a spectrum of BMIs [9]. Several gene markers associated with the UPR, including GRP78, ATF6α, PERK, and XBP1 mRNA splicing, were positively correlated with BMI after controlling for contributions made by macrophages using CD68 expression. The final study examined adipose tissue in

morbidly obese subjects (BMI 51.3 ± 3 kg/m^2, $n = 11$) before and 1 year after gastric bypass surgery [10]. Subjects lost ~40 % of body weight at the 1-year follow-up at which time significant reductions were observed in adipose tissue GRP78 mRNA, XBP1 mRNA splicing, and phosphorylation of eIF2α. Genetic and dietary murine models of obesity are also characterized by increased markers of ER stress in adipose tissue [11–13].

Physiologic Signals That Provoke ER Stress in Adipose Tissue

Several perturbations associated with obesity, such as endotoxemia, insulin resistance with hyperglycemia, elevated circulating free fatty acids, overnutrition, and local tissue hypoxia, may serve as initiating signals for ER stress in adipose tissue [14–18]. Lipopolysaccharides (100 mg/ml), high glucose (25 mM), and saturated fatty acids (2 mM) increased markers of ER stress (e.g., phosphorylation of eIF2α, calnexin) in primary human adipocytes [19]. In addition, a mixture of fatty acids (myristic, lauric, arachidonic, oleic, and linoleic acid, final concentration of 0.5 mM) increased markers of ER stress in 3T3-L1 adipocytes [20]. Whether endotoxemia, glucose, and free fatty acids at levels observed in obesity can induce ER stress in adipose tissue in vivo is presently unclear. Calorie restriction reduced ER stress in adipose tissue of ob/ob mice, suggesting that overnutrition may be an in vivo signal that provokes ER stress in adipose tissue [13]. Adipocyte expansion in obesity may exceed the diffusion limit of oxygen, resulting in adipose tissue hypoxia [21, 22]. In 3T3-L1 adipocytes, hypoxia increased markers of ER stress, and adipose tissue taken from obese mice was characterized by hypoxia and ER stress [21]. Thus, several physiologic responses to obesity and/or adipocyte expansion may influence ER homeostasis and elicit activation of the UPR. The extent to which these responses influence homeostasis of adipose tissue ER in vivo and the mechanisms that mediate disruption of homeostasis require further study.

A Conceptual Model for Obesity-Related Adipose Tissue ER Stress

ER stress is typically defined as the accumulation of unfolded proteins in the ER lumen [2, 23]. Activation of the UPR in response to ER stress functions to remove these unfolded proteins and restore ER homeostasis. Based on this fundamental view, the presence of adipose tissue ER stress in obesity implies that unfolded proteins have accumulated in the ER lumen due to an imbalance in the protein load presented to the ER lumen and/or the ability to fold and degrade these proteins.

Most proteins that are secreted from the cell are synthesized on membranes of the ER and transported through the membrane to the ER lumen. Proteins in the secretory pathway that are destined for compartments other than the ER or Golgi eventually interact with the trans-Golgi network. From this network, proteins can be loaded onto one of three types of vesicles. The first type of vesicle that buds from the trans-Golgi network is directed to the lysosome, an organelle responsible for the intracellular degradation of macromolecules. Soluble proteins delivered by this pathway include lysosomal digestive enzymes, such as proteases, and membrane proteins, such as V-class protein pump proteins. The second type moves to and fuses with the plasma membrane, releasing its contents by exocytosis in a continuous or constitutive manner. Examples of proteins released by this type of vesicle include collagen by fibroblasts and serum proteins by hepatocytes. The third type, secretory vesicles, is stored within the cell until a signal for exocytosis causes release of their contents at the plasma membrane. Examples of proteins released in this manner include insulin and glucagon from the pancreas, milk proteins from the mammary gland, and various protein secreted by white adipose tissue [24].

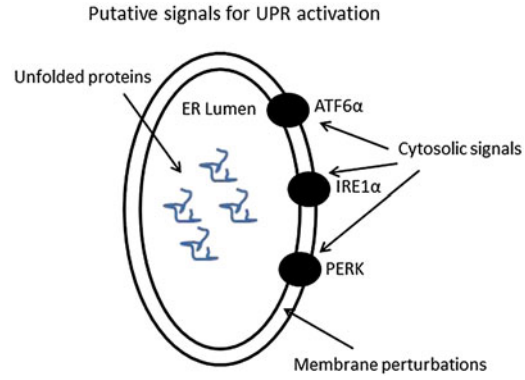

Fig. 8.2 Working model of signals that do or may result in the activation of the unfolded protein response. *ER* endoplasmic reticulum, *ATF6α* activating transcription factor-6α, *IRE1α* inositol-requiring 1α, *PERK* protein kinase-like ER kinase. See text for additional details

Adipose tissue and resident macrophages are a source for a number of secreted proteins. The secretion of several of these proteins including leptin, resistin, retinol binding protein-4, and tumor necrosis factor-α is increased in response to adipocyte expansion [25]. Thus, it is possible that the increased demand placed on the ER to process these secreted proteins leads to transient or chronic accumulation of unfolded proteins. In this scenario, obesity-related ER stress in adipose tissue would result from an excessive load of proteins presented to the ER for processing (Fig. 8.2).

In contrast, adiponectin secretion is reduced in obesity [25]. Adiponectin is synthesized as a 32-kDa monomeric protein and is then assembled in to low molecular weight trimers, medium molecular weight hexamers, and high molecular weight multimers (HMW) [25]. HMW adiponectin is thought to be the active form in plasma [26]. Not only are posttranslational modifications, such as lysine hydroxylation and glycosylation, important to the formation of HMW complexes but the distribution of circulating adiponectin oligomers depends on ER chaperone function [25]. Adiponectin secretion is controlled by the ER chaperones, ER resident protein-44 (ERp44) and ER oxidoreductin-1 (Ero1-1α), which cooperate to regulate adiponectin retention and release, respectively [27, 28]. Decreased expression of ERp44 and Ero-1α in adipose tissue of ob/ob mice is associated with a reduced ratio of HMW to total adiponectin in the circulation [28]. In addition, disulfide-bond A oxidoreductase-like protein (DsbA-L) also appears to play an important role in adiponectin folding and processing [29, 30]. Hypoxia and impairments to mitochondrial function reduce adiponectin transcription and synthesis [21, 31]. Perhaps more relevant to the present discussion, the treatment of db/db and diet-induced obese mice with tauroursodeoxycholic acid, a chemical chaperone that can alleviate ER stress [12], increased cellular and serum levels of adiponectin [30]. In addition, DsbA-L is negatively correlated with obesity in mice and humans and protects against ER stress-mediated adiponectin downregulation in 3T3-L1 adipocytes [29, 30]. Thus, obesity-mediated reductions in circulating adiponectin may result from signals that impair the ability of the ER to process this protein and in turn result in the accumulation of its unfolded protein product (Fig. 8.2).

Physiologic signals may also induce ER stress and activate the UPR in adipose tissue through mechanisms that operate in concert with or distinct from the accumulation of unfolded proteins. For example, the physiologic environment leading to and resulting from obesity may lead to changes in the composition of the ER membrane, which in turn may influence the function of any or all of the proximal UPR-membrane bound sensors (Fig. 8.2). A recent study demonstrated that membrane factors (i.e., depletion of membrane inositol or deletion of genes involved in lipid homeostasis) and unfolded proteins activate IRE1α via different mechanisms in yeast [32]. There is also increasing evidence that IRE1α activation and signaling involves the formation of a complex protein platform at the ER membrane [33]. Therefore, obesity and the associated physiologic environment may influence the composition of this protein platform and therefore the activity of IRE1α.

Cytosolic signals may interact with proximal UPR sensors and lead to selective activation of components of the UPR (Fig. 8.2). Indeed, previous studies have identified links between growth factors and PERK [34], and between PI3K signaling and double-stranded RNA-activated protein kinase (PKR, discussed in more detail below) [35], that may be independent of unfolded protein accumulation.

Consequences of ER Stress in Adipose Tissue

ER Stress and the UPR: Additional Outcomes and Connections

In vivo, the diversity of ER stress-mediated UPR signaling likely yields outcomes that are specific to the stress imposed and the needs of the involved cell but may be broadly grouped into three potential outputs: adaptation (ER stress → UPR activation → reestablishment of ER homeostasis), alarm (ER stress → UPR activation → activation of signaling pathways involved in inflammation, antioxidant defense, and/or insulin action → reestablishment of ER homeostasis or mild, chronic ER stress), and apoptosis (ER stress → UPR activation → failure to resolve severe ER stress → cell death) [36]. This diverse set of potential UPR-mediated outputs suggests that the UPR influences a broad spectrum of cellular events that extend beyond restoration of homeostasis within the ER lumen.

PERK is one of the four protein kinases that can phosphorylate eIF2α; the other three are PKR which is activated in response to viral infection, general control non-derepressible 2 kinase (GCN2) which is activated in response to amino acid deprivation, and heme-regulated inhibitor kinase (HRI) which is primarily expressed in reticulocytes and appears to coordinate globin polypeptide synthesis with heme availability [2]. Protein kinase-mediated p-eIF2α not only regulates translation but also the activation of nuclear factor kappa-β (NFKβ), via reduction in the abundance of its inhibitor IKβ [4, 37, 38]. PERK can also phosphorylate nuclear erythroid 2 p45-related factor 2 (Nrf2) triggering the nuclear import of Nrf2 [39]. Nrf2 not only promotes cell survival in response to ER stress but also confers cytoprotection against oxidative stress and exogenous xenobiotics [40, 41]. Thus, PERK-mediated p-eIF2α links the UPR to inflammation, via NFKβ and redox balance, via Nrf2.

IRE1α, in addition to catalyzing XBP1 splicing, has additional functions related to cellular signaling. Activated IRE1α can interact with the adaptor protein TNFR-associated factor 2 and lead to activation of c-Jun-NH_2-terminal kinase and NFKβ [42]. IRE1α activation has also been linked to the activation of p38 mitogen-activated protein kinase and extracellular-regulated kinase [33, 43, 44]. These interactions suggest that the IRE1α branch of the UPR regulates not only adaptation to ER stress and cell survival via XBP1 splicing but also activation of signaling pathways involved in inflammation, insulin action, and apoptosis.

ATF6α and XBP1s have been linked to lipid biosynthesis and ER membrane expansion via mechanisms that are partially distinct [45, 46]. Recent studies have also demonstrated that the transcriptional activity of XBP1s can be modified by acetylation/deacetylation and SUMOylation [47, 48]. The ability to modify XBPs transcriptional activity is a logical mechanism to regulate the magnitude and/or selectivity of IRE1α-XBP1-mediated outputs.

Physical and functional links between the ER and mitochondria have been demonstrated [49, 50]. ER–mitochondrial coupling may promote mitochondrial respiration and be influenced by ER stress and UPR activation [50]. Chronic or severe ER stress may, in turn, modify cellular metabolism [51]. Mitochondrial energy metabolism may also support ER function [52]. Mitochondrial function is closely aligned with the development and/or exacerbation of chronic, metabolic diseases, including obesity [53, 54]. It is likely that the alignment of mitochondrial function with chronic, metabolic diseases also involves the ER.

These examples serve to emphasize that not only does the induction of ER stress in the context of chronic diseases likely go beyond the accumulation of unfolded proteins but also the consequences of UPR activation likely involves multiple cellular signaling and nutrient metabolic pathways [23, 55].

The UPR in Adipose Tissue

UPR-mediated eIF2α phosphorylation results in transient attenuation of protein synthesis and selective translation of a select group of mRNAs, including ATF4 [4]. ATF4 is a member of the ATF family of basic leucine zipper (bZIP) transcription factors and can heterodimerize with multiple bZIP transcription factors including CCAAT/enhancer-binding protein [56]. At least one study has demonstrated that ATF4 regulates several aspects of mammalian metabolism, including fat storage, energy expenditure, and glycemic control. ATF4 is expressed in the Drosophila fat body (dATF4), and flies with insertions into the dATF4 locus have a lean phenotype with reduced circulating lipids [57]. Thus, ATF4 is linked to adipose tissue lipid accumulation, in part via effects on energy expenditure.

Within the lumen of the ER, protein chaperones and folding enzymes such as GRP78, glucose-regulated protein 94 (GRP94), protein disulfide isomerase, calnexin, and calreticulin assist and promote the folding of newly synthesized polypeptides and prevent aggregation of unfolded proteins [58]. GRP78$^{+/-}$ mice are resistant to diet-induced obesity, and adipose tissue inflammation and insulin resistance [59]. This resistant phenotype was suggested to result from upregulation of other ER chaperones (e.g., GRP94) and proteins involved in ER-associated degradation, thus improving ER quality control and folding capacity in white adipose tissue. Thus, the UPR in adipose tissue is highly adaptive and ER protein quality control likely plays an important role in adipose tissue function under conditions of fat expansion.

Free fatty acids derived from adipose tissue play an important role in obesity-related insulin resistance, inflammation, and hepatic steatosis [60, 61]. A recent study in *Caenorhabditis elegans* suggested that IRE-1 and HSP-4, the nematode IRE1 and GRP78 homologs, respectively, regulate the expression of the fasting-induced lipases, FIL-1 and -2 [62]. These lipases were both necessary and sufficient for fasting-induced fat granule hydrolysis. Whether ER stress and the UPR can regulate mammalian adipocyte lipolysis is unclear [63, 64].

Closing Remarks

The ER plays a role in diverse cellular functions due to its ability to regulate protein processing and lipid synthesis, serve as a calcium reservoir, and house proteins involved in glucose metabolism. The ER is equipped with a protein quality control system, the UPR, which appears to be activated in obesity. The UPR interacts with a number of cellular signaling pathways (e.g., inflammatory and insulin signaling) that are also activated in obesity. Thus, there is great interest in understanding the factors that cause ER stress and the consequences that result from activation of the UPR. The ER likely plays a critical role in both the metabolic and endocrine roles of adipose tissue. Several signals associated with adipose tissue dysfunction, such as hypoxia, may also serve as inducers of ER stress. Obesity-related adipose tissue inflammation and insulin resistance may involve ER stress and the UPR. Identification of the signals that mediate adipose tissue ER stress and the role played by the UPR in obesity-related adipose tissue dysfunction will provide important clues related to fundamental adipose tissue biology and obesity-related complications.

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Chapter 9
Autophagy in White Adipose Tissue

Scott G. Goldman, Hanlin Tao, and Shengkan 'Victor' Jin

Keywords Autophagy • Adipogenesis • Mitochondria • Oxidative stress • ER stress • Adipocyte

Key Points

- We summarize molecular components of autophagy
- We describe cellular functions of autophagy
- We review autophagy functions at organismal level
- We describe the role of autophagy in white adipose differentiation
- We describe autophagy in mature white adipocytes
- We postulate potential role of autophagy in physiopathological conditions related to white adipose tissue

Introduction

Macroautophagy (referred to hereafter as autophagy) is a highly conserved process of intracellular degradation characterized by the formation of autophagosome, a structure consisting of a double-membrane that envelopes cytoplasmic content, and transports it to a lysosome for destruction. Autophagy, once thought to function as an indiscriminate recycler of cellular content, has come to be better understood as a highly complex process that plays a role in a variety of physiologic and pathologic processes.

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To date, autophagy has been shown to be involved in immunity [1, 2], tumorigenesis [3, 4], programmed cell death [5], the selective degradation of organelles [6, 7], aging [8], cellular stress [9], a host of neurodegenerative conditions [10], and differentiation [11].

Core Autophagy Machinery

Autophagy activation is a complex cellular process and requires highly coordinated interactions among the autophagy proteins. The molecular machinery of autophagy is conserved throughout evolution from yeast to humans [12–14]. The genes encoding the basic components of the machinery are named *atg* (*au*toph*a*g*y*-related) genes. The core process of autophagy is the formation of the double-membrane structure of autophagosome and the translocation of autophagosome to lysosome, which is carried out by the core autophagy machinery (Fig. 9.1). The molecular components of the mammalian core autophagy machinery can be functionally divided into five groups: (1) the unc-51-like (ULK) kinase complex made up of ULK1 (or ULK2, both are yeast Atg1 homologs), Atg13, and Atg17; (2) the Beclin1/PI3K complex made up of Vps34, Vps15, and Beclin1 (the yeast Atg6 homolog);

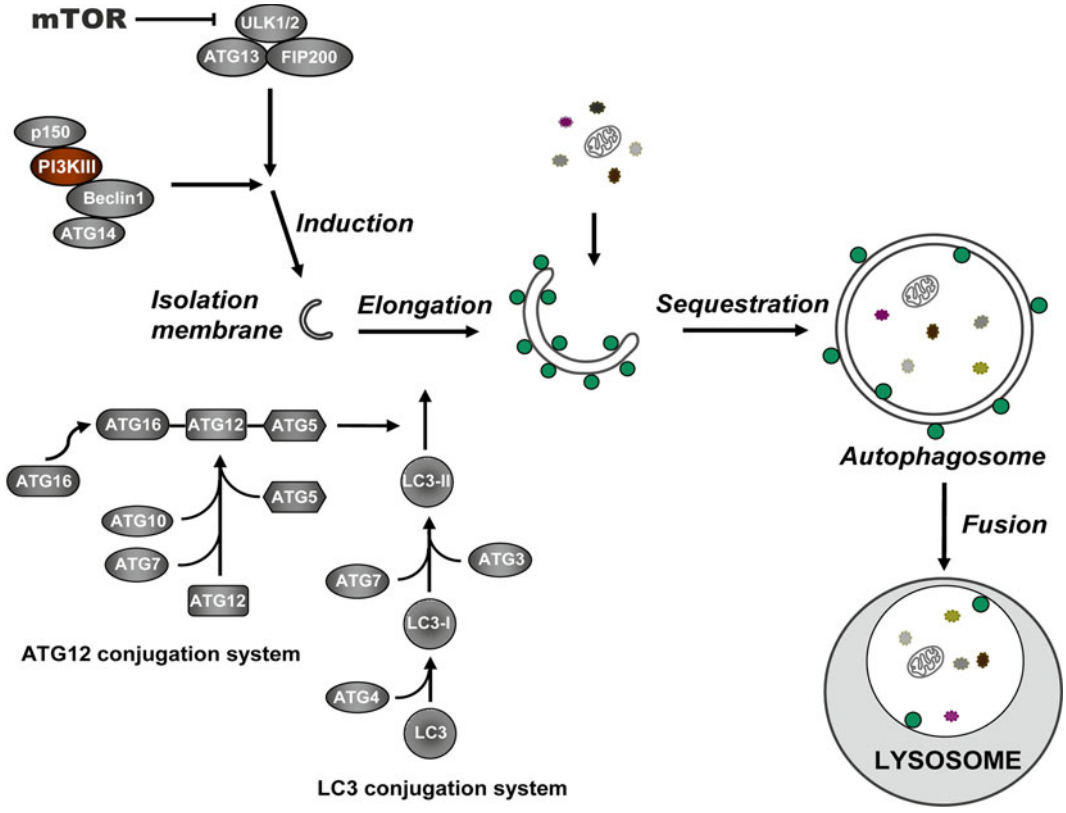

Fig. 9.1 Schematic overview of the core autophagy machinery. The molecular process of autophagy comprises an initiating stage to form a ULK complex; a vesicle nucleation (pre-autophagosome (PAS) formation) stage initiated by the formation of a complex including Atg6/beclin1 and its binding partner class III phosphoinositide 3-kinase (PI3K); vesicle elongation and autophagosome formation stage that requires two ubiquitin-like conjugation system (Atg12 and Atg8/LC3 conjugation system respectively); in the end an auto(phago)lysosome formation stage completed by the fusion of the mature autophagosome with the lysosome. : isolation membrane, : Atg8/LC3 protein, : mitochondrion, : protein aggregates [12–16]

(3) the ubiquitin-like protein conjugation systems of Atg12 and LC3 (Atg8 homolog); (4) the transmembrane proteins Atg9 and VMP1; and (5), proteins associated with autophagosome-lysosome fusion.

ULK1 and ULK2 are the mammalian orthologs of the yeast kinase Atg1. The ULK kinase complexes transduce upstream signals, such as signal from mTOR, to induce autophagosome formation [15, 16]. The best understood of these complexes is the ULK1 complex, consisting of Atg13, FIP200, and Atg101 [17]. At least two ULK complexes (containing either ULK1 or ULK2) exist in mammalian cells that regulate autophagy [9]. ULK complex components are direct substrates of mTOR [18]. Under starvation conditions, mTOR activity is inhibited, leading to reduction of ULK-Atg13 phosphorylation, which activates the ULK kinase activity. In turn, the ULK complexes lead to the initiation of autophagy via a process that is still not understood [18].

The Class III PI3K complex is made up of Vps34, Beclin1 (the mammalian homologue of Atg6), p150 (the mammalian homologue of Vsp15), and either Atg14-like protein (Atg14L or Barkor) or the ortholog of Vsp38 known as ultraviolet irradiation resistance-associated gene (UVRAG). Ambra1 has also been suggested as a member of this complex [19]. This complex, anchoring to membrane by the phosphatidylinositol-3-phosphate (PI3P)-generating kinase activity of Vsp34, is responsible for the recruitment of additional autophagy-related proteins such as the DFCP1 [20] and WIPI family proteins [21] which are necessary for the formation of the nascent phagophore, or early autophagosome [18].

The two ubiquitin-like protein-conjugation systems in mammals are believed to play a role in the elongation of the membrane of the phagophore [22, 23]. The first system consists of an ubiquitin-like protein, Atg12, conjugating with Atg5 via the activity of Atg7 (an E1-like enzyme) and Atg10 (an E2-like enzyme) [24]. The Atg12–Atg5 complex undergoes non-covalent interaction with Atg16L. Together they form oligomers that may provide the scaffold and curvature for autophagosome formation [24]. The second ubiquitin-like protein system is characterized by the involvement of Atg8, also known as Microtubule-Associated Protein 1 Light-Chain 3 (MAP1LC3, or simply LC3). Atg8/LC3 undergoes C-terminal cleavage to form LC3-I by the protease Atg4. Then through the E1-like enzyme, Atg7, and the E2-like enzyme, Atg3 LC3-I is conjugated to phosphatidylethanolamine (PE) [18]. At least four mammalian orthologs of Atg4 and six mammalian orthologs of Atg8 have been discovered thus far [9]. The lipidated LC3 is termed LC3-II, and it attaches to both the internal and cytoplasmic surfaces of the phagophore double-membrane. LC3-II is ultimately released or removed from the outer autophagosomal membrane following the fusion of the autophagosome with a lysosome. This process has long been used to monitor cellular autophagy levels [25, 26].

The transmembrane protein Atg9 is conserved among species [27] and consists of six trans-membrane domains with both the C- and N-terminus exposed on the cytosolic side of the membrane. This protein resides in the *trans*-Golgi network and has been shown to redistribute to peripheral locations in the cell that coincide with the distribution of LC3-bearing autophagosomes [18]. It is believed that Atg9 may play a role in the delivery of lipids to the elongating autophagosomal membrane [9]. VMP1, meanwhile, has been shown to be necessary for the translocation of Beclin1 and LC3 to the autophagic membrane [9].

Identification of the molecular components of autophagy machinery allows generation of the various genetic models that are deficient in autophagy. Characterization of these model systems with targeted deletion of autophagy genes have led to the elucidation of the autophagy functions at the cellular and organismal levels.

General Functions of Autophagy at the Cellular Level

Survival Under Nutrient Starvation

Some of the earliest studies of autophagy noted an increase in the number of autophagosomes in cells subjected to nutrient deprivation [28]. The implication of this finding was that autophagy played a role in cellular survival during periods of depleted resources. Indeed, a multitude of studies since have

shown that denying cells the nutrients particularly amino acids, will inevitably lead to a significant up-regulation in autophagic activity [28–32]. It is believed that the purpose of this general increase in autophagy is to provide amino acids essential for cell survival by "recycling" proteins and organelles through the autophagosomal-lysosomal pathway [31, 32]. It is now well established that the process of autophagy serves a key role in cell survival during periods of nutritional stress. One most striking phenotype of autophagy-deficient cells is accelerated death, either through necrosis or apoptosis, under nutrient starved conditions [33]. Proteins are not the only nutrition-related molecules liberated by autophagy. Under the correct conditions, autophagy has been shown to play a role in the rapid release of glycogen stores, thus allowing survival of both the cell and, through the process of the export of glucose into the blood, the entire animal [34]. Conditions which favor so-called glycogen autophagy include the neonatal starvation period which occurs in mammal just after parturition and prior to nursing, and prolonged periods of intense aerobic physical activity that lead to a demand for glucose in excess of that freely available in the blood [34].

Mitochondrial Quality Control

Another observation that dates back to the earliest days of autophagy research is the presence of mitochondria in autophagosomes [35–37]. Initially, it was believed that mitochondria were being enveloped by autophagosomes by mere happenstance, but more recent work has led to the belief that mitochondria are specifically targeted by autophagosomes under appropriate circumstances in a process now referred to as mitophagy. A series of studies have shown that mitochondrial damage can induce the formation of autophagosomes. This induction is a process separate from general macroautophagy and it is a "targeting" mechanism for the identification and envelopment of damaged mitochondria by autophagosomes [38]. A number of hypotheses exist regarding what particular indicator of mitochondrial damage triggers mitophagy. Various studies have implicated loss of mitochondrial membrane potential as mechanisms for autophagy induction. One pathway by which the depolarized mitochondria are selectively degraded is the Pink1-Parkin pathway. Depolarization of mitochondria interferes with the normal mitochondrial internalization of PINK1 (kinase PTEN-induced putative kinase protein 1) [39, 40]. PINK1 recruits the E3 ligase Parkin to mitochondria [41], leading to ubiquitination of VDAC and probably other mitochondrial outer membrane proteins [42]. The polyubiquitin chain attracts p62, which is able to recruit the autophagy machinery through its interaction with the autophagy protein MAP-LC3 [43–45]. One caveat to this working model is that the mice with Parkin gene deletion do not appear to show major defect in selective degradation of mitochondria. This suggests that the PINK1-Parkin pathway might not be the only mitophagy pathway and the pathway can be effectively compensated by other alternative mechanisms.

Alleviate ER Stress and Degrade Protein Aggregates

Multiple physiological and pathological conditions may perturb protein folding in the endoplasmic reticulum, leading to a condition known as ER stress. ER stress has been shown to be an important stimulus of autophagy [46, 47]. The primary pathway through which the ER attempts to regain homeostasis is the unfolded protein response (UPR), which induces the inhibition of general protein synthesis but promotes the production of proteins that ameliorate ER stress. Recent studies have shown a strong link between activation of UPR and increased autophagy induction. In mammals the UPR has at least three canonical effectors, inositol-requiring transmembrane kinase and endonuclease1 (IRE1), PERK (PKR-like eIF2a kinase, also known as EIF2AK3) and ATF6 (activating

transcriptional factor 6). Upon ER stress, these three effectors initiate different signaling pathways leading to transcription of a large profile of genes, among which are many genes that induces autophagy [9, 46, 47]. Besides UPR, other mechanisms have also been shown to be involved in the ER stress-induce autophagy, such as the inhibition of Akt/mTOR signaling pathway [48] and the calcium-dependent activation of protein kinase C theta [49].

Considering that the ubiquitin-proteasome and autophagy-lysosome systems are the two major degradation routes in eukaryotic cells, activation of autophagy upon ER stress represents an alternative pathway for the elimination of unfolded protein aggregates and provides a secondary protection mechanism for the cells. Studies have shown that activation of autophagy alleviate ER stress induced by proteasome inhibitors and therefore diminishes the death signal [46]. Autophagy deficiency cells show significantly increased vulnerability to ER stress and they die through rapid activation of apoptosis [50]. In addition, it was also suggested that autophagy during ER stress may contribute to the degradation of the damaged ER [51], therefore help to maintain the ER plasticity, replenishment, and homeostasis. Recently ER stress has been linked to many metabolic conditions, especially it has been shown ER stress may contribute to the development of insulin resistance [52]. Taking into account the general role of autophagy in ameliorating ER stress, exploration of the role of autophagy in these metabolic conditions is warranted.

Alleviate Oxidative Stress

Oxidative stress is another important stimulus of induction of autophagy [53, 54]. A number of mechanisms have been reported to mediate the oxidative stress-induced autophagy. First, H_2O_2, which represents a relatively stable and long-lived ROS, can directly modify thiol-containing proteins. Atg4, which is an essential protease in the autophagic pathway, has been shown to be a direct target for oxidation by H_2O_2 [55]. Some research suggested that the Atg4 oxidation directly regulates autophagy [54, 55]. In addition, mTOR signaling pathway has been shown to be extensively involved in the autophagy activation by oxidative stress [56]. In some malignant cells, it was shown that ROS initiates autophagy through inhibiting Akt/mTOR signaling in a BNIP3-dependent manner [57].

More recently, the NF-E2-related factor 2 (NRF2)—p62/SQSTM1 pathway has been reported to mediate oxidative stress-induced autophagy [58–60]. NRF2 protein is an important transcriptional factor that mediates transcriptional activation of cytoprotective genes, including those encoding antioxidant proteins, detoxification enzymes, and proteasome subunits. Under normal conditions NRF2 is ubiquitinated by the CUL3/RBX1-dependent E3 ubiquitin ligase complex (KEAP1). This leads to the constant degradation of NRF2 by proteasome thereby keeps the pathway shutting down. In response to oxidative stress, NF-E2-related factor 2 (NRF2) induces the transcription of p62. The p62 protein binds to the Keap1 protein at the site, which impedes the interaction between KEAP1 and NRF2. As a result, NRF2 is further accumulated. These interactions constitute a positive feedback loop that amplifies the oxidative stress response [58, 59]. Importantly, p62 is not only a protein that is subjected to autophagic degradation but also a strong inducer of autophagy. Thus, oxidative stress can strongly induce autophagy through upregulating p62; on the other hand, autophagy also modulates cellular response to oxidative stress by regulating the NRF2-p62 circuit through degrading p62.

Manifestation of the General Cellular Functions of Autophagy at Organismal Level

Although initially considered simply a degradation pathway, multiple levels of regulation and selection mechanism have enable autophagy numerous cellular functions, which elicit profound implications for higher organisms and play complex roles in human health and disease.

As a selective degradation mechanism, autophagy has been shown to play a beneficial role in certain neurodegenerative diseases by contributing to the clearance of abnormal proteins aggregates [61], for example, Huntingtin protein (associated with Huntington disease), α-synuclein (associated with Parkinson disease), and tau (associated with Alzheimer's disease). Studies on neurodegenerative animal models have shown that genetic inhibition of autophagy enhances degeneration symptoms, while pharmacologic induction of autophagy alleviates degeneration by preventing neuronal cell death [62].

Autophagy also plays a comprehensive role in tumorigenesis. It is well known that one major cause of tumorigenesis is the DNA damage and mutations due to oxidative stress, radiation, aging, and some other stressors. As an essential antistress and cytoprotective mechanism, autophagy has been shown to protect cells from the damage caused by these stress and thus prevent tumorigenesis [54, 63]. However, once the tumor is formed, it was also found that the same cytoprotective mechanism of autophagy can promote tumor cell survival during later stages of tumor progression as well under chemotherapy or radiotherapy conditions. Autophagy is also an important innate immune defense mechanism. A wide range of bacteria, viruses, and parasites have been shown to be targets of autophagy, although some of them have evolved strategies to either evade autophagy recognition or even use autophagy for their survival and replication [64].

Autophagy Functions in Special Tissue Types: The Role in Cell Differentiation

In addition to the general functions of autophagy that probably can apply to all cell types, the identification of autophagic machinery as a mechanism for the removal of organelles, combined with the understanding that autophagy serves as an apparatus for bulk degradation of cytoplasm, led to inquiries about the possible role of autophagy in cellular remodeling during differentiation of some highly specialized tissues. Significant evidence has now been accumulated in support of this hypothesis. For instance, autophagy has been identified as playing a key role in reticulocyte development. Early investigations established the removal of mitochondria via autophagy as a key process in the development of mature erythrocytes [65]. More recent works have identified the BH3-only protein Nix as a mitochondrial receptor for selective mitophagy in the developing red blood cell [66–69].

Erythrocytes are not the only blood cells to require mitophagy for normal development. Experiments on T-lymphocytes have shown that these cells utilize mitophagy for the removal of unneeded mitochondria [70]. The implications of this finding have not been fully explored, but it does suggest that mitophagy plays a role in T-cell development. Additionally, there is evidence of the importance of mitochondria clearance in lens cells as well [71] during periods of differentiation.

White adipose tissue in normal mammals consists of cells occupied almost entirely by a single lipid droplet, with minimal cytoplasm and few observable organelles. Mature adipocytes are differentiated from fibroblast-like pre-adipocytes. Recent studies have revealed another role for autophagy in mitochondrial removal and cytoplasmic remodeling of adipocytes during adipogenesis, which will be described in detail in the next section. In addition, the general function of autophagy in mature adipocytes and its possible implications in adipocyte physiopathology will also be discussed.

White Adipocytes

In mammals, white adipose tissue (WAT) was originally identified as a repository for excess lipids, but it is now understood that WAT is also an endocrine organ which contributes to energy homeostasis

not only through the storage and release of lipids but via the secretion of adipokines that exercise effects on other tissues [72, 73]. Although many tissues are capable of storing lipids under the right circumstances, the bulk of lipid storage and release in adult mammals is handled by WAT. In this tissue, lipogenesis and lipolysis are primarily regulated by the hormone insulin, although a number of other factors also influence the balance between fatty acid storage and release which allow for energy homeostasis in an environment of unstable nutrition. Adipocytes also assist in metabolic regulation by releasing hormones like leptin and adiponectin [72, 74]. At the local level, these cells can influence other tissues at the local level through the expression of cytokines, such as inflammation-associated tumor necrosis factor alpha and interlukin-6 [73]. Together, these molecules, also known as adipokines, are active molecules which regulate neurological activities such as appetite and behavior as well as metabolic activities of peripheral tissues. Evidence is mounting to support a model in which pathological aspects of obesity may be due to the altered adipokine secretion profile exhibited by hyperplastic WAT [75].

White adipocytes possess a unique and highly differentiated cellular structure. Mature white adipocytes are often described as having an "engagement ring" profile in which the cell is almost completely occupied by a single, large lipid droplet, with the nucleus sandwiched between the droplet and the cell membrane representing the "diamond" on the ring when viewed under light microscopy. When viewed under the higher magnification of electron microscopy, the mature white adipose cell is observed to contain a small number of mitochondria distributed thinly along the periphery, sandwiched between the lipid droplet and the cell membrane. Meanwhile, brown adipocytes possess a significant amount of cytoplasm heavily interspersed with mitochondria, other organelles, and a large number of small lipid droplets.

It is believed that white adipocytes are derived from mesenchymal stem cells [76, 77] and differentiate from fibroblastic pre-adipocytes via a two-step process. In the first step, pluripotent mesenchymal stem cells (MSCs) undergo a process known as "determination." Determination results in the formation of fibroblast-like cells that are morphologically identical to pluripotent MSCs but are now destined to differentiate only into adipocytes. These cells are now referred to either as "pre-adipocytes" or "adipoblasts." The second stage of differentiation is referred to as "adipogenesis" and consists of the formation of mature adipocytes from fibroblast-like pre-adipocytes. This stage of development is largely dependent on levels of PPARγ, which is referred to as the "master regulator" or adipogenesis. PPARγ is required both for the initiation of adipogenesis, and for the maintenance of the mature adipocyte [78, 79].

Autophagy in WAT Differentiation

As early as 1980, morphological studies of pre-adipocyte 3T3-L1 cells stimulated to undergo adipogenesis by electron microscopy revealed massive autophagy activation as well as the engulfment of mitochondria by autophagosomes [80]. More recent studies with molecular and genetic approaches have supported the idea that autophagy plays a significant role in adipogenesis [81, 82]. Multiple cell culture models have illustrated that adipocyte progenitor cells lose their efficiency to differentiate into normal white adipocytes when autophagic activity is inactivated or inhibited [83]. For instance, primary mouse embryonic fibroblasts (MEFs) obtained from mice with the homozygous deletion of an essential autophagy gene, *atg5*, cannot undergo efficient adipogenesis Instead, most *atg5* knock-out cells develop multiple small lipid droplets and then die prematurely [83]. This observation has been buttressed by similar findings obtained from experiments using primary MEFs obtained from *atg7*$^{-/-}$ mice [81]. Additional experiments have shown that knocking down autophagy genes in pre-adipocytic 3T3-L1 cells has a similar effect in reducing the efficiency of adipocytic differentiation [82].

In vivo studies have showed that a lack of functional autophagy results in significant changes of WAT at the tissue and cellular levels. Two mouse models in which the *atg7* gene is conditionally knocked out in adipose tissue have been developed and studied thus far [81, 82]. Both reports indicate that the mutant mice exhibited decreased white adipose tissue mass. Histologic analyses showed that white adipocytes in *atg7* conditional knock-out mice were smaller in size, contained multiple small lipid droplets, a greater volume of cytoplasm, and a more mitochondria than observed in wild-type WAT cells. The most significant changes observed in *atg7* knockout white adipocytes were the presence of more mitochondria compared to the relatively few mitochondria observed in normal WAT cells and the presence of multiple small lipid droplets rather than a single large droplet. During normal adipogenesis, biogenesis of mitochondria is increased in the early stages. Mitochondrial content increases by 20–30-fold during early adipogenesis to support the energy needs and lipogenesis of differentiation [84]. The morphology of mature adipocytes, which have very limited mitochondrial content, indicates that the mass degradation of excess mitochondria must take place prior to full adipocyte maturity. Thus, the accumulation of larger numbers of mitochondria observed in autophagy-deficient adipocytes provides strong genetic evidence that autophagy is responsible for the removal of excess mitochondria during normal adipogenesis. A working model is presented in Fig. 9.2a on the role of autophagy in WAT differentiation.

Changes of WAT were not limited to morphology, but also were reflected in systemic metabolic data. Notably, the autophagy-deficient conditional knock-out mice were more sensitive to insulin, highly resistant to high-fat diet induced obesity, and exhibited decreased levels of blood triglycerides and cholesterol, even though the mutant mice had the same rate of daily food intake as the wild-type mice. At this point, it is not clear how the inactivation of autophagy in adipose tissue leads to the above-mentioned systemic metabolic features. One proposed working model is that autophagy-deficient WAT may influence peripheral tissue metabolism through enhanced free fatty acid catabolism. Autophagy-deficient white adipocytes exhibit increased fatty acid β-oxidation rates [81, 82]. This might be caused by the massive accumulation of mitochondria which might have decreased energetic efficiency (e.g., partially uncoupled, Fig. 9.2a). An altered adipokine profile has also been implicated in the development of apparently beneficial metabolic outcomes in mutant mice.

Autophagy in Mature White Adipocytes and Its Association with Obesity and Insulin Resistance

Recent publications have indicated that autophagy is not only necessary for adipocyte differentiation, but appears to play a role in homeostasis regulation in mature adipocytes as well. WAT samples from a large cohort of obese and nonobese individuals and a large cohort of type II diabetic patients and nondiabetic individuals were compared for autophagy activities [85]. The protein and mRNA levels of autophagy genes as well as the flux of autophagy were quantified. Interestingly, a clear correlation was observed that the WAT samples from the obese or diabetic patients have significantly elevated autophagy activity as compared to the nonobese or nondiabetic counterparts [85]. Why autophagy levels correlate with WAT hypertrophy and insulin resistance in humans is unclear. Given the general role of autophagy in alleviating stress from oxidative stress and ER stress, as described in the previous section, and the well-established pathological phenotypes such as ER and oxidative stress observed in hypertrophic WAT, a working model is proposed in Fig. 9.2b. In this model, autophagy is induced by elevated ER stress, or oxidative stress resulting from white adipocyte hypertrophy. In turn, autophagy activation functions to alleviate the cellular stress.

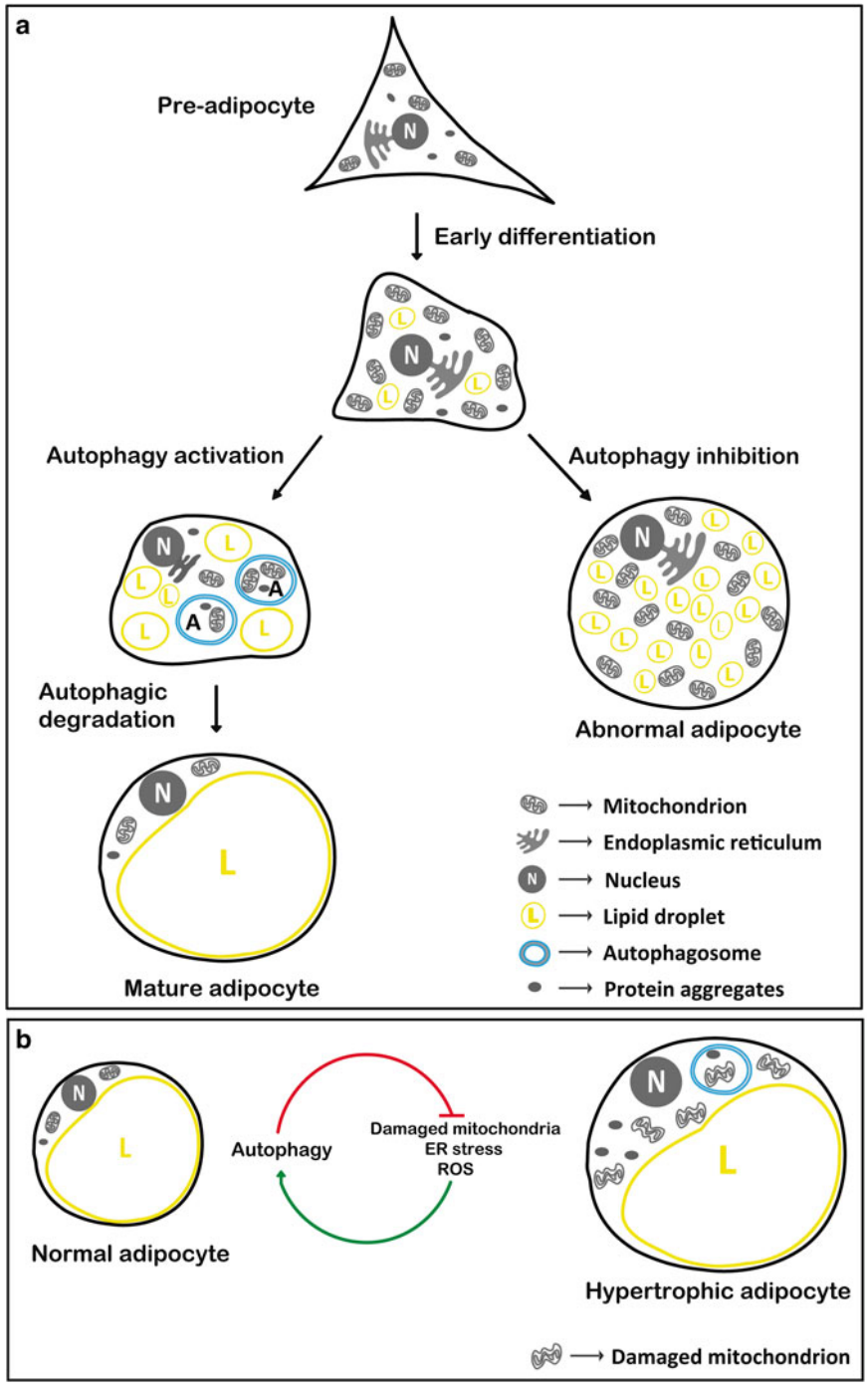

Fig. 9.2 Schematic representation of the role of autophagy in adipogenesis (**a**) and in mature adipocytes (**b**). (**a**) Autophagy is responsible for removal of excess mitochondria and other cytoplasmic components during late differentiation stages. Autophagy inhibition in adipogenesis leads to accumulation of energy inefficient mitochondria and excess cytosol. (**b**) Autophagy is activated in mature adipocyte in response to various types of cellular stress and autophagy activation alleviates the stress by degrading the causal factors such as protein aggregates and damaged mitochondria

Conclusion

The field of autophagy has experienced unparalleled growth in recent years. Where once an ancillary mechanism of random cellular recycling was observed, molecular methods have revealed an extraordinarily complex, highly regulated process essential to the maintenance of cellular homeostasis and survival. As autophagy in specific tissues is further investigated, the list of physiological and pathological events with which autophagic machinery interacts has grown at a staggering rate and shows no signs of slowing. In adipocytes, for instance, we have moved from a system in which we know little about the role of autophagy, to a system in which normal adipogenesis and adipocyte homeostasis are dependent upon it.

In the nascent adipocyte we now know that normal autophagic machinery is necessary for the proper development of morphologically and physiologically normal white and brown adipocytes. Mounting evidence indicates that autophagy is necessary for the removal of excess mitochondria, removal of other unneeded cytoplasmic content and organelles, and normal formation of the lipid droplet. Adding to this, we have observed that adipocytes generated from autophagy-deficient preadipocytes appear to impart insulin sensitivity upon their hosts, in addition to a number of other beneficial metabolic traits.

Meanwhile, in mature wild-type adipocytes, it appears that autophagy activity is also associated with adipocyte hypertrophy (obesity) and insulin resistance (type II diabetes). However, this association likely reflects the general functions of autophagy, i.e., cellular maintenance, in adipocytes. Most likely, the elevation of autophagy activity is the consequence of the pathological conditions of adipocytes rather than the cause of these conditions. In response to the stress conditions, autophagy is activated in these cells and helps alleviate the stress.

The above interpretations, if correct, would have interesting implications in the development of potential interventions targeting autophagy in adipose tissue for treating obesity or its associated metabolic diseases. First, inhibition of autophagy in adipose tissue would affect the natural turnover of white adipose tissue, which is estimated to turnover 10 % of total adipocytes annually in a normal adult human. This may have salutary effects on the various metabolic traits as demonstrated in the mouse genetic model [81, 82], including reducing energy efficiency, increasing insulin sensitivity, and reducing blood triglyceride and cholesterol levels. However, autophagy inhibition may exacerbate the pathological conditions in mature hypertrophic adipose tissues such as oxidative stress and ER stress. In addition, autophagy inhibition may have adverse effects in other organs and tissues, which would increase the risk of cancer and neurodegenerative diseases. Nevertheless, epidemiologic studies have widely reported that patients treated with hydroxychloroquine, an FDA approved drug for malaria and rheumatoid arthritis and a potent autophagy inhibitor [86], have much low risk to develop type II diabetes [87]. Future comprehensive studies are necessary to dissect the overall benefit and adverse effects of this new strategy.

A second approach is to activate autophagy in adipose tissues. From reviews in other chapters of this book we know that inflammation in WAT plays a causal role in the development of insulin resistance and other metabolic symptoms. Oxidative and ER stress in hypertrophic adipose tissue clearly contributes to development of inflammation. Therefore, autophagy activation in mature white adipocytes might be able to reduce the cellular stress and is expected to have beneficial impact. Most likely pharmacological activation of autophagy in WAT will not have any meaningful effect on adipogenesis, since the autophagy activity in those differentiating cells are already very high. Moreover, it is less likely that this strategy will have adverse effect on other organs and tissues. However, the potential salutary effect at systemic level has yet to be proven experimentally.

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Chapter 10
Obesity and Micronutrient Deficiencies

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Keywords Obesity • Micronutrients • Chronic disease • Deficiency

Key Points

- Obese individuals have a relatively high incidence of deficiency and insufficiency for several micronutrients, a phenomenon particularly prevalent in those with class III obesity (BMI > 40.0 kg/m^2).
- Obesity may perpetuate micronutrient deficiency and insufficiency through increased need in relation to body size; decreased absorption; altered metabolism as a result of an underlying low-grade inflammatory process; and increased sequestration within adipose tissue.
- Micronutrient deficiency may lead to physiologic changes that promote increased fat deposition and insufficiency and activation of an inflammatory response both of which are associated with increased risk of several chronic diseases including cardiovascular disease, type 2 diabetes, and several cancers.
- In this chapter, the relationship between obesity and status of several micronutrients including vitamins A, C, and D, iron, selenium, and zinc is discussed.

Introduction

Obesity is a serious public health threat affecting millions worldwide in both the developed and developing world [1]. In 2009, approximately 40 % of US adults were classified as obese, with a body mass index (BMI) ≥ 30.0 kg/m^2 [2]. A myriad of genetic, environmental, and behavioral factors are associated with the global rise in obesity [3]. Particularly, increased availability of inexpensive, energy-dense, and low-nutrient value foods are considered major contributory components

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associated with the rise in obesity [3]. Approximately 27–30 % of the daily caloric intake of US children and adults is comprised of low nutrient-dense foods, with sweeteners and desserts contributing 18–24 % of total calories [4, 5]. Meanwhile, modern farming and food processing techniques have led to a reduction in the micronutrient content of many common foods [6].

Micronutrients are necessary for a multitude of bodily processes including macronutrient metabolism, immune function and response, tissue function, and optimal growth and development [7–10]. Despite excess calorie intake, obese individuals have a relatively high incidence of deficiency and insufficiency for several micronutrients; a phenomenon particularly prevalent in those with class III obesity (BMI$\geq$40.0 kg/m^2) [11]. However, there is no clear consensus, and it remains uncertain whether the association is causal and if so the direction of causality [12]. Several hypotheses have emerged describing obesity's role in perpetuating micronutrient deficiency and insufficiency including: increased need in relation to body size; decreased absorption; altered metabolism as a result of an underlying low-grade inflammatory process; as well as sequestration within adipose tissue [12]. While others suggest that micronutrient deficiency and insufficiency may lead to physiologic changes that promote increased fat deposition and activation of an inflammatory response both of which are associated with increased risk of chronic diseases including cardiovascular disease, type 2 diabetes, and several cancers [12–14]. In this chapter, the relationship between obesity and the status of several micronutrients including vitamins A, C, and D, iron, selenium, and zinc is discussed. These micronutrients were selected because of the high prevalence of deficiency observed in obese persons as reported in the epidemiologic and clinical literature [15]. Also, to facilitate the discussion, the function, metabolism, and pathologic characteristics of deficiency for each micronutrient is briefly reviewed.

Obesity and Vitamin A

Vitamin A Function, Food Sources, and Homeostasis

Vitamin A, a generic term used to categorize a subclass of retinol derivatives, is an essential nutrient that cannot be synthesized by the body, and must be obtained via dietary means [16]. Vitamin A derived from animal sources, including fish, eggs, and liver is predominately in the form of retinyl esters while plant sources contain the carotenoid β-carotene, a pro-vitamin form commonly found in sweet potatoes, carrots, pumpkin, and mangos [17, 18]. Dietary-sourced retinyl esters and β-carotene can be converted to retinol in the intestinal lumen and repackaged as retinyl esters prior to absorption [17]; β-carotene can also be converted to retinaldehyde, a vitamin A derivative, in adipose tissue [19]. The most common form of vitamin A, in mammalian tissues, is retinol, a vitamin A alcohol [17]. Another metabolite, retinoic acid, is formed by an irreversible reaction and plays an important role in cellular differentiation [20]. Vitamin A and its metabolites are also required for numerous bodily processes including visual acuity [21], reproduction [22], bone growth, reducing oxidative stress and immunity [23].

Vitamin A Deficiency

Vitamin A deficiency is common world-wide with the greatest incidence in the developing world [18]. Persons at greatest risk for vitamin A deficiency or insufficiency include those who are malnourished or with chronic diarrhea, premature infants, infants, and young children in developing countries, people with cystic fibrosis due to impaired fat absorption, and pregnant and lactating women living in

developing counties [16, 24–26]. However, insufficiency has also been observed in individuals with obesity [27–29]. Deficiency of vitamin A is linked to adverse vision changes, increased susceptibility to infection, cellular damage, and impaired immune response [16, 17]. The clinical pathologies presented with vitamin A deficiency are mainly a result of its effects on the immune system specifically mucosal integrity and decrease in the T-helper cell response [30, 31].

Vitamin A and Adipose Tissue

Although the liver is the primary storage site for vitamin A in mammals, several studies have demonstrated that adipose tissue plays an active role in the metabolism and homeostasis of this micronutrient [32–34]. Also, vitamin A and its derivatives play an important role in adipocyte metabolism [27]. Vitamin A and its metabolites are actively involved in adipocyte differentiation; PPARγ transcription (the major regulator of adipogenesis); and adipocyte production of anti-inflammatory cytokines [32, 35]. Approximately 10–20 % of total body retinoids, in the form of retinyl esters, are believed to be located in adipose tissue [36]. However, it remains uncertain the exact distribution of vitamin A and it derivatives, across the specific adipose depots, or what the physiologic impact of differing concentrations may have on adipocyte function [32].

Obesity and Vitamin A Status

Human Studies

Several epidemiologic and clinical studies have investigated associations between obesity and vitamin A status, measured as serum retinol. In many of these studies, low serum retinol concentrations were observed in obese individuals. In obese Turkish adults, serum retinol, adjusted for cholesterol and triglyceride concentrations, was significantly lower in obese compared to normal weight individuals [29]. Also, adjusted serum retinol was negatively correlated with BMI ($r=-0.27$; $p<0.01$) and waist circumference ($r=-0.20$; $p<0.05$) in the obese group. In morbidly obese Spanish adults, BMI was negatively correlated with serum retinol concentrations ($r=-0.33$; $p=0.002$) [28]. It was also low in morbidly obese Brazilian adults with nonalcoholic fatty liver disease [37]. In a preoperative investigation of 144 Brazilian adult bariatric candidates, vitamin A deficiency, defined as serum retinol concentration less than 1.05 μmol/l, was present in 14 % of the patients and was inversely correlated with between BMI ($r=-0.21$; $p=0.02$) and waist circumference ($r=-0.53$; $p=0.001$) [38, 39]. An inverse correlation was observed between weight, BMI, and hip circumference and serum retinol in overweight/obese Thai adults [40]. However, some studies, conducted in both adults and children, have reported no association between serum retinol and BMI [41–45]. Discrepancies in the literature may be partly related to the weight variation of the individuals studied. Class III obesity appears to be most closely linked to lower serum retinol concentrations as opposed to those with a lower body mass index.

Preclinical and Animal Studies

Studies utilizing adipocyte cell lines, dietary studies with rodents, and research conducted using genetically modified mice, have allowed for a more mechanistic assessment regarding the associations between obesity and vitamin A [46]. Studies in adipocyte cell lines have demonstrated that retinoic acid and retinaldehyde are both capable of inhibiting adipogenesis [47]. In obese rats, supplementation of vitamin A resulted in significant weight loss compared to a control group

receiving a lower dosage [48]. In other studies, supplementation with retinoic acid resulted in weight loss, decreased adiposity, and increased insulin sensitivity in both lean and obese mice [49–53]. Also, rats supplemented with vitamin A had decreased leptin and increased uncoupling protein-1 expression both of which are involved in energy balance and expenditure [54]. Conversely, mice fed a diet deficient in vitamin A had increased adiposity [52]. In a study using genetically modified mice, knockout of the enzyme retinaldehyde dehydrogenase-1, which is involved in retinoid metabolism, allowed for intracellular levels of retinoic acid and retinaldehyde to increase, resulting in leaner and more glucose tolerant animals compared to controls [47]. Taken together, results suggest that vitamin A is tightly linked to body fat accumulation as well as metabolic control.

Mechanisms Linking Obesity to Vitamin A Status

Effect of Obesity on Vitamin A Status

There are several hypotheses describing mechanisms linking obesity with vitamin A deficiency and insufficiency. One hypothesis describes a causal pathway in which obesity precedes vitamin A deficiency. Vitamin A has been well recognized for its favorable effect on immune response and oxidative stress [31]. Obesity is associated with chronic inflammation that generates and promotes increased free-radical production and oxidative stress [55]. As a result, antioxidants, like vitamin A, may become more quickly depleted suggesting obese persons may have a greater requirement for antioxidants, including vitamin A [28, 29]. Others have suggested that dysregulation or decreased availability of other micronutrients including iron, copper, and zinc, which can be altered in obesity, may play a role in vitamin A stability, conversion, and metabolism [56]. Also, inadequate dietary vitamin A intake may be associated with decreased levels although results from several studies suggest otherwise [28, 44]. In another study [48], results suggested a defect in vitamin A absorption given that supplementation did not raise hepatic retinol levels when compared to lean animals receiving the same dose; this could also reflect a shift in storage into extrahepatic tissues like adipose.

Effect of Vitamin A Deficiency on Adiposity

Results from preclinical and animal studies suggest that vitamin A deficiency may promote the deposition of adipose tissue and precede obesity although the precise mechanism is unclear [12]. Reduced retinoic acid can promote adipogenesis at the early stages of adipocyte formation, favoring upregulation of PPAR-λ and CEBP-α, both of which are adipogenic factors [35, 52]. Vitamin A may also be associated with diminished adipocyte apoptosis [57]. Retinoic acid is also involved in the regulation of several adipokines including leptin and resistin which are important in regulating energy balance and body composition [58]. In animals, a vitamin A deficient diet was associated with increased leptin gene expression which could promote dysregulation of energy balance and favor weight gain [48, 59].

Obesity and Vitamin A Supplementation

No studies have looked specifically at the effect of vitamin A supplementation in obese populations [14]. Although some have looked at the impact of a mixed supplements, which included vitamin A, on adiposity. Mexican women supplemented with fortified low-fat milk, including vitamin A, reduced their body weight, BMI, and total body fat compared to a control group [60]. Similarly,

supplementing obese women with micronutrients, that included vitamin A, resulted in decreased body weight and body fat than women receiving a supplement without vitamin A [61]. In both studies, it was unclear if vitamin A and/or the other micronutrients were responsible for this shift in adiposity, and neither study investigated the impact of supplementation on adipokine or cytokine production. More studies, in humans, are required to determine if increased vitamin A intake would have a beneficial impact on obesity and chronic disease risk reduction.

Obesity and Vitamin C

Vitamin C Function, Sources, and Homeostasis

Vitamin C is a water-soluble vitamin involved in a multitude of biologic processes. It acts as a cofactor for enzymes involved in collagen synthesis, neurotransmitter conversion, fatty acid oxidation, and protein metabolism [62]. Vitamin C is also critical to the intestinal uptake of non-heme iron through the reduction of ferric iron to the ferrous form [63]. Notably, also through its reducing potential, vitamin C acts as a potent antioxidant, scavenging free radicals, and lessening oxidative damage in the body [64].

Humans are unable to endogenously produce vitamin C so dietary sources are essential [62]. Vitamin C can exist in two forms, ascorbic acid (reduced form) or dehydro-ascorbic acid (oxidized form) [65]. All fruits and vegetable contain some vitamin C, but the richest sources included oranges, kiwi, red peppers, spinach, and broccoli [66]. Because vitamin C is readily taken up by the intestinal enterocytes [62]. However, the molecular mechanisms involved in vitamin C homeostasis are not well understood [65].

Vitamin C Deficiency

Prolonged vitamin C deficiency (serum levels <11.4 μmol/l) can lead to scurvy which is associated with impaired collagen synthesis and wound healing, inflamed and bleeding gums, fatigue, and capillary fragility [67]. Deficiency is also associated with iron deficiency as non-heme iron absorption is diminished [63]. Vitamin C deficiency in developed countries is rare but persons at risk for insufficiency include smokers, individuals with limited food variety, those with malabsorption, and the obese [68, 69]. Because of its antioxidant effect, more recent studies suggest that vitamin C insufficiency is associated with the development of several chronic diseases including macular degeneration, cardiovascular disease, and cancers [62].

Obesity and Vitamin C Status

Several epidemiologic and clinical studies have examined the relationship between systemic vitamin C concentrations, measured as circulating ascorbic acid, and obesity/adiposity. In a large cohort of British adults, central adiposity, determined by waist-to-hip ratio, was significantly inversely associated with plasma ascorbic acid concentrations in both men and women [69]. Furthermore, the effect of central adiposity was independent of BMI and observable in non-obese individuals.

Studies investigating the association between BMI and plasma ascorbic acid levels have been mixed. In a Norwegian study, morbidly obese adults had significantly lower concentrations of ascorbic acid compared to healthy controls [70]. Similarly, in US adults, there was a modest inverse

relationship between BMI and circulating ascorbic acid levels [71]. In a small clinical study of young men, obese participants had 38 % lower plasma ascorbic acid compared to lean controls [72]. However, in a French study, BMI was not correlated with to plasma ascorbic acid concentrations [73].

Mechanisms Linking Obesity with Lower Systemic Vitamin C Concentrations

A couple theories have emerged linking obesity with vitamin C deficiency. One theory supports the notion that adiposity leads to a reduction in vitamin C concentrations while others suggest, that vitamin C deficiency is associated with lipid accumulation in adipose tissue thus promoting obesity.

Obesity is associated with increased oxidative stress and free radical production [74]. Thus, given that vitamin C is an antioxidant and scavenger of free radicals [75], obese persons or those with increased central adiposity may more quickly deplete their antioxidant reserves as reflected by lower circulating vitamin C concentrations. Similarly, other populations with increased oxidative stress including smokers and diabetics also present with lower plasma vitamin C concentrations [71, 76].

Another theory is that vitamin C deficiency may impair fatty acid oxidation promoting fat deposition. Vitamin C is an essential cofactor in the synthesis of carnitine which is required for the oxidation of fatty acids [77]. A deficiency in carnitine could result in lipid accumulation in tissues. In fact, in persons with an in-born error of carnitine metabolism, tissue lipid accumulation is present [78]. It is clear that both theories are closely linked, but it remains uncertain if vitamin C deficiency causes fat or if obesity depletes the available pool of as a result of increased oxidative stress.

Obesity and Vitamin C Supplementation, Human Studies

A few human trials have investigated the impact of vitamin C supplementation on lipid oxidation and weight status. In one study, participants replete for vitamin C had increased fat oxidation during exercise compared to depleted participants [78]. In another study, a once daily high dose of vitamin C was associated with greater weight loss during a 6 week weight reduction trial compared to placebo (2.5 kg vs. 1.0 kg; $p<0.05$) [79]. Together these findings support the theory that vitamin C concentrations may be closely linked to fatty acid metabolism and fat deposition. However, in a small clinical trial, vitamin C supplementation was not associated with greater weight loss [80]. Unfortunately, none of the studies reported how supplementation impacted circulating ascorbic acid concentrations.

Adipose Tissue and Vitamin C, Preclinical and Animal Studies

Researchers investigated the role of supplemental vitamin C on body weight, lipolytic response, and glucocorticoid metabolism in early stages of diet-induced overweight in rats fed a cafeteria-style diet compared to rats fed standard chow [81]. In the cafeteria-style fed animals, vitamin C supplementation was associated with reduced weight gain and normalization of plasma insulin levels. Furthermore, the supplemented animals were more active and had decreased adipocyte lipolysis. In another study, treatment with vitamin C was associated with a dose dependent decrease in leptin secretion, reactive oxygen species production, and inhibition of glucose uptake and glycerol release by adipocytes [82]. Furthermore, vitamin C has been shown to inhibit the adipocyte-macrophage cross-talk associated with increased low-grade inflammation through inhibition of nitric oxide production and monocyte chemoattractant protein-1 expression and secretion [83]. Cumulatively, findings from the preclinical

and animal studies suggest that vitamin C is intricately involved in adipocyte glucose and fat metabolism, reactive oxygen species production, and modulation of the inflammatory process [12].

Obesity and Vitamin D

Vitamin D Function, Sources, and Homeostasis

Vitamin D is a fat-soluble vitamin that plays an important role in bone metabolism and calcium homeostasis [84]. More recently, vitamin D has been recognized as a pleiotropic molecule given that many tissues including the brain, prostate, breast, and colon, as well as immune cells, have a vitamin D receptor and respond to systemic levels of the bioactive form of vitamin D, 1,25 dihydroxy vitamin D [85]. The bioactive form of vitamin D is involved in many bodily processes including regulation of cell proliferation, differentiation, and apoptosis, and angiogenesis, immune function, insulin secretion, and muscle strength [86–88].

Vitamin D can be present in two hormone precursor forms, ergocalciferol (vit D2), found in plants and some fish, and cholecalciferol (vit D3) synthesized by the skin in response to sunlight exposure [89]. Few foods naturally contain vitamin D, but many including milk, yogurt, and cheese are fortified with vitamin D during processing [90]. Vitamin D manufactured by the skin or obtained from the diet is metabolized in the liver to 25-hydroxy vitamin D, the form used to assess a person's systemic vitamin D status. 25-hydroxy vitamin D is then further converted, primarily by the kidneys, to the bioactive form, 1,25 dihydroxy vitamin D [85].

Vitamin D Deficiency

Vitamin D deficiency or insufficiency is estimated to impact one billion people world-wide [91, 92]. There is no consensus on the optimal serum 25-hydroxy vitamin D concentration, although most clinicians would define insufficiency as serum 25-hydroxy vitamin D less than 20 ng/ml and deficiency as less than 12 ng/ml [93]. Classically, vitamin D deficiency is associated with disorders of bone metabolism including rickets, osteomalacia, and osteoporosis [85, 94, 95]. Research has also emerged associating vitamin D deficiency with the development of several chronic conditions including cardiovascular disease, type 2 diabetes and several cancers [96–98]. Risk factors for insufficiency/deficiency include reduced synthesis from the skin due to seasonality, aging, darker pigmentation, and excessive use of sunscreen; inadequate dietary intake; decreased absorption; liver disease; kidney disease; and obesity [99, 100].

Adipose Tissue and Vitamin D

Several genes related to vitamin D metabolism are present in adipose tissue including a gene that encodes the enzyme responsible for converting 25-hydroxy vitamin D to its bioactive form [101], as well as a gene involved in its catalysis [102]. This suggests that adipose tissue may be involved in local synthesis and degradation of bioactive vitamin D [103]. Furthermore, human adipose tissue expresses a vitamin D receptor and bioactivation and release of 1,25 dihydroxy vitamin D from human mammary adipocytes has been observed [103]. This is notable because it suggests that like the kidney, adipose tissue may be able to convert 25-hydroxy vitamin D and release the bioactive form to adjacent

tissues and cells. A few preclinical studies suggest that vitamin D may also be involved in adipogenesis and adipose tissue lipid metabolism [104–106].

Obesity and Vitamin D Status

Numerous epidemiologic and clinical studies have evaluated vitamin D status, measured as serum 25-hydroxy vitamin D, in overweight and obese children and adults. Many of the studies found that overweight and obesity is associated with reduced serum 25-hydroxy vitamin D concentrations which has been linked to increased risk of hyperparathyroidism, insulin resistance, metabolic syndrome, nonalcoholic fatty liver disease, and some cancers in these populations [107–111]. In a large sample of premenopausal women, overweight and obesity, based on BMI, was independently associated with increased risk of vitamin D insufficiency and obesity with vitamin D deficiency [112]. Similarly, in US adolescents, serum 25-hydroxy vitamin D levels were inversely associated with body weight [113].

Other measures of adiposity including total fat mass, body fat percentage, and waist circumference show an even stronger inverse association with serum 25-hydroxy vitamin D concentrations in both adults and children [114–117]. In fact, recent work suggests that patterns of fat deposition, particularly in the visceral adipose depot, may play an integral role in negatively modulating serum 25-hydroxy vitamin D concentrations more so than deposition in other adipose compartments [118]. However, it remains unclear mechanistically how increased visceral adipose tissue is related to reduction in serum 25-hydroxy vitamin D concentrations.

Proposed Mechanisms Linking Obesity with Lower Serum 25-Hydroxy Vitamin D Concentrations

The mechanisms underlying reduced serum 25-hydroxy vitamin D concentrations in overweight and obese individuals are currently unknown although several hypotheses have been proposed. One hypothesis suggests that obese individuals have underexposure to sunlight as a result of engaging in less outdoor activity or wearing additional clothing which would result in less endogenous vitamin D production [119]. However, if this were the case, pre-vitamin D would also be expected to be lower but studies report no significant differences in cutaneous pre-vitamin D concentrations between non-obese and obese individuals [120].

Another theory is that obese individuals have increased circulating concentrations of the bioactive form of vitamin D which leads to decreased hepatic production of serum 25-hydroxy vitamin D and subsequently lower serum levels of this metabolite. However, serum levels of 1,25 dihydroxy vitamin D are also inversely correlated with BMI which does not provide support for this hypothesis [121].

It has been suggested that vitamin D is sequestered within adipocytes [120, 122]. This is an attractive theory because the vitamin D is a fat soluble vitamin although few studies have investigated the vitamin D content of human adipose tissue. One small human study reported that serum and subcutaneous adipose levels of vitamin D were positively correlated in obese individuals, consistent with the theory that fat tissue can act as a storage reservoir [123]. However, it can't be concluded from this study if adipose concentrations of vitamin D are higher in obesity given the lack of a non-obese comparison group.

Other lesser explored theories include greater catabolism as a result of expression of a vitamin D specific hydroxylase in adipose tissue [101]; reduced liver synthesis of 25-hydroxy vitamin D as a result of hepatic fat infiltration [124]; and increased total body clearance of vitamin D as a result of a chronic pro-inflammatory state and vitamin D's proposed anti-inflammatory effect [125].

Obesity and Vitamin D Supplementation

A few studies have looked at response to oral vitamin D supplementation in obese persons. In one study, overweight and obese premenopausal women were randomized to vitamin D supplementation (25 μg/day cholecalciferol) or placebo for 12 weeks [126]. Women taking vitamin D had a significant increase in serum 25-hydroxy vitamin D compared to women randomized to placebo (38.2 ± 32.7 nmol/l vs. 4.6 ± 14.8 nmol/l; $p < 0.001$). One study compared response of obese and lean individuals to UVB exposure and a large oral dose of vitamin D (50,000 IU) [120]. The obese group had lower serum 25-hydroxy vitamin D levels following UVB exposure and oral supplementation compared to the lean individuals. Furthermore, BMI was inversely correlated with serum vitamin D concentrations following supplementation ($r = -0.56$, $p = 0.007$). Another study, using computerized medical records, investigated response to vitamin D supplementation in over 16, 000 adults with baseline serum 25-hydroxy vitamin D levels less than 20 ng/nl [127]. Higher BMI was associated with a poorer response to supplementation compared to those with lower BMI. Supporting this, Forsythe et al. [131] reported that BMI was inversely associated with vitamin D status following supplementation in older adults. Collectively, the findings support the theory that sequestration of vitamin D in the larger adipose mass in obesity is likely. However, the need for a higher dosage of vitamin D for repletion or differences in vitamin D metabolism between the obese and lean individuals could not be deduced from these studies.

Weight Loss and Vitamin D Status

A few studies have assessed vitamin D status following bariatric surgery or participation in a behavioral weight management intervention. In a study of 20 severely obese women, circulating 25-hydroxy vitamin D concentrations were acutely increased 1 month post gastric bypass surgery followed by a decreasing trend thereafter [128]. This finding supports the theory that adipose tissue may sequester vitamin D given systemic levels transiently increased following initial weight loss. Similar findings were reported in another bariatric study [70]. However, Pramyothin et al. [129], did not observe a rise in serum 25-hydroxy vitamin D concentrations at 3, 6, 9, or 12 months post-surgery. Collectively, these studies suggest that vitamin D stored in the adipose tissue is not sufficient to maintain vitamin D concentrations after gastric bypass surgery. This may be further compounded by vitamin D malabsorption post-operatively. Following participation in a behavioral weight loss intervention, weight loss and reduction in body fat mass was associated with increased serum 25-hydroxy vitamin D concentrations in a dose-dependent manner and was independent of changes in fitness level or vitamin D supplement use [130]. It is theorized that reduction in fat mass reduces the adipose compartment in which vitamin D can be sequestered [130]. A theory strongly supported by the supplement studies [120, 131].

Obesity and Iron

Iron Function, Dietary Sources, and Uptake

Iron is an essential element that is a key component of oxygen-carrying proteins, a vital player in cellular metabolism, and is essential to cell growth and differentiation [132]. Dietary iron exists in two forms, heme and non-heme. Heme iron is found in animal-based foods like meats that contain hemoglobin and myoglobin [133]. Non-heme iron can be found in plant-based foods including kidney beans and spinach, while the supplement form commonly exists as ferrous sulfate [133]. Regardless

of source, any iron taken up by the duodenal enterocyte joins the labile iron pool and is transferred into plasma by the basolateral iron transporter ferroportin-1 or stored as ferritin within the enterocyte [134]. If not utilized, the enterocyte-stored iron will be lost through intestinal sloughing [135]. Cytosolic iron that is exported into plasma binds to transferrin, which then delivers iron to erythroblasts and other tissues [135].

Iron Regulation/Hepcidin

Tight regulation of iron is necessary because iron is highly toxic and humans lack a regulated pathway to excrete large amounts [136]. Therefore, effective communication between key sites of iron use, absorption, and storage, at both the cellular and systemic level, is necessary to maintain appropriate iron balance [137]. Hepcidin is a small peptide hormone that functions as both the homeostatic regulator of systemic iron metabolism and mediator of host defense and inflammation [132, 138]. The sensing of circulating iron and iron stores is thought to occur in the liver, which is the primary site of hepcidin production and secretion [132, 139]. It controls the movement of iron into plasma by regulating the ferroportin-1 exporter. The ferroportin-1 exporter facilitates iron export into plasma from iron-handling tissues including enterocytes, hepatocytes, reticuloendothelial macrophages, and the placenta [132, 140]. When hepcidin binds to ferroportin-1 the two proteins are internalized and degraded within lysosomes [132]. Hepcidin expression is simultaneously regulated by the interplay of pathways controlled by iron status, erythropoietic activity, and inflammation, and the relative strength of each signal [139–141]. When body iron levels are elevated, or if inflammation or infection is present, liver hepcidin production is increased resulting in diminished ferroportin-1 expression. Down-regulation of ferroportin-1 results in reduced iron export from the iron-handling tissues [132, 139, 140]. Conversely, when body iron levels are depleted or anemia or hypoxia exists, hepcidin expression is minimal, allowing for increased dietary iron absorption and mobilization from body stores via active ferroportin-1 transporters. Hepcidin is also expressed to a lesser degree in the adipose tissue, heart, placenta, and kidneys where it is presumably regulated by inflammation and not body iron [140].

Iron Deficiency

Iron deficiency remains the most common nutritional deficiency and cause of anemia worldwide [142]. Populations in developing countries, premenopausal females, pregnant women, children, vegetarians, and frequent blood donors are largely affected by iron deficiency due to low dietary intake, inadequate iron bioavailability, increased iron demand required for growth and development, iron losses, and changes in blood volume [142–144]. Iron deficiency is a condition in which there is inadequate iron to maintain normal function of bodily tissues [145]. The health impact of iron deficiency can include fatigue, weakness, decreased work capacity, palpitations, pallor, and alterations in immune function [146, 147]. In persons with iron deficiency, hepcidin is suppressed to very low or undetectable levels allowing for the flux of iron into plasma from diet and storage sites [148, 149].

Obesity and Iron Status

As early as the 1960s, researchers observed a strong correlation between decreased serum iron concentrations and increased adiposity in children and adolescents [150, 151]. Decades later, results from

National Health and Nutritional Examination Survey III showed that overweight children and adolescents were two times more likely to be iron deficient, based on two of three clinical indicators of iron status including transferrin saturation, ferritin, and erythrocyte protoporphyrin, than those of normal weight [152]. Similar results were reported in several other studies. A cross-sectional study of 321 Israeli children and adolescents reported that those with a BMI above the 85th percentile were 1.75 times more likely to have decreased serum iron levels than those below this threshold [153]. In obese Iranian children between 11 and 17 years of age, iron deficiency, defined as serum ferritin <12 ng/ml and transferrin saturation <16 %, was three times more prevalent compared to those of normal weight [154]. Furthermore, in Chinese adolescent males, iron deficiency anemia, defined as hemoglobin <12.0 g/dl for boys <14 years old and <13.0 g/dl for boys aged 14 years and older, was higher in obese (26.3 %) compared to the normal weight group (19.0 %) [155].

The relationship between obesity and depleted iron status has also been confirmed in adults but with less consistency. In several studies, markers of iron status including hemoglobin, transferrin saturation, and serum iron were significantly lower in obese compared to normal weight adults [156–160]. Notably, in two of the studies, the association between obesity and low iron status was much weaker for men [156, 160]. Another study reported a significant inverse association between central ($r = -0.19$; $p < 0.05$) and total fat mass ($r = -0.19$; $p < 0.05$) with serum iron concentrations in Hispanic women, but similar associations were not observed for men or women from other racial/ethnic groups [161]. When the nutritional status of obese men and women was assessed before bariatric surgery, low levels of both serum iron and hemoglobin were observed [162, 163]. Additionally, in a case–control study of obese and non-obese postmenopausal women obesity was associated with significantly higher soluble transferrin receptor levels (Obese: 1.38 mg/dl vs. Lean: 1.16 mg/dl; $p < 0.001$), a marker of early cellular iron depletion; BMI was positively correlated with the receptor levels ($r = 0.48$; $p < 0.001$) [164].

Mechanisms Linking Obesity with Iron Deficiency

Early Hypotheses

In overweight and obese children and adolescents, it has been hypothesized that rapid growth, increased blood volume, early onset of menstruation, poor diet, minimal physical activity, and genetics may be contributing to the iron deficiency observed in this population [152, 153]. However, one study reported that iron deficiency was positively associated with BMI and inflammation (measured as C-reactive protein (CRP)) but not with race, age, dietary iron intake, years since beginning menstruation, or physical activity [165]. Similarly, in adults, inflammation, (measured as CRP) and BMI were both independently associated with iron deficiency whereas dietary iron intake, dietary factors that can enhance or inhibit iron absorption, and physical activity were not associated [166, 167]. Although not measured in any of the preceding studies, findings suggest that obesity may be associated with an upregulation of systemic hepcidin, likely triggered by low-grade chronic inflammation, resulting in diminished ferroportin-1 activity at sites of iron flux, ultimately reducing iron bioavailability.

Obesity and Hepcidin

Indeed, several researchers have demonstrated that serum hepcidin is significantly elevated in obese compared to non-obese women and children [168–170]. In one study, overweight children had higher serum hepcidin concentrations and poorer iron status, despite similar dietary iron intake, when compared to normal weight children [169]. Serum hepcidin was positively correlated with BMI and

body iron but surprisingly no relationship was observed with several inflammatory factors including CRP, and IL-6. In another study, overweight children were found to have higher serum hepcidin and lower serum iron and transferrin saturation compared to normal weight children and serum hepcidin was inversely correlated with iron absorption [170].

Adipose Tissue and Hepcidin

A seminal study reported that hepcidin gene expression was significantly higher in visceral and subcutaneous adipose tissue and was positively correlated with BMI and inflammation (as measured by IL-6) in obese compared to non-obese premenopausal women [171]. Also, *ex vivo* protein expression was detected in the subcutaneous and visceral adipose tissue explants from the same obese women suggesting that adipose-derived hepcidin could play a significant role in systemic iron regulation. However, serum hepcidin concentrations and active secretion of the protein from adipose tissue was not assessed. Recently, a study reported that serum hepcidin was positively correlated with liver ($r=0.61$; $p=0.04$) and weakly correlated with subcutaneous ($r=0.01$; $p=0.95$) or visceral ($r=-0.19$; $p=0.43$) adipose hepcidin gene expression in severely obese women, and liver gene expression was 700 times greater than that expressed in the subcutaneous or visceral adipose tissue depots [168]. Furthermore, findings from an in vivo vein drainage study, conducted in obese and non-obese adults, reported no net secretion of hepcidin from the subcutaneous adipose depot. This suggests that the adipose tissue depot many not actively secrete hepcidin and contribute negligibly to circulating levels [172]. The mechanisms linking obesity to elevated hepcidin concentrations and iron depletion remain unclear. However, it is attractive to speculate that liver-derived hepcidin, stimulated by inflammation, plays an important role in this phenomenon although additional research is required to confirm this.

Obesity and Iron Supplementation

Very few studies have examined the impact of iron supplementation on repletion efforts in iron deficient obese individuals. Notably, in a study of Thai women and children, independent of iron status, both BMI and inflammation (as measured by CRP) were negatively correlated with absorption of isotopically labeled iron [173]. Additionally, those with greater adiposity were unable to improve their iron status by oral repletion when compared to non-obese controls. Given the paucity of elegantly designed iron absorption studies in obese populations, it remains unclear if there is any benefit to providing oral iron supplements to obese individuals. Furthermore, given the oxidative potential of iron, one could surmise that exposing the gastrointestinal tract to higher levels of unabsorbed iron could have pathologic consequences.

Weight Loss and Iron Status

Findings from several studies, investigating the impact of diet-induced weight loss on iron status in both adults and children are inconsistent. In a cohort of obese children, no significant change in serum iron, serum ferritin, or transferrin saturation was observed after a 13-week hypocaloric diet and significant reduction of body weight [174]. Similarly in adults, diet-induced weight loss was associated with maintenance or improvement of serum iron, transferrin saturation, and hemoglobin [175, 176]. Conversely, a significant decrease in transferrin saturation was observed after just 1 week of adherence to a very low calorie diet [177]. Similarly, in a group of obese women, a significant decline in

hemoglobin, hematocrit, and red blood cell count was reported after engaging in a 15 week hypocaloric diet [178].

The results detailing the impact of weight loss on iron status following bariatric surgery are also inconsistent. Several studies have reported increased incidence of iron deficiency, decreased hemoglobin and as decreased iron absorption following gastric bypass surgery [179, 180]. Bariatric surgery is thought to negatively impact iron status through decreased gastric acid secretion (needed to convert dietary ferric iron to the ferrous state which is necessary for absorption); reduced tolerance to red meat; and decreased intestinal surface area for the absorption of iron [181]. However, Anty and colleagues [182] reported 6 months after bariatric surgery (94 % gastric bypass), in a cohort of premenopausal women, that transferrin saturation was significantly higher (18 % vs. 25 %; $p<0.0001$). Also, several other studies have reported no significant change in iron status following gastric bypass or gastric banding surgery [183, 184]. While another study reported a significant decline in soluble transferrin receptor concentrations 18 months post-surgery (baseline: 1.40 vs. follow-up 1.27 mg/l; $p<0.05$) [185].

Weight Loss, Hepcidin, and Iron Status

There is some evidence that serum hepcidin concentrations decrease with weight loss. In one study, 6 months following restrictive bariatric surgery, premenopausal women lost a significant amount of weight, had significantly decreased serum hepcidin (baseline 111.25 ng/ml vs. 6 months post-op 31.35 ng/ml; $p<0.0001$) and inflammatory protein concentrations, as well as improved functional iron status [186]. Similarly, in children, following a 6 month weight loss program, serum hepcidin concentrations declined significantly (2.1 nmol/l vs 1.1 nmol/l; $p=0.003$) and iron absorption was substantially improved [187]. Collectively, these findings suggest weight loss results in reduced inflammation, lower serum hepcidin concentrations, and improvement in both iron absorption and iron status in persons with obesity. However, due to contrasting results regarding the impact of weight loss on iron status, it is important that more prospective studies, examining the effects of weight reduction (by dietary and surgical means), are conducted in adults and children with obesity so that the relationship between excess weight and iron regulation can be better understood.

Obesity and Selenium

Selenium Function, Sources, and Homeostasis

Selenium is a trace mineral that is used by the body for the production of proteins called selenoproteins, that are is essential to human health, but can be toxic if consumed in excess [188]. There are 25 known selenoproteins critical to bodily processes including immune function and hormone production [188]. The importance of selenoproteins to human health is demonstrated by single nucleotide polymorphisms in selenoprotein genes. This defect in selenoprotein genes is associated with increased risk of cardiovascular disease, type 2 diabetes, and several cancers [189].

The selenium content of food varies greatly and is highly dependent on soil selenium concentrations [188]. Organ meat, muscle meat, seafood, some nuts, and cereals and grains are the richest sources of selenium in the food supply [188]. Supplemental forms of selenium include inorganic sodium selenite as well as organic selenomethionine [190]. Selenium in both the organic and inorganic form appear to be actively absorbed, with minimal homeostatic control in the small intestine

[191]. Absorption studies suggest selenium metabolism may be dependent on the form ingested (organic vs. inorganic) and is intricately linked to endogenous amino acid and protein metabolism [192]. Urinary excretion of selenium regulates systemic homeostasis and is highly dependent on dietary intake [193]. There is no evidence of a specific storage form or site for selenium in the human body [194], although the skeletal muscle contains the largest pool of organic selenium in the body under conditions of adequate intake; inorganic forms are thought to be in a separate pool and readily available for selenoprotein synthesis [195, 196].

Selenium Deficiency

In the US, selenium deficiency is rare but has been observed in other countries, particularly China, where soil levels of the trace mineral are low [197]. Selenium deficiency (serum selenium <0.75 μmol/l) is associated with a form of cardiomyopathy called Keshan's disease, hypothyroidism, weakened immune system, and skeletal disorders [198–200]. Persons receiving total parenteral nutrition and those with severe gastrointestinal problems like Crohn's disease or removal of a portion of the stomach such as with bariatric surgery may experience selenium deficiency [161, 201]. Also, individuals with systemic inflammation or infection including those with obesity have decreased circulating selenium levels [202, 203]. Lower blood levels of selenium have been associated with increased cancer mortality in large epidemiologic studies [204, 205].

Adipose Tissue and Selenium

Relatively, few studies have investigated expression or regulation of selenium or selenoproteins in adipose tissue. One study using both cell culture and animal models showed that adipose tissue expresses selenoproteins and reduced levels were observed in obese animals [206]. In culture, reducing selenoprotein concentrations was associated with impaired adipocyte differentiation, increased oxidative stress and inflammation, and insulin resistance. Early findings suggest that selenoproteins play a critical role in adipogenesis and the inflammatory process in adipose tissue.

Obesity and Selenium Status

Several epidemiologic and clinical studies have investigated the relationship between obesity/adiposity and circulating selenium concentrations in adults and children, but with inconsistent findings. In an epidemiologic study of US adults, among both men and women, higher BMI was associated with lower serum selenium compared to those with a lower BMI [207]. Also, in a large study of French adults, BMI was inversely associated with serum selenium concentrations in woman but not in men [203]. However, in Spanish adults, there was no association between serum selenium concentrations and BMI or other measures of adiposity including waist circumference and waist to hip ratio [208].

In a smaller clinical study, circulating selenium concentrations were not different between obese and non-obese adults [209]. However, selenoprotein concentrations were significantly lower in the obese males. In a clinical study of Spanish school-children, serum selenium was significantly lower in overweight and obese compared to normal weight children (BMI > 85th percentile: 64.6 ± 16.8 μg/l vs. normal weight: 75.3 ± 12.2 μg/l; $p < 0.001$) and inversely correlated with BMI [210]. Furthermore, in female adults seeking bariatric surgery, serum selenium was significantly lower than in non-obese women [211].

Mechanisms Linking Obesity with Lower Selenium Concentrations

The hypothesized mechanisms linking obesity to decreased systemic selenium concentrations is related to selenium's role as an antioxidant [206]. As mentioned previously, increased fat mass is associated with increased oxidative stress [212]. Increased energy substrates including glucose and lipids, can increase mitochondrial activity and generation of reactive oxygen species in bodily tissue, particularly in adipocytes [213, 214]. Consequently, the increase in oxidative stress would lead to antioxidants, including selenium, to become depleted as oxidative stress exceeds the antioxidant capacity, and would likely manifest as lower circulating antioxidant concentrations including selenium [12, 28, 29]. This is significant because selenium depletion is associated with increased chronic disease risk.

Obesity and Selenium Supplementation

Several studies have investigated the impact of selenium supplementation and increased consumption of selenium-rich foods on weight/adiposity, oxidative stress, DNA damage, blood lipids, and circulating selenium levels in overweight and obese individuals. In a randomized placebo-controlled trial, overweight participants received a hypocaloric legume-based diet enriched with arginine, selenium, or arginine and selenium for 6 weeks [215]. For those randomized to the hypocaloric diet plus selenium, there was no significant effect on body fat, BMI or oxidative stress. However, in participants randomized to the hypocaloric diet supplemented with both arginine and selenium a significant reduction in subscapular skinfold thickness was observed. In another small randomized placebo-controlled crossover trial, overweight participants ($n = 10$) and normal weight participants ($n = 10$) were randomized to selenium or placebo supplementation for 3 weeks and were challenged to a bout of exercise [216]. Selenium supplementation was associated with decreased post-exercise oxidative stress, in the overweight participants but no effect of was observed at rest or 30 min post-exercise in either group.

Two studies investigated the effect of Brazil nut consumption, a source of highly bioavailable selenium [217], on oxidative stress and circulating selenium concentrations in obese females and adolescents. Thirty-seven obese women consumed one Brazil nut, approximately 290 μg of selenium, for 8 weeks [217]. Post-intervention, plasma and erythrocyte selenium and selenoprotein activity increased significantly and DNA damage decreased markedly. In the other study, obese adolescent females were provided three to five Brazil nuts per day, processed and capsulized, or placebo for 16 weeks [218]. Post-intervention, the Brazil nut consuming group had higher serum selenium, lower cholesterol, and decreased oxidation compared to the placebo group; weight did not change in either group. Cumulatively, findings suggest that in the short term, increased selenium intake reduces oxidative stress and improves the lipid profile in overweight and obese individuals but has minimal effect on weight status. Little is known regarding the long-term impact of increased selenium intake on reducing chronic disease risk in overweight and obese individuals suggesting the need for further study.

Obesity and Zinc

Zinc Function, Sources, and Homeostasis

Zinc is an essential trace metal that plays an important role in many bodily processes including macronutrient metabolism, immune function, growth and development; and insulin storage and release [219]. Zinc also functions as an antioxidant [220].

The primary food sources of zinc include oysters, meats, liver, and eggs [221]. Whole grains are relatively high in zinc although other components in these foods cause a marked reduction in its bioavailability [222]. Zinc can also be endogenously released into the intestine through pancreatic and intestinal secretions or sloughed mucosal cells [223].

Zinc absorption from dietary or endogenous sources takes place in the small intestine and several factors enhance zinc absorption including the presence of picolinic acid, vitamin B6, and some amino acids [224]. Conversely, fiber, oxalic acid, tannins, selenium, iron, and calcium can inhibit absorption [224]. Zinc homeostasis is controlled at the intestinal level and is excreted in feces if not absorbed [219]. Zinc is primarily transported attached to albumin. Zinc is then taken to the liver where it can be stored bound to hepatic metallothionein or shuttled to other tissues to participate in a variety of biologic processes [224].

Zinc Deficiency

Zinc deficiency is somewhat uncommon in the US but it is observed in both developing and developed countries [23]. Zinc deficiency is associated with growth retardation, impaired immune function, and loss of taste and appetite [225–227]. Determining an individual's zinc status can be difficult given there is no single reliable method to determine body zinc concentrations [228]. Zinc status is most commonly evaluated in serum and plasma but also as plasma metallothionein, erythrocyte zinc, and leukocyte and neutrophil zinc concentrations [219]. Groups at risk for zinc deficiency or insufficiency include those with gastrointestinal disorders associated with malabsorption, alcoholics, those with liver cirrhosis or chronic kidney disease, pregnant and lactating women, those with inadequate dietary zinc intake, and obese individuals [229–231].

Zinc and Adipose Tissue

Several studies have investigated zinc's role in adipogenesis and adipocyte glucose metabolism. Zinc is intricately involved in insulin secretion and action which when upregulated, promotes adipocyte differentiation [232]. The addition of zinc into a culture medium resulted in insulin-like effects that enhanced adipogenesis in 3T3-L1 adipocytes [233]. Also, zinc supplementation in cattle significantly increased adipocyte differentiation and resulted in increased fat marbling in these animals [233]. Furthermore, zinc deficiency in a murine model was associated with impaired adipocyte leptin secretion, increased neuropeptide Y concentrations, and reduced body fat [234]. Leptin secretion and gene expression is induced by insulin [235, 236]. Therefore, given zinc's proposed role in insulin synthesis and secretion, zinc deficiency in these animals may partially explain the reduction in leptin concentrations observed [234].

Some zinc containing proteins may also play a role in adipocyte metabolism. Zinc finger proteins, which contain one or more zinc ions, are associated with regulation of adipogenesis and adipocyte glucose metabolism [237–239]. Furthermore, zinc α-2 glycoprotein, a protein expressed in human visceral and subcutaneous adipose tissue, can stimulate lipolysis suggesting that zinc α-2 glycoprotein may be highly involved in local regulation of adipose tissue metabolism [240, 241].

Obesity and Zinc Status

Several epidemiologic and clinical studies have investigated the relationship between obesity/adiposity and zinc concentrations in both adults and children, although findings have been somewhat

inconsistent. In an adult urban Indian population, greater central adiposity was associated with zinc deficiency [242]. Zinc deficiency was also coupled with greater prevalence of both coronary artery disease and insulin resistance in this population. In a cohort of male adults, plasma zinc was significantly lower in obese compared to non-obese controls (512.35 vs. 831.5 μg/dl; $p=0.0001$) [160]. Furthermore, erythrocyte copper–zinc superoxide dismutase, a potent antioxidant defense enzyme, was significantly lower in the obese males (373.41 vs. 532.46 U/ml; $p=0.0001$). In a small clinical study, obese participants had significantly lower plasma zinc concentrations compared to normal weight controls (13.5 ± 1.0 vs. 18.1 ± 0.9 μmol/l; $p<0.005$) [243]. While in a group of obese and lean Brazilian women, BMI and waist circumference were negatively associated with erythrocyte zinc levels [244]. Similarly, plasma and erythrocytes zinc levels were lower in obese compared to non-obese children; urinary zinc excretion was also significantly higher in the obese group [245]. In a study evaluating zinc concentrations from hair, children that were heavier or had greater body fat had significantly lower zinc concentrations in their hair [246]. In a study of rural Mexican women, BMI, percent of body fat and waist circumference were not associated with lower plasma zinc concentrations when compared to controls [14].

Mechanisms Linking Obesity with Lower Zinc Concentrations

There are several theories linking obesity with decreased zinc concentrations. One hypothesis is that obesity-induced inflammation stimulates the expression of metallothionein and zinc transporters promoting zinc accumulation in the liver and periphery including the adipose tissue in turn decreasing bioavailability [244, 247, 248]. Another theory is that zinc deficiency can increase oxidative stress and enhance the inflammatory response in obese individuals [160, 249]. However, the opposite effect may also be true in which obesity-induced inflammation and oxidative stress deplete systemic zinc status given its potent antioxidant role [250]. Nevertheless, in persons with severe inflammation or HIV, minimal shifts in plasma zinc concentrations have been observed, refuting this hypothesis [251, 252]. Lastly, zinc is involved in regulation of leptin which is integral to the neuroendocrine pathway responsible for appetite control and energy balance [253, 254]. Zinc deficiency is associated with decreased leptin concentrations which could significantly impact body composition and adipose tissue mass promoting obesity [253, 255].

Obesity and Zinc Supplementation

Several human and animal studies have investigated the impact of zinc supplementation on adiposity and other physiologic parameters obese populations. In obese men, 1 month of zinc supplementation was associated with increased leptin concentrations compared to men randomized to placebo [256]. This suggests that zinc may play a significant role in body energy balance and body weight regulation. However, in obese women, randomized to 4 weeks of zinc supplementation, serum leptin remained unchanged [257]. Given the inconsistency, additional studies evaluating the impact of zinc supplementation on leptin concentrations in obese compared to lean individuals is warranted.

In a recent meta-analysis, investigating the impact of zinc supplementation on glycemic control in healthy, diabetic, or obese individuals, zinc supplementation was associated with a significant decline in blood glucose concentrations with the most dramatic decline observed in diabetic and obese individuals [258]. This suggests that zinc supplementation may promote better glycemic control. Furthermore, in obese children, following an 8 week double-blind placebo controlled zinc supplementation trial, weight, BMI, CRP, and insulin decreased significantly during zinc supplementation whereas these values increased during placebo treatment [259]. In a similar study, conducted in obese Iranian

children, zinc supplementation was associated with decreased fasting plasma glucose and insulin but no change in weight or BMI [260].

In animals, zinc supplementation ameliorated body fat accumulation and some of the metabolic effects of sucrose-induced obesity including reduced glucose and insulin concentrations and higher systemic leptin and zinc levels [255]. Specifically, authors suggest that zinc-induced increases in leptin may suppress some of the metabolic effects of obesity in these animals. Another study, investigated the effect of zinc deficiency and supplementation on adiposity and serum leptin in mice fed a high and low-fat diet [261]. High-fat fed mice had high body fat and lower systemic zinc concentrations compared to low-fat fed animals. Furthermore, leptin was negatively correlated with adipose zinc concentrations suggesting a relationship between adiposity, leptin, and zinc concentrations. Given the inconsistencies in the literature, additional trials are necessary before broader public health recommendations for zinc supplementation can be made for the prevention of obesity or reduction of metabolic disorders in those with obesity [258].

Conclusions

In summary, the current literature suggests that obesity is associated with deficiency and insufficiency of several micronutrients. Deficiency and insufficiency of many of these micronutrients is independently linked to increased risk of several chronic diseases including cardiovascular disease, type 2 diabetes, and several cancers [12–14]. However, it remains uncertain the direction of causality and if obesity perpetuates micronutrient deficiencies or if deficiencies increase the risk for obesity [12]. For the human trials, the heterogeneity in study design, the measure of adiposity used, ethnicity, age, gender, and health status of the participants may have resulted in some of the differences observed making it difficult to draw conclusions regarding the relationship between obesity, micronutrient deficiencies, and the health impact of micronutrient supplementation on obesity and chronic disease risk reduction [12]. Furthermore, it remains unclear the physiologic mechanisms connecting obesity with several micronutrient deficiencies. Additional research is needed to further clarify these mechanisms and how they relate to the disease process. Such understanding may help to facilitate widespread treatment and prevention strategies that can more effectively reduce the public health burden of obesity and chronic diseases stemming from excess weight [12].

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Part III
Obesity

Chapter 11
Epidemiology of Obesity in Children

Youfa Wang and Hyunjung Lim

Keywords Child • Adolescent • Obesity • Overweight • Body mass index

Key Points

- Childhood obesity has become a serious public health threat worldwide. The prevalence has reached a high level in many countries but large variations exist across countries and population groups within countries.
- The prevalence of childhood obesity is higher in developed countries than developing countries. However, it has been increasing dramatically in many developing countries, particularly in urban settings and among high socioeconomic status groups in the past two decades.
- Various references and standards have been used to define obesity and overweight in children and adolescents over time and across countries although in general most are based on age-sex-specific BMI cut points. They can give different estimates of the rates. The International Obesity Task Force (IOTF) BMI reference. The 2006 WHO Growth Standards for preschool children, and US 85th and 95th BMI percentiles have been used widely.
- Childhood obesity has many immediate, intermediate and long-term health consequences. Overweight and obese children are likely to maintain their status into adulthood and are at higher risks for developing chronic diseases. A good understanding of the childhood obesity epidemic will help guide intervention efforts and develop effective population-based programs and policies.

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Abbreviations

BMI	Body mass index
CDC	Centers for Disease Control and Prevention
NHANES	National Health and Nutrition Examination Survey
WHO	World Health Organization
IOTF	The International Obesity Task Force

Introduction

The rising childhood obesity prevalence is a serious public health problem in many countries worldwide. It becomes a major public health challenge of the twenty-first century [1–3]. Recent studies indicate that approximately 20 % of school age-children in European countries are overweight or obese and 5 % are obese. In North America, these figures are 30 % and 15 %, respectively. It is estimated that 155 million, or one in ten school-age (5–17 years old) children are overweight or obese [4]. During recent years, overweight and obesity have been increasing dramatically in many developed and developing countries [1, 5, 6].

Although current understanding of the health consequences of overweight and obesity is predominately based on adult studies, increasing evidences suggest that childhood obesity has a number of immediate, intermediate, and long-term health consequences. Childhood obesity has long-term effects on mortality and morbidity [7, 8]. Overweight and obese children are likely to maintain their status into adulthood and are at higher risks for developing chronic diseases such as hypertension, dyslipidemia, type 2 diabetes, heart disease, stroke, gallbladder disease, osteoarthritis, sleep apnea and respiratory problems, and certain cancers [9]. A good understanding of the childhood obesity epidemic will help guide intervention efforts and develop effective population-based programs and policies.

Classification of Childhood Overweight and Obesity

Various measures and references have been used to define obesity and overweight in children and adolescents over time and across countries at present. This has affected prevalence estimates across studies and populations. Current consensus is that body mass index (BMI = weight [kg]/height [m]^2) is a good measure of adiposity in children and adolescents [10–14]. However, BMI varies substantially by age and gender in children. Thus, unlike in adults, BMI cut-off points used to classify obesity in children should be sex-age-specific. For adults, based on the World Health Organization (WHO) recommendations, BMI of 25 and 30 are widely used to defined overweight and obesity, respectively [15].

Different references based on weight-for-height indexes, such as BMI and weight-for-height, have been used to classify body weight status for children. However, application of these measures varies considerably [10–12, 14, 16]. For example, in the USA, the sex-age-specific 85th and 95th BMI percentiles have been used. Other countries, such as China, France, the UK, Singapore, and the Netherlands, have developed their own BMI references using local data. The BMI cut-points in these references differ considerably. A universal reference will help facilitate international comparisons and to monitor the global obesity epidemic. Following are several references that have been used widely.

1. *The International Obesity Task Force (IOTF) reference*. The reference supports a series of sex-age-specific BMI cut-points for children age 2–18 years, which correspond to the BMI cut-points of 25 and 30 for adulthood overweight and obesity, respectively [14]. The cut-points were derived based on sex-specific BMI-age curves with BMI of 25 and 30 at age 18, which were fit based on large data sets from six countries, namely, Brazil, Britain, Hong Kong, the Netherlands, Singapore, and

the USA. The reference has been recommended for international use based on its unique strengths. It is simple to use and is consistent for children and adolescents. However, the reference data sets may not adequately represent non-Western populations, and there are large differences in the prevalence of overweight and obesity across the six source nations [17].

2. *The 2006 WHO Growth Standards for preschool children*. The WHO released new growth standard for children from birth to the age of 60 months in 2006 [18]. These were developed based on the Multicentre Growth Reference Study (MGRS), which recruited affluent, breast-fed, and healthy infants/children whose mothers did not smoke during or after delivery from six cities in Brazil, Ghana, India, Norway, Oman, and the USA. The data showed great similarity in growth across all sites with only about 3 % of the total variation in growth attributable to race/country. Data were pooled to generate cut-off points. These standards include anthropometric indicators such as height-for-age (length-for-age), weight-for-age, weight-for-height (weight-for-length), and BMI-for-age. BMI *Z*-score ≥2 was recommended to classify "obesity" and BMI *Z*-score ≥1 to classify "overweight." These standards have been widely used.
3. *The 2007 WHO growth reference for school-age children and adolescents*. In 2007, the WHO released another set of growth references for children and adolescents aged 5–19 years [19]. The references were derived based on the same US dataset for the 1978 WHO/National Center for Health Statistics (NCHS) growth references, but used different growth curve smoothing techniques. The references include three indicators: BMI-for-age, weight-for-age, and height-for-age. Overweight and obesity cut-points were based on BMI-for-age *Z*-scores. A *Z*-score of 1 was found to be equivalent to a BMI-for-age of 25.4 for boys and 25.0 for girls in 19-year olds. As these values are equal or close to the WHO BMI cut-points of 25 used in adults, it was recommended to use a *Z*-score of 1 to classify "overweight" and a *Z*-score ≥2 to classify "obesity". BMI-for-age *Z*-scores <−2 and <−3 were set as the cut-points for thinness and severe thinness, respectively. The reference is not widely used.
4. *BMI references used in the USA*: In 2000, the US NCHS and the Centers for Disease Control and Prevention (CDC) updated growth charts (including BMI percentiles) using data from five national health examination surveys from 1963 to 1994 including the National Health and Nutrition Examination Survey (NHAENS) [10]. Before the release of the 2000 CDC Growth Charts, 85th and 95th percentiles based on data from the First NHANES (1971–1974) were used in the USA as well as in many other countries to classify childhood obesity and overweight [20, 21]. They recommended the use of sex- and age-specific 95th and 85th BMI percentiles to classify childhood obesity and overweight, respectively, in children over age 2 years old. These cut-points are not directly linked to health risks.

The Global Epidemic of Childhood Overweight and Obesity

1. *Recent and current prevalence of overweight and obesity*. Obesity has become an epidemic in children worldwide, but large regional differences exist. The combined prevalence of overweight and obesity is high in many regions and countries around the world (see Tables 11.1, 11.2, and 11.3). Table 11.1 shows the projected prevalence rates in WHO-defined regions. Our previous work to project combined prevalence for 2006 yielded a range from 17 % in South East Asia to 40 % in the Americas [1]. In general, combined prevalence is much higher in developed countries than in developing countries. There are also considerable age- and gender differences in many populations. Based on our estimations [1] and the findings of others [22–25], approximately 26 % of school-age children in European countries were overweight or obese in 2006, and 5 % were obese. In Americas, these figures were 28 % and 10 %, respectively.

There are large between-country variations in the prevalence across and within world regions (see Fig. 11.1 and Table 11.2). Figure 11.1 shows a worldwide view of combined prevalence of overweight and obesity in childhood. Combined prevalence is high in Western and industrialized countries, such

Table 11.1 Prevalence (%) of overweight and obesity in school-age children based on available data and IOTF criteria, and estimated for 2006 and 2010

WHO region (dates of most recent surveys)	Most recent surveys		Projected 2006[a]		Projected 2010[a]	
	Overweight and obesity	Obesity	Overweight and obesity	Obesity	Overweight and obesity	Obesity
Africa (1987–2003)	1.6	0.2	[b]	[b]	[b]	[b]
Americas (1988–2002)	27.7	9.6	40.0	13.2	46.4	15.2
Eastern Mediterranean (1992–2001)	23.5	5.9	35.3	9.4	41.7	11.5
Europe (1992–2003)	25.5	5.4	31.8	7.9	38.2	10.0
South East Asia (1997–2002)	10.6	1.5	16.6	3.3	22.9	5.3
West Pacific (1993–2000)	12.0	2.3	20.8	5.0	27.2	7.0

Data source: Wang and Lobstein, 2006

[a]Based on population weighted annualized increases in prevalence

[b]There were insufficient data on school age children in the WHO African Region to make estimates of projected prevalence rates

Table 11.2 Combined childhood overweight and obesity prevalence (%) based on data collected since around the year 2000 for selected countries by WHO Region[a]

	Year of survey	Age (years)	Boys	Girls	BMI reference
WHO Africa region					
Algeria	2006	6–10	7.4	7.4	IOTF
Seychelles	2004/2005	9–15	16.5	21.0	IOTF
South Africa	2001–2004	6–13	13.6	17.7	IOTF
WHO Americas region					
Brazil	2002	7–10	23.1	21.1	IOTF
Canada	2004	2–19	28.9	26.6	2000 CDC
Chile	2002	6	28.6	27.1	IOTF
Mexico	2006	2–19	28.4	27.3	2000 CDC
USA	2009–2010	2–19	33.0	30.4	2000 CDC
WHO Eastern Mediterranean region					
Egypt	2005	10–17	23.4	29.6	85th percentile
Iran	2003/2004	6–18	14.4	14.0	IOTF
Kuwait	1999–2000	10–14	44.7	44.9	NCHS
Saudi Arabia	2002	1–18	16.7	19.4	IOTF
United Arab Emirates (UAE)	1998–1999	5–17	32.4	32.4	IOTF
WHO European region					
England	2007	5–17	22.7	26.6	IOTF
France	2006/2007	3–17	13.1	14.9	IOTF
Germany	2008	4–16	22.6	17.7	IOTF
Netherlands	2003	5–16	14.7	17.9	IOTF
Switzerland	2007	6–13	16.7	13.1	IOTF
WHO South East Asia region					
India	2007–2008	2–17	20.6	18.3	IOTF
India	2005–2006	<5	1.7	1.4	2006 WHO growth standard
Malaysia	2002	7–10	9.7 (obesity)	7.1 (obesity)	WHO
Sri Lanka	2003	10–15	1.7	2.7	IOTF
Vietnam	2004	11–16	11.7 (boys and girls)		IOTF
WHO Western Pacific region					
Australia	2007	2–16	22.0	24.0	IOTF
China	2005	7–18	14.9	8.9	Chinese ref.
Japan	1996–2000	6–14	16.2	14.3	IOTF
New Zealand	2007	5–14	28.2	28.8	IOTF
South Korea	2005	10–19	21.7	17.1	Korean ref.

[a]Some prevalence data was limited by data availability; many rates presented here may not be nationally representative. Only data collected since 2000 were used and we report statistics for those countries with large population sizes within each region as examples. We also added some additional data. (Main data Source: IASO 2012)

Table 11.3 Time trends in the combined prevalence (%) of overweight and obesity in preschool-age children aged 0–5 years for years from 1990 to 2010 and projections for 2015 and 2020, by United Nations (UN) Region[a–d]

UN region and sub-region	1990	1995	2000	2005	2010	*2015*	*2020*
Global	4.2	4.6	5.1	5.8	6.7	*7.8*	*9.1*
Developing countries	3.7	4.0	4.5	5.2	6.1	*7.2*	*8.6*
Developed countries[e]	7.9	8.8	9.7	10.6	11.7	*12.9*	*14.1*
Africa	4.0	4.7	5.7	6.9	8.5	*10.4*	*12.7*
Eastern	3.9	4.4	5.1	5.8	6.7	*7.6*	*8.7*
Middle	2.5	3.4	4.7	6.4	8.7	*11.7*	*15.5*
Northern	6.1	8.0	10.3	13.3	17.0	*21.4*	*26.6*
Southern	10.2	9.5	8.8	8.2	7.6	*7.0*	*6.5*
Western	2.2	2.9	3.8	4.9	6.4	*8.3*	*10.6*
Asia[f]	3.2	3.4	3.7	4.2	4.9	*5.7*	*6.8*
Eastern	4.8	4.9	5.0	5.1	5.2	*5.3*	*5.4*
South Central	2.3	2.6	2.9	3.2	3.5	*3.9*	*4.3*
Southeastern	2.1	2.6	3.1	3.8	4.6	*5.6*	*6.7*
Western	3.0	4.5	6.8	10.1	14.7	*21.0*	*29.1*
Latin America and Caribbean	6.8	6.8	6.8	6.9	6.9	*7.0*	*7.2*
Caribbean	4.6	5.1	5.6	6.2	6.9	*7.6*	*8.3*
Central America	4.8	5.3	5.9	6.5	7.2	*8.0*	*8.8*
South America	8.0	7.7	7.4	7.1	6.8	*6.5*	*6.3*
Oceania[g]	2.9	3.1	3.2	3.3	3.5	*3.6*	*3.8*

Reproduced, with permission, from *American Journal of Clinical Nutrition*, November 2010
Data source: de Onis et al. 2010
[a]All surveys included both boys and girls
[b]Cross-sectional data on the prevalence of overweight and obesity were obtained from national nutrition surveys. A total of 450 nationally representative surveys were available from 144 countries. Of the 450 surveys, 413 were conducted in developing countries and 37 in developed countries. About 38 % of the surveys (171 surveys) were conducted between 1991 and 1999, 16 % (70 surveys) were conducted before 1991, and 46 % (209 surveys) after 1999
[c]Linear mixed-effects models were fit to estimate prevalence rates and numbers of affected children by region from 1990 to 2020
[d]Overweight and obese statuses were defined based on >2 SDs (standard deviations) from the weight-for-height median
[e]Including Europe, Northern America, Australia, New Zealand, and Japan
[f]Excluding Japan
[g]Excluding Australia and New Zealand

as the USA, Canada, some European countries, some countries in South America, some nations in the Middle East, some nations in North Africa, and in the Asia-Pacific region (e.g., Indonesia and in New Zealand) [26]. According to a recent study examining combined prevalence by WHO region [26], the Region of the Americas (approximately 25–30 %) and Eastern Mediterranean Region (approximately 20–40 %) had higher prevalence than the South East Asian and Western Pacific Regions including nations such as India, Malaysia, Vietnam, China, Australia, South Korea, and Japan. Africa had the lowest prevalence (about 10 %). There were also differences between countries within the same WHO region. In the Eastern Mediterranean Region, the combined prevalence in Egypt and Kuwait were about 30 % and 45 % among girls, respectively, while the prevalence was only 14.0 % amongst Iranian girls. Self-reported information in a 2001–2002 international school survey of 11-, 13-, and 15-year-olds from 35 countries in Europe and North America ($N = 162{,}305$) showed large between-country difference in the obesity/overweight prevalence in adolescents, which ranged from 3.5 % in Lithuanian girls to 31.7 % in boys from Malta [27].

The International Association for the Study of Obesity recently reported the combined prevalence of overweight and obesity among childhood based on findings of many researchers by six WHO regions across the world [28]. Some of the data were shown in Table 11.2, which also includes other data. The following countries had the highest combined rate in their respective WHO Region: the USA (32 %), Kuwait (44 %), England (25 %), New Zealand (28 %), India (19 %), Seychelles (18 %).

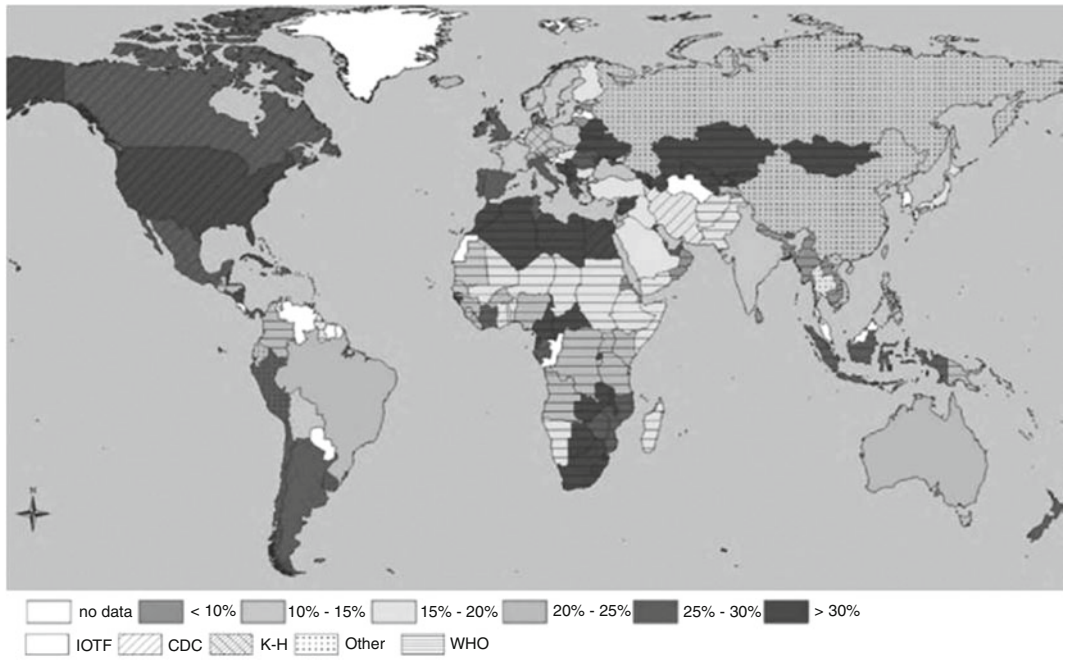

Fig. 11.1 Worldwide combined prevalence of overweight and obesity in children and adolescents. The prevalence estimates were calculated as the arithmetic mean of the age-specific estimates. Difference references (e.g., those recommended by the IOTF, WHO, and US CDC) were used to classify overweight and obesity across countries (Data Source: Pigeot et al. 2011)

2. *Time trends in the prevalence of childhood obesity*. Many countries have data collected over the past two decades allowing for the examination of time trends in obesity in both adults and young people. We studied the global trends in childhood obesity in a comprehensive meta-analysis of studies published between 1980 and 2005 from over 60 countries [1]. The combined prevalence of overweight and obesity increased in almost all countries for which trends data were available. From the 1970s to the end of the 1990s, the combined prevalence doubled or tripled in several large countries in North America (i.e., Canada and the USA), the Western Pacific Region (i.e., Australia), and Europe (i.e., Finland, France, Germany, Italy, and Spain). We estimated the prevalence based on the IOTF BMI cut points (Table 11.1).

One recent study examined trends in the combined prevalence in preschool age children (0–5 years old) between 1990 and 2010, and projected worldwide rates for 2015 and 2020 (Table 11.3) [29]. It estimated 43 million children (35 million in developing countries) were overweight or obese in 2010, and 92 million were at risk of overweight; and the global combined prevalence increased from 4.2 % in 1990 to 6.7 % in 2010. If such trends continue, these numbers may reach 9.1 % (or approximately 60 million children) in 2020. For developing countries alone, the combined prevalence was estimated at 6.1 % in 2010 and is expected to rise, perhaps as high as 8.6 % by 2020. 2010 rates were lower in Asian than in Africa (4.9 % vs. 8.5 %), but a much larger number of children are affected (17.7 million vs. 13.3 million) in Asia compared to Africa. Given the dramatic increases in combined prevalence since 1990, the study concluded that effective interventions starting as early as infancy are necessary to reverse anticipated trends.

Among major industrialized countries, the USA has the highest prevalence and the largest number of overweight and obesity individuals. The prevalence in children has increased for all ages between 2 and 19 years, but the increase in obesity leveled off in recent years. Figure 11.2 shows time trends in the prevalence of obesity (BMI ≥95th percentile) by age between 1971–1974 and 2009–2010.

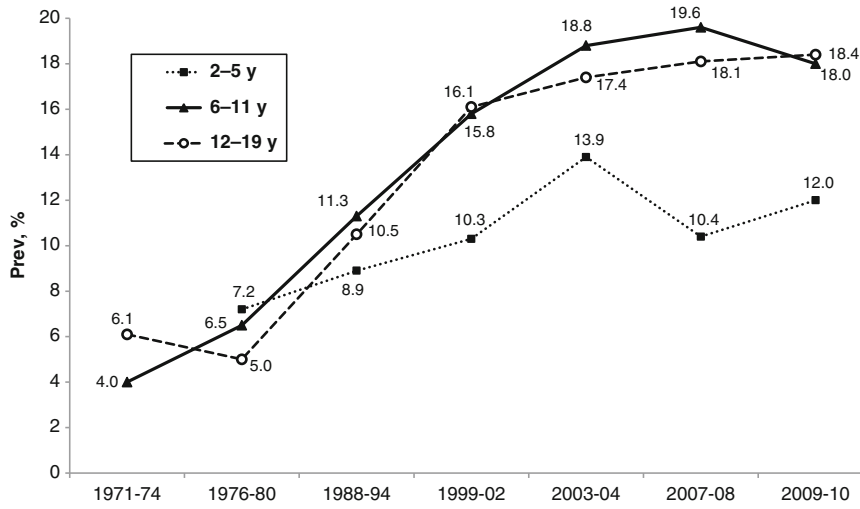

Fig. 11.2 Trends in the prevalence (%) of obesity (BMI ≥ 95th Percentile) in US children and adolescents, by age: 1971–1974 to 2009–2010. Based on US national data collected in NHANES (Wang and Lim, 2012). Reprinted, with permission, from the *International Review of Psychiatry*, 2012, 24(3), p. 176

Between NHANES II (1976–1980) and 2003–2004, the average annual rate of increase in obesity prevalence was approximately 0.5 % point in children aged 2–19. However, the NHANES data shows a decrease in the prevalence of obesity among children aged 2–5 years, from 13.9 % in 2003–2004 to 10.4 % in 2007–2008, although the prevalence in both children aged 6–11 years and adolescents was slightly increased [30]. In 2009–2010, the national prevalence of obesity (16.9 %) was similar to that in 2007–2008; and it was 12.0 %, 18.0 %, and 18.4 % in children aged 2–5, 6–11, and 12–19 years, respectively [31].

In some developing countries, the prevalence of child overweight and obesity has increased alarmingly over the past two decades, with the combined prevalence within some sub-regions and population groups being similar to that in some industrialized countries. This is especially the case in countries that are in the midst of rapid social economic transitions (e.g., China, Brazil, and Mexico). China, in particular, is illustrative of dramatic increases in obesity in children and adults [6, 32].

In China, the combined prevalence of obesity and overweight nationwide increased between 1985 and 2005. Figure 11.3 shows overall trends in prevalence based on data collected through a series of representative school-based cross-sectional survey that collected health data amongst school-age children [33]. The combined prevalence has increased approximately tenfold since 1985. The combined prevalence has risen more rapidly (from 2.8 to 19.3 %) in boys than in girls (from 2.4 to 10.8 %), and in the more developed regions and in high income groups.

Nevertheless, our understanding of the current global childhood obesity epidemic and time trend data remains limited due to the lack of up-to-date, comparable and representative data from different countries. In addition, past studies have included dissimilar study samples and used different criteria to define obesity. This makes it difficult to compare findings. Furthermore, there are large within-country differences in many countries for both the prevalence and trends [34].

Discussion

Obesity is a serious threat to global health in the twenty-first century. The prevalence of obesity and overweight in children has tripled in many countries since the 1980s, and the number of people affected is expected to continue to rise. Obesity has many short- and long-term health and financial

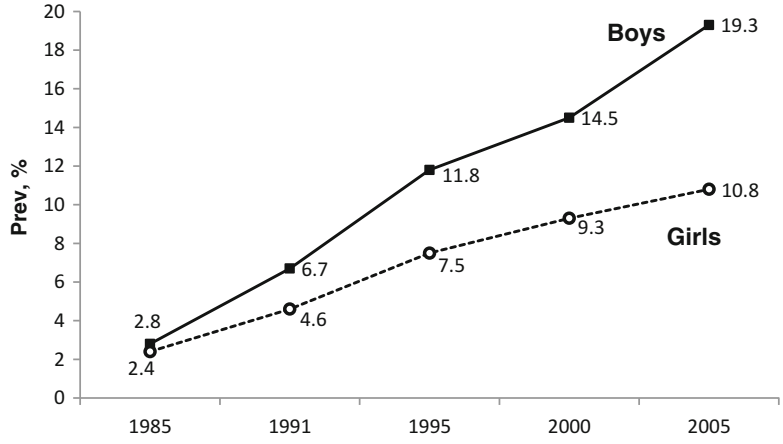

Fig. 11.3 Trends in the combined prevalence (%) of overweight and obesity in Chinese school-age children, by gender: 1985–2005. Overweight and obesity were classified based on Chinese BMI cut points; the prevalence was based on nationwide survey data (Ji and Cheng, 2009). Reprinted, with permission, from the *American Journal of Clinical Nutrition*, 2010, 92(5)

consequences for individuals, families and the society. For example, obesity is already responsible for 2–8 % of health costs and 10–13 % of deaths in parts of Europe, and it was even worse in the USA, may reach 17 % of health costs in 2030 [35]. However, various references and standards have been used over the past two decades and at present to define overweight and obesity in children, although in general most are based on age-sex-specific BMI cut points (percentile or other cut points), and they can give different estimates of the rates.

The prevalence of childhood obesity has reached a very high level in many world regions, but large variations exist across countries and population groups within countries. In general, the prevalence is much higher in developed countries than developing countries. However, overweight and obesity have been increasing dramatically in many developing countries, particularly in urban settings and among high socioeconomic status (SES) groups during recent years. For example, in 2010, the combined prevalence was 31.8 % in US children and adolescents [31], while it was <5 % in many developing countries [29]. Nevertheless, overweight and obesity rates have been increasing dramatically in many countries and population groups. In recent years, the prevalence has increased at a much faster rate in some developing countries, such as China, compared to other industrialized countries. Different from industrialized countries, in developing countries, often urban residents and those of higher SES are more likely to be overweight or obese than their counterparts.

The rising epidemic reflects the profound changes in society and in individuals' behavioral patterns during recent decades. Economic growth, modernization, urbanization and globalization of food markets are some of the forces contributing to the epidemic. The impact of global exchanges of trade, information and culture, made possible by new information technologies, on health-related behaviors such as dietary intakes are likely considerable as well, though are not yet well understood. Obesity is related to SES, however, the associations vary by gender, age, and countries. Previous studies suggest that SES groups with greatest access to energy-rich diets are likely to be at increased risk. In general, low-SES groups in industrialized countries and high-SES groups in developing countries are at higher risk of being overweight than their counterparts. For example, a recent study showed that the prevalence of overweight was higher among children from less affluent families in 21 of 24 Western and 5 of 10 Central European countries compared to children from more affluent

families. However, children from more affluent families were at higher risk of overweight in some countries (i.e., Croatia, Estonia, and Latvia. In Poland, Lithuania, Macedonia, and Finland), girls from less affluent families were more likely to be overweight while the opposite was found for boys [27]. For US children and adolescents, our research shows that the patterns of SES disparity of overweight varied across age, ethnic, and gender groups, and have changed over time [36]. Disparities have decreased since the early 1990s with the rise of the obesity epidemic, but African American children with a high SES were at increased risk. As a results of growing obesity prevalence, it is possible that the association between SES and obesity in some industrialized countries may tend to become weaker even disappear, while may change direction in some developing countries.

Conclusions

In conclusion, we are facing a growing global obesity epidemic that influence both industrialized and developing countries. The growing obesity crisis calls on timely and effective interventions. Obesity as well as the related diseases is largely preventable. The development of effective population-based programs and policies for the prevention of obesity in children should be a priority as obesity is difficult to cue once develops and has many long-term health problems, social and economic consequences. A good understanding of the scope of the childhood obesity problem will help guide intervention efforts.

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Chapter 12
Genetics of Obesity

Beatrice Dubern and Patrick Tounian

Keywords Obesity • Genetics • Monogenic • Leptin • Melanocortins • Polygenic • Epigenetics

Key Points

- Obesity results from the phenotypic expression of a genetic susceptibility under the pressure of an obesogenic environment.
- Genetic contribution is variable from severe and rare monogenic forms (<1 % to 2–3 % of cases) to more common forms of obesity with numerous genes involved, each gene having a minor contribution in determining phenotype (polygenic obesity).
- The melanocortin pathway plays a pivotal role in the control of food intake. Mutations in genes encoded proteins in this pathway (leptin, leptin receptor, proopiomelanocortin, …) are responsible for severe early-onset obesity with severe hyperphagia and endocrine anomalies such as hypogonadotrophic hypogonadism.
- The MC4R gene is also a major candidate gene for human obesity due to its pivotal role in control of food intake. More than 90 different mutations are described in approximately 2–3 % of obese children and adults.
- Lost or duplicated segments of chromosomes named copy number variants (CNVs) encompassing genes involved in weight regulation have been recently involved in human obesity.
- In polygenic obesity, 3 main approaches (candidate gene studies, genome-wide linkage and genome-wide association studies (GWAS)) have been used to identify novel gene variants with variable success since 15 years.

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- Around 20 loci consistently associated with obesity-related traits have been discovered in obese adults using GWAS. In children, the most important locus was discovered in the FTO or "fat mass and obesity-associated" gene.
- Environmental factors probably also induce a fetal programming towards obesity (undernutrition or overnutrition and maternal smoking during pregnancy, or gestational diabetes), but the putative role of postnatal factors (formula feeding rather than breastfeeding, excess in $n-6$ polyunsaturated fatty acids or protein intakes) needs to be further explored.

Introduction

Obesity is characterized by a high phenotype heterogeneity linked most notably to differences in the stages of weight evolution. However, it is now well accepted that its development stems from interaction of multiple environmental factors (abundant food availability and/or reduction in physical activity) with genetic factors. During the last decades, the increased availability of palatable food and the conditions of decreased physical activity (motorization/sedentary games) dramatically increased the prevalence of childhood obesity in industrialized countries. More recently, occurrence of this obesogenic environment in developing nations led to a rapid increase in its prevalence in urban regions, whereas a lot of children still suffer from undernutrition in rural regions of the same countries [1]. In addition, numerous epidemiological and intervention studies carried out in different cohorts (twins brought up together or separately, adopted children, nuclear families, etc.) have recognized the role of individual genetic and biological susceptibilities in response to the current weight-gain promoting environment [2].

While obesity was first thought to be a disease that obeys the rules of Mendelian inheritance, new technologies paint a far more complicated picture of this metabolic disease. The forms of obesity due to a single, naturally occurring dysfunctional gene (i.e., monogenic obesity) identified to date are both severe and rare (from <1 % to 2–3 % depending of gene). It is however likely that most of the other severe forms are also monogenic and still remain to be identified. The more common forms of obesity are polygenic, with numerous genes involved, each gene having a minor contribution in determining phenotype [3]. Recent findings suggest a more complex genetic contribution to the occurrence of obesity than previously thought up.

Syndromic Obesity

There are around 30 Mendelian disorders in which patients are clinically obese and additionally distinguished by mental retardation, dysmorphic features, and organ-specific developmental abnormalities. These syndromes arise from discrete genetic defects or chromosomal abnormalities and are both autosomal and X-linked disorders. The most common disorders known are Prader–Willi (PWS) and Bardet–Biedl (BBS) syndromes, but many others have been reported (Table 12.1) [4]. The Online Mendelian Inheritance in Man database provides access to their clinical descriptions (OMIM; http://www.ncbi.nlm.nih.gov/omim/).

The PWS is one of most common genetic diseases linked to obesity (1 in 20,000–25,000 births). The PWS is characterized by hypotonia at birth, hyperphagia and severe food impulsivity, behavioral problems, dysmorphic features, mental retardation, progressive obesity, and hypogonadism. It is due to physical (microdeletion) or functional (uniparental maternal disomy) absence of the paternal 15q11.2-q12 chromosomal segment [5, 6]. At least three genes in this region have been recognized and encode different proteins which functions are not fully understood. It is believed that several

Table 12.1 Syndromic obesities [10]

	Obesity associated phenotypes	Genetics
Prader–Willi	Neonatal hypotonia Hyperphagia, food impulsivity Dysmorphy; height retardation Hypogonadism Mental retardation	Physical (microdeletion) or functional (uniparental maternal disomy) absence of the paternal region 15q11.2-q12
Bardet–Biedl	Retinal dystrophy Hexadactyly; mental retardation Hypogonadism; renal abnormalities	BBS1 (11q13); BBS2 (16q21); BBS3 (3p12-13); BBS4 (15q22); BBS5 (2q31); BBS6 (MKKS); BBS7 (4q27); BBS8 (TTC8); BBS9 (7p14); BBS10 (12q); BBS 11 (TRIM32); BBS 12 to 18
Cohen	Retinal dystrophy Dysmorphy, mental retardation Neutropenia	Autosomal recessive Gene COH1 (chr 8q22-q23)
Alström	Retinal dystrophy Deafness Renal abnormalities	Autosomal recessive Gene ALMS1 (chr 2p13-p14)
Borjeson-Forssman-Lehmann	Severe mental retardation, hypotonia, microcephaly, facial dysmorphy, hypogenitalism	X-linked Gene PHF6 (Xq26-q27)
Albright hereditary osteodystrophy	Facial dysmorphy; brachymetacarpy; variable mental retardation; resistance to hormones	Autosomal dominant Mutations in the GNAS1 gene encoding for the alpha subunit of the Gs protein

genes are affected explaining the heterogeneity of phenotype. The genes implicated are for example small proteins ribosomes, zinc finger proteins involved in gene transcription (ZNF127AS, par5, PARSN, IPW, and PAR1) and necdine involved in cell growth. The genetic basis of hyperphagia remains undefined. However, the hormone ghrelin may be implicated [7], via its regulation of hunger and stimulating growth factor hormone (GH) secretion [6].

The BBS is a rare autosomal recessive disease (1 in 100,000 births, with an increased prevalence in Arab and Bedouin populations with 1 in 13,500 births). It is characterized by obesity associated to retinal dystrophy, polydactyly, renal abnormalities, hypogonadism, and sometimes learning disabilities [8]. The genetic approach in affected families revealed that at least 12 different genes are involved, but they are all related to primary cilia function introducing the concept of ciliopathy disease [9]. These cilia are involved in mammalian development and used to transmit signaling messages from the outside to the inside of the cell. Alström syndrome has clinical similarities with BBS and is now recognized also as a ciliopathy [6, 9].

Albright hereditary osteodystrophy, also known as the type 1A pseudohypoparathyroidism is another example with autosomal dominant transmission by the mother. It is characterized by short stature, obesity, brachydactyly, skull and face malformations, resistance to various hormones including resistance to parathyroid hormone, and sometimes developmental abnormalities [10]. Mutations in the GNAS1 gene encoding for the alpha subunit of the Gs protein are identified and some of them may be involved in the hypothalamic circuits controlling energy balance. Cohen syndrome is characterized by late-onset obesity (after the age of 10 years) associated to moderate mental retardation, typical craniofacial features, progressive pigmentary retinopathy, severe early-onset myopia, and intermittent neutropenia [10]. It is an autosomal recessive disorder with variable clinical manifestations. Mutations in the gene COH1 (chromosome 8q22) encoding a transmembrane protein presumably involved in intracellular protein transport, are described [11, 12].

These examples highlight the need for complementary studies in affected families in order to characterize the genes responsible for these rare diseases. Although some of them have been identified, it

is necessary to describe the pathophysiological links between the protein products and the development of diseases with multiple clinical features. Finally, identification of such genes may help to recognize novel candidate genes for common obesity.

Recessive forms of Monogenic Obesity

Since the last 15 years, significant success has been derived from studies of candidate genes implicated in rodent models of monogenic obesity. Those studies based on genes encoded proteins known to cause severe obesity in rodents have shown that they also contribute to human early-onset obesity especially for those involved in the leptin pathway. This hypothalamic pathway is activated following the systemic release of the adipokine leptin (LEP) and its subsequent interaction with the leptin receptor (LEPR) located on the surface of neurons of the arcuate nucleus region of the hypothalamus (Fig. 12.1). The downstream signals that regulate satiety and energy

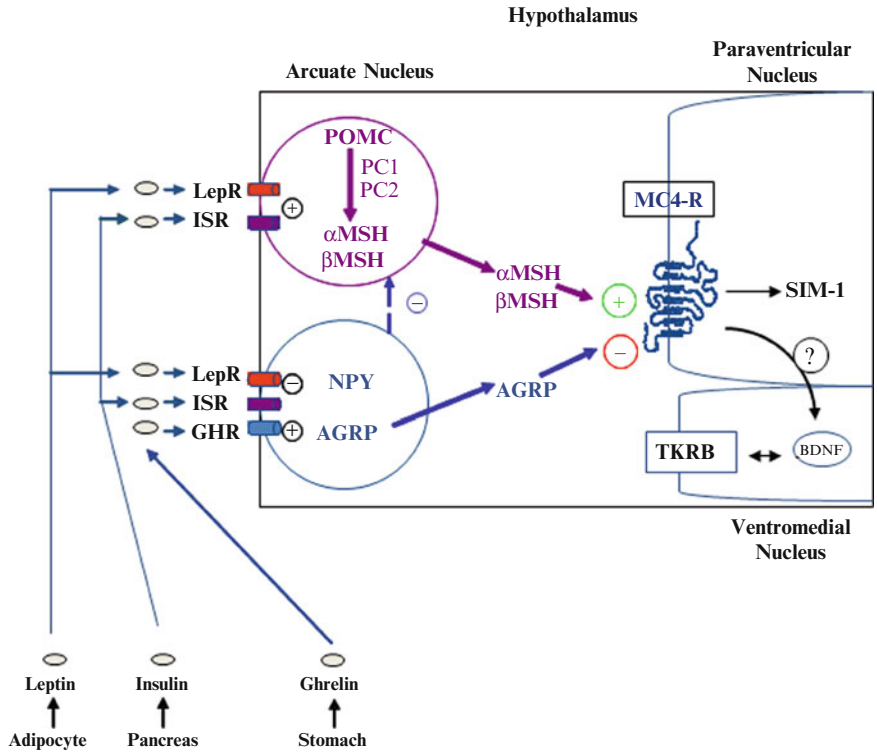

Fig. 12.1 The leptin/melanocortin pathway. Neuronal populations propagate the signaling of various molecules (leptin, insulin, ghrelin) to control food intake and satiety. POMC-neurons in the arcuate nucleus are activated by leptin and insulin and produce the α-melanocyte stimulating hormone (α-MSH), which then activates the MC4R receptor in the paraventricular nucleus resulting in a satiety signal. The downstream roles of SIM1, BDNF, and TKRB are currently being explored. A separate group of neurons expressing NPY and AGRP produce molecules that act as potent inhibitors of MC4R signaling. Several mutations of those genes involved in the leptin/melanocortin pathway are responsible for early-onset and severe obesity. *POMC* proopiomelanocortin, *LepR* leptin receptor, *ISR* insulin receptor, *GHR* ghrelin receptor, *NPY* neuropeptide Y, *AGRP* agouti-related protein, *SIM1* single-minded 1, *BDNF* brain-derived neurotropic factor, *TRKB* tyrosine kinase receptor, *PC1 and 2* proconvertase 1 and 2

homeostasis are then propagated via proopiomelanocortin (POMC), cocaine-and-amphetamine-related transcript (CART), and the melanocortin system [13]. While POMC/CART neurons synthesize the anorectic peptide α-melanocyte stimulating hormone (α-MSH), a separate group of neurons express the orexigenic neuropeptide Y (NPY) and the agouti-related protein (AGRP), which acts as a potent inhibitor of melanocortin 3 (MC3R) and melanocortin 4 (MC4R) receptors. Mutations in human genes coding for LEP [14–17] and LEPR [18–20] lead to severe early-onset obesity (Table 12.2) with a rapid and dramatic increase in weight soon after birth.

Congenital Leptin Deficiency

Close to *ob/ob* mice [21], less than 20 individuals carrying a mutation in the LEP gene with undetectable serum leptin levels (<1 ng/ml) have been identified since 1997 [14–17, 22–24].

Almost all patients are characterized by severe early-onset obesity with severe hyperphagia and endocrine abnormalities such as hypogonadotrophic hypogonadism. Especially LEP mutation carrier adults failed to undergo pubertal development but showed plasma cortisol levels in the normal range in contrast to *ob/ob* mice [24]. Concerning body weight, BMI is higher than 40 kg/m^2 with extremely high fat mass (more than 50 % body fat) and relatively normal resting energy expenditure. Feeding behavior is characterized by major hyperphagia and ravenous hunger [25]. Surprisingly, an Austrian girl has been recently described with less severe obesity (BMI 31.5 kg/m^2, Zscore BMI 2.46 SD), extremely low energy intake in everyday life despite an increased consumption of calories in a test meal [17]. Even if one takes into account a substantial underreporting, this observation might suggest that despite leptin deficiency, it is possible to control energy intake and thus to prevent extreme obesity especially in case of parents provided a favorable environment by controlling the patient's eating behavior vigorously from early infancy onward [26]. A further explanation might be related to the different genetic backgrounds of different subjects with LEP deficiency. Despite this specific case, severe early-onset obesity with major hyperphagia is still recognized as the main clinical presentation of LEP deficiency and justifies measurement of circulating leptin [22, 25]. High rate of infection is also described with deficiency in T cell number and function suggesting the implication of leptin in the immune system [27, 28], as abnormalities of sympathetic nerve function due to defects in the efferent sympathetic limb of thermogenesis [24].

LEP deficiency is a unique situation of extreme obesity where a therapeutic option is available. Indeed, leptin deficient children and adults benefit from subcutaneous daily injection of leptin, resulting in weight loss, mainly of fat mass, with a major effect on reducing food intake and on other dysfunctions including immunity as described previously [22]. After leptin therapy, the detailed microanalysis of eating behavior of three leptin-deficient adults before and after leptin treatment, revealed reduced overall food consumption, slower rate of eating and diminished duration of eating of every meal in the three subjects. Leptin treatment is also able to induce features of puberty even in adults [25].

Leptin Receptor-Related Monogenic Obesity

In case of LEPR deficiency (*db/db* mice, fatty Zucker and Koletsky rat models), the phenotype is very close to that of *ob/ob* mice but unresponsive to endogenous or exogenous leptin [29]. In humans, three obese subjects homozygous for a mutation generating a LEPR lacking both transmembrane and intracellular domains were firstly reported [18]. The mutant receptor circulates at high concentrations, binding leptin and resulting in very elevated serum leptin levels. Phenotypic similarities with the LEP-deficient subjects were noticed. Especially, LEPR deficient subjects exhibited rapid weight gain in the

Table 12.2 Rare monogenic forms of human obesity

Gene (references)	Mutation type	Obesity	Associated phenotypes
Leptin [14–17]	Homozygous mutation	Severe, from the first days of life	Gonadotropic and thyrotropic insufficiency
Leptin receptor [18–20]	Homozygous Mutation	Severe, from the first days of life	Transient gonadotropic, thyrotropic, and somatotropic insufficiency
Proopiomelanocortin (POMC) [33, 107]	Homozygous or compound heterozygous	Severe, from the first month of life	ACTH insufficiency Mild hypothyroid and ginger hairs if the mutation leads to the absence of POMC production
POMC but in the α-MSH coding region [36, 108, 109]	Heterozygous non synonymous mutations	Severe obesity occurring in childhood	Rapid size growth
Single-minded 1 (SIM1) [39]	Translocation between chr 1p22.1 and 6q16.2 in the SIM 1 gene	Severe obesity occurring in childhood	–
Neurotrophic tyrosine kinase receptor type 2 (NTRK2) [42]	De novo heterozygous mutation	Severe from the first months of life	Developmental delay Behavioral disturbance Blunted response to pain
Melanocortin 4 receptor (MC4R) [44–46]	Homozygous mutation	Severe from the first months of life	Rapid size growth

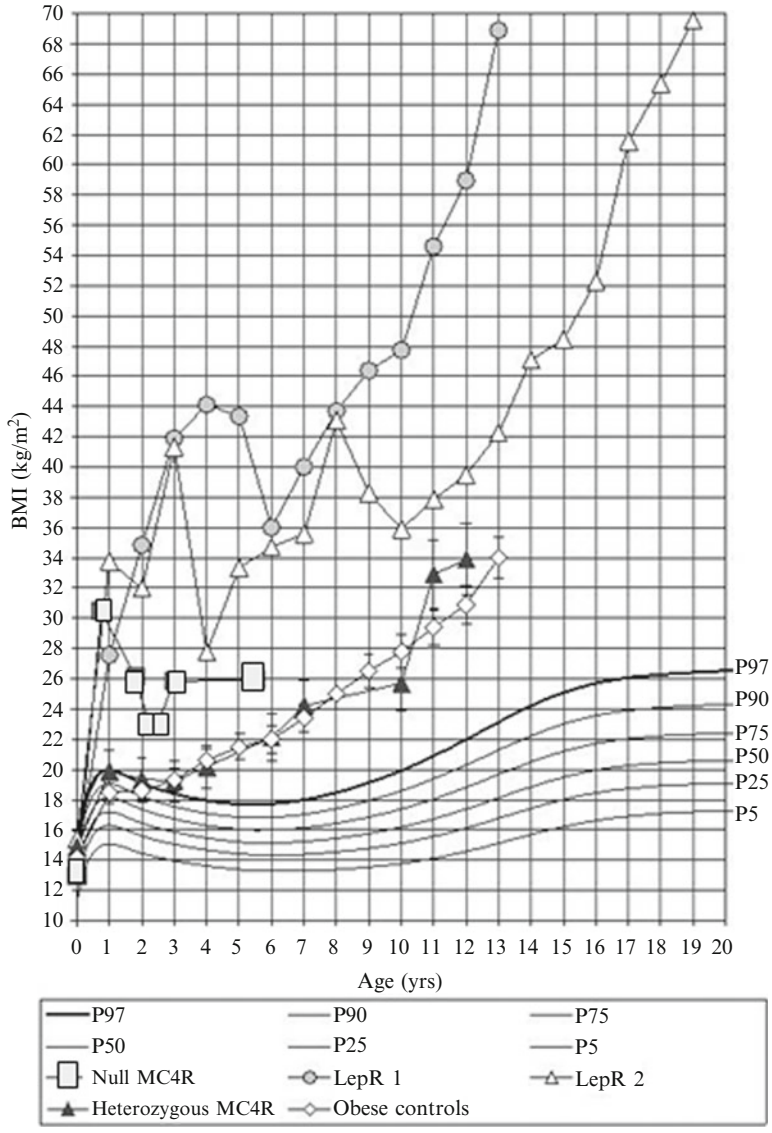

Fig. 12.2 BMI curves of 2 homozygous null LEPR mutants (LEPR 1 and 2), 1 homozygous null MC4R patient, 6 heterozygous MC4R carriers, and 40 non-mutated obese controls. The reference curves are the standard French/Institut National de la Santé et de la Recherche Médicale percentile curves

first few months of life (Fig. 12.2) with large amount of total body fat mass (>50 %) and resting energy expenditure related to the level of corpulence, severe hyperphagia associated to aggressive behavior when denied food and endocrine abnormalities (hypogonadotrophic hypogonadism, insufficient somatotrophic or thyreotropic secretions). Surprisingly, there is evidence of spontaneous pubertal development in 2 individuals with this LEPR mutation, (K Clément, unpublished observation). The follow-up revealed also the normalization of thyroid mild dysfunction at adult age (K Clément, unpublished observation). At adulthood, one LEPR deficient patient was able to give birth to a healthy boy at the age of 26 years. Her total weight gain was +50 kg through pregnancy, with a progression of BMI from 68.6 to 87.6 kg/m^2. She did not develop gestational diabetes or pregnancy-induced complications. She delivered by elective cesarean section under epidural anesthesia for breech presentation

and suspected macrosomia. The neonate anthropometry was in the normal range (birth weight 3,720 g, birth length 50 cm, and head circumference 36.5 cm). Thus despite major weight gain, no major clinical or metabolic complication occurred in this LEPR-deficient patient during pregnancy, emphasizing the need for reproductive counseling for these women. Importantly, the observation of a natural pregnancy in a LEPR deficient woman raises questions about the role of leptin in human reproductive functions [30].

Since this first description, 5 unrelated subjects from the Reunion French Island (homozygous deletion of exons 6, 7, and 8) and two Egyptian cousins (homozygous P316T mutation) were diagnosed with a close clinical presentation as described in LEPR deficient subjects (B Dubern, J Le Bihan and K Clement, unpublished observations) [20]. Even if LEPR mutations seem to be rare, up to 3 % of patients with severe obesity have also been found to harbor loss-of function mutations in the LEPR gene [19]. Affected subjects were characterized by hyperphagia, severe obesity, alterations in immune function, and delayed puberty due to hypogonadotropic hypogonadism. Serum leptin levels were within the range predicted by the elevated fat mass in these subjects. Their clinical features were less severe than those of subjects with congenital leptin deficiency or the first family described with LEPR mutation [19].

Because of the nonfunctional LEPR, leptin treatment is useless in these subjects and factors that could possibly bypass normal leptin delivery systems are not yet currently available (ex: ciliary neurotrophic factor activating downstream signaling molecules such as STAT-3 in the hypothalamus area) [31, 32].

Others Cases of Monogenic Obesity Downstream Leptin Pathway

Mutations of genes located downstream of the leptin pathway are also responsible for monogenic obesity with similarities with LEP and LEPR mutations carriers. Especially, endocrine abnormalities are always present. Obese children with complete POMC deficiency have ACTH deficiency which can lead to acute adrenal insufficiency from birth. These children display a mild central hypothyroidism that necessitates hormonal replacement [33]. Children have ginger hair due to the absence of αMSH, which activates the peripheral melanocortin receptor type 1 (involved in pigmentation). Several observations suggest that the skin and hair phenotype might vary according to the ethnic origin of POMC mutation carriers [34–36]. Carriers of a PC1 mutation have, in addition to severe obesity, postprandial hypoglycemic malaises explained by the accumulation of proinsulin through lack of PC. Severe diarrhea is also described maybe due to alterated processing of digestive prohormones derived from entero-endocrine cells [37].

Haploinsuffficiency for brain-derived neurotrophic factor (BDNF), single-minded homolog 1 (SIM1) and its receptor TrkB coded by the neurotrophic tyrosine kinase type 2 gene (NTRK2) has been associated with severe hyperphagic obesity, accompanied by syndromic features in humans [38]. Implication of single-minded 1 (SIM1) gene was identified in a girl with early-onset obesity and a *de novo* chromosomal translocation [39]. Her early weight gain was comparable to LEP and LEPR-deficient children. SIM1 is a transcription factor playing a major role in neuronal differentiation of the paraventricular nucleus of the hypothalamus [40, 41]. A de novo heterozygous mutation in NTRK2 gene was also described in a 8 year old boy with early-onset obesity and a mental retardation, developmental delay and anomalies of higher neurological functions like the impairment of early memory, learning, and nociception [42]. In vitro studies of some but not all mutations have suggested that they could impair hypothalamic signaling processes [43]. Considering the pivotal role of the melanocortin pathway in the control of food intake, mutations in the MC4R gene located downstream the leptin pathway are also responsible for severe early-onset obesity in case of rare homozygous mutations [44–47]. However, in contrast to the alterations in leptin pathway described above, no endocrine abnormalities have been described in these patients.

Partial Gene Deficiency and Obesity

The MC4R gene is considered as a major candidate gene for human obesity due to the pivotal role in control of food intake (Fig. 12.1). Since 1998, its genetic evaluation revealed that MC4R-linked obesity is the most prevalent monogenic form of obesity identified to date. Its represents approximately 2–3 % of childhood and adult obesity with more than 90 different mutations described in different populations (European, North American and Asian) [47, 48]. They include frameshift, in-frame deletion, nonsense and missense mutations located throughout the MC4R gene. The frequency of such heterozygous carriers in nonobese controls or in the general population is about 10 fold lower than in obese patients [49, 50].

In contrast with the rare monogenic obesities, even a meticulous clinical analysis does not easily detect obesity stemming from MC4R mutations because of the lack of additional specific phenotypes. In families with MC4R-linked obesity, obesity tends to have an autosomal dominant mode of transmission, but the penetrance of the disease can be incomplete and the clinical expression variable underlying the role of the environment and of other potentially modulating genetic factors [49, 50]. In contrast with homozygous null MC4R mutations, obesity onset and severity are variable in heterozygous MC4R mutation carriers and are related to the severity of the functional alteration (abnormal MC4R membrane expression, defect to the agonist response and disruption in the intracellular transport of the protein [48–54]). It is accepted that MC4R mutations are responsible for haploinsufficiency rather than a dominant negative activity. While the roles of homo and hetero-dimerization in G protein synthesis and maturation are emphasized, some dominant negative effects of MC4R mutations might not be excluded.

Authors agree on that MC4R mutations facilitate early-onset obesity. In addition, MC4R mutations carriers display increased linear growth, in particular in the first 5 years of life [55] but do not appear to be taller as adults [49, 51]. Assessment of body composition in these patients demonstrates increase in both fat and lean mass [56]. One study performed in English children has suggested increased bone mineral density and size [55] partly due to decreased bone resorption [57, 58]. Obese children carrying MC4R mutations have a marked hyperphagia that decreases with age when compared to their siblings [56]. In both children and adults, no evidence has been found for decreased metabolic rate, increased frequency of "binge eating" disorder [49, 50, 59], diabetes or other obesity complications [45, 46, 49, 55, 60]. Finally, hypothalamo-pituitary axis and reproductive axis as well as thyroid function are normal [45, 46, 51, 55]. Finally, obesity related to heterozygous MC4R mutations can be placed between the exceptional forms of monogenic obesity with complete penetrance and the polygenic forms of common obesity.

MC3R, another receptor activated with POMC-derived peptides, has an important complementary role in the regulation of energy homeostasis next to MC4R. Recently, several rare mutations with functional alterations have been described to be associated with severe obesity in children [61, 62]. However, further epidemiological and functional research regarding the importance of MC3R mutations are necessary in order to confirm the importance of MC3R mutants and their potential combined effects with other genes in severe early-onset obesity [63].

Genome Structural Variations

Recently, advances in genome-scanning technologies has led to discover that genetic differences among people can derive from lost or duplicated segments of chromosomes named copy number variants (CNVs) [64]. Rare deletions in the region p11.2 of the chromosome 16 have been reported in about 0.5 % of severe obese individuals with a link between these deletions and obesity [65–69]. The 16p11.2 deletion encompasses about 30 genes including the SH2B adapter protein 1 (SH2B1) which

is known to be involved in leptin and insulin signaling. SH2B1 knock-out mice develop hyperphagia and obesity [70]. In addition, the SH2B1 locus was recently associated with common obesity by genome-wide association studies (GWAS) [71, 72].

A recent study identified 17 rare CNV loci only found in obese but not in lean children of European ancestry. Eight of them were also found in obese children of African ancestry, but not in lean control subjects [73]. Finally, rare CNVs > 2 Mb have been described to be present in 1.3 % of obese subjects but absent in lean controls [68]. Several CNVs disrupt known candidate genes for obesity, such as NAP1L5, UCP1, and IL15 [68] offering novel insights into the genetic architecture of obesity.

Polygenic Forms of Obesity

The common obesity is much more complex than monogenic and syndromic forms described above. Three main approaches have been used to identify novel gene variants associated with polygenic obesity with variable success: candidate gene studies, genome-wide linkage and genome-wide association studies (GWAS).

Candidate Gene Studies

The choice of a candidate gene is based on several factors including the physiological role of its product, its chromosomal location in one obesity linked region (called regions or QTL Quantitative Trait Loci) and the consequences of its invalidation or overexpression (transgenic) in rodent or the in vitro functional consequences of mutations or changes in DNA. To date, several hundred of candidate genes for obesity have been selected for association studies [74]. Polymorphisms in genes known to code for proteins involved in the regulation of energy balance in animal models or human monogenic obesity were tested for association with obesity related traits at the population level. Among these genes, polymorphisms in LEP and LEPR as well as different variants known to play a role in feeding behavior (PCSK1, POMC, or BDNF), neural signaling, e.g., cannabinoid receptor (CNR1), dopamine receptor (DRD2), or serotonin receptor (2C HTR2C), or function (SLC6A4) have been shown to be associated with obesity or predictive of BMI [74].

Interestingly, rare polymorphisms in MC4R have been associated with a protective effect against obesity. Two gain-of-function MC4R polymorphisms (I251L and V103I) have been negatively associated with obesity [75, 76]. In a meta-analysis including 39,879 European subjects, the V103I variant was associated with a 20 % lower risk for obesity as in another meta-analysis of 3,526 individuals from East Asia with a 31 % lower risk of obesity [76, 77]. The I251L was also associated with a 50 % lower risk for obesity in a meta-analysis of 11,435 European subjects [76].

Candidate gene studies have provided suggestive evidence that multiple genes are involved in the predisposition to obesity. However, these genes were supported by data from underpowered studies; thus, replication in large-scale studies and meta-analytical approaches could not conclude a definitive effect for most of the variants cited previously [78].

Genome-Wide Linkage Studies

Genome-wide linkage scans consists to genotype families recruited for the high recurrence of a disease using highly polymorphic microsatellite markers that are regularly located across the whole genome, followed by a calculation of the degree of linkage of the marker to a disease trait.

This approach led to the successful identification of >1,200 genes involved in human disease, but its application in complex genetic traits as obesity has been more controversial [79].

In obesity, Comuzzie et al. performed one of the first genome-wide linkage scans [80] using multipoint linkage analysis in a population of Mexican Americans. They identified a region located on chromosome 2p21 associated with circulating leptin levels and that contains the POMC gene. One year later, Hager et al. reported another region strongly associated with obesity on chromosome 10p in French families [81]. Then, several teams reported multiple loci associated with obesity or related phenotypes in different populations (Pima Indians, Finnish subjects, African Americans, …) [82–84]. Promising candidate genes were identified using this approach, such as GAD2 (glutamate decarboxylase 2) at the origin of the γ-aminobutyric acid that upregulates food intake, ENPP1 (ectonucleotide pyrophosphatase/phosphodiesterase 1), which plays a part in brain insulin sensing, and SLC6A14 (solute carrier family 6 membrane 14), a tryptophan transporter implicated in appetite regulation [78].

However, if more than 80 linkage studies for obesity-related traits have been reported, the regions associated with obesity were not systematically confirmed in independent populations and most of the genes explaining these associations had not been identified. In addition, a meta-analysis of 37 published studies including more than 31,000 individuals was unable to confirm a major locus for obesity [85]. Two possible explanations are possible for these findings: (a) genes influencing adiposity have a very small effect with substantial genetic heterogeneity and variable dependence on environmental factors; (b) rare variants with high disease penetrance explain the linkage peaks [34, 86].

Genome-Wide Association Studies

GWAS have revolutionized the field of genetic epidemiology since they screen the whole genome at a very high resolution (hundreds of thousands of genetic variants). Thus, they make it possible to identify precisely genetic variants robustly linked to obesity while they have relatively small effect sizes. So far, around 20 loci consistently associated with obesity-related traits have been discovered in adults [87]. In children, the most important locus was discovered in the FTO or "fat mass and obesity-associated" gene located on chromosome 16q12.2 [88] with a strong association between its variant rs9939609 and BMI. Indeed, children (and adults) carrying two copies (16 % of the sample) of this allele had approximately 1.67 more risk to be obese than noncarriers. Four large GWAS meta-analyses in general populations of European descent confirmed this strong association with BMI and identified 35 additional SNPs in 33 loci robustly associated with BMI. Multiple independent association signals were also reported at the FTO, MC4R, and BDNF loci [71, 89]. Altogether these loci explained only 1.45 % of the variance in BMI suggesting that many additional common genetic variants associated with BMI remain to be discovered [89].

In conclusion, extensive genetic studies conducted over the past 15 years in large populations of obese and lean populations showed that (1) there are multiple genetic factors that contribute to common obesity in close interaction with environment, (2) the search for genomic regions (or loci) associated to obesity was somewhat disappointing since the role of only 20 genes was confirmed in different populations with generally very low effects on phenotypes, (3) each allele may be associated with a different phenotype due to ethnic or environmental factors (type of food consumption, degree of physical activity, hormonal conditions, gender, medications, etc.), (4) the use of these polymorphisms alone or in combination in order to predict the evolution or the risk of obesity is almost impossible. So the refining of methodological approaches, the use of large-scale cohorts with standardized phenotypes, and the development of more accurate specific statistical tools will certainly enlighten the involvement of unsuspected loci and determine the role of those already identified, especially in terms of gene–gene interactions. Moreover, identifying the respective contribution of genetic predisposition and lifestyle in the determination of individual weight is definitely the task to focus on.

The Role of Epigenetics

Since recently, some observations suggest that fetal and early postnatal environment may play a role in programming towards overweight or obesity later in life (Table 12.3). These early determinants represent constitutive factors which are not inscribed in the genetic patrimony and, generally, not transmitted across generations. Most of them are certainly still to be discovered. Classically three types of mechanisms are discussed: (a) programming of the endocrine system and hormones—glucocorticoids in particular—and their effect on the hypothalamic-pituitary axis, (b) programming related to the oxygen supply which is associated to the development of cardiovascular system and (c) nutritional programming when caloric restriction and protein deprivation lead to a reduction in placental and umbilical flow, causing a real famine in the fetus. For example, studies of individuals exposed to famine in utero during World War II show that mothers who suffered famine periconceptually and in the first trimester of pregnancy gave birth to children with a normal weight at birth but exhibited increased risk of later obesity [90]. Conversely, in individuals whose mothers were exposed to famine during the last trimester of pregnancy the risk of obesity was absent [90]. The proposed mechanisms underlying this relationship are a dysfunction in the hypothalamic nuclei that control energy balance. It is hypothesized that undernutrition during central nervous system development generates a process favoring a better metabolic efficiency to compensate for the energetic deficit [91]. This acquired energy-sparing system would lead to an excessive fat storage when enough food is available. The absence of an effect when mothers are deprived of food only during the last trimester could be explained by the fact that the development of the central body weight regulatory system was achieved when undernutrition occurred. Prepregnancy obesity in mothers and gestational weight gain are also positively associated with obesity in offspring from childhood to adulthood [92]. This is likely to primarily reflect the genetic effect on obesity. However, maternal weight loss through bariatric surgery prevents transmission of obesity to children compared with the offspring of mothers who did not

Table 12.3 Early programming in childhood obesity: summary of the possible constitutive factors

Factors	Relevance to childhood obesity risk/etiology
Fetal programming	
Undernutrition or overnutrition	Nutritional state of the mother may alter the methylation of genes, resulting in effects on fetal/offspring development in later life [94]
	Effects of exposure to undernutrition seen only in the first trimester of pregnancy [90]
	Maternal weight loss via bariatric surgery prevented transmission of obesity [93]
Smoking	Mechanisms not yet well characterized
	Data suggest children of mothers who smoked during pregnancy are at elevated risk for becoming overweight/obese [110]
Gestational diabetes	Several confounding factors exist (i.e., trait of familial predisposition)
	Associated with a small increase in the rate of childhood obesity risk [95, 96]
	Possible metabolic imprinting through exposure to maternal hyperglycemia, altering the development of regulatory centers in the brain [97]
Early postnatal programming	
Elevated weight at birth	>4 kg may be associated with an increased risk; however, confounding factors exist owing to maternal characteristics
Rapid weight gain	Not an issue in small-for-gestational-age neonates: genetic markers suggest rapid early infancy weight gain only represents a phenotypic expression and not an etiology [111]
	Elevated risk for later cardiovascular diseases or metabolic syndrome is more likely [112]
Feeding practices	Studies demonstrating the putative protective effects of breast feeding against later obesity may have inherent bias or confounding factors [101]
	The role of excess intake of $n-6$ polyunsaturated fatty acids in lactating mothers [113] and the possible link between high protein intake in infancy and risk of later obesity [104, 105, 114] are inconclusive to date

undergo the surgery and remained obese [93]. The mechanism underlying the relationship between the nutrition state of the mother and the predisposition to obesity in children still obviously needs to be elucidated. Epigenetic factors may be suggested since maternal intake of nutrients has been shown to alter the methylation of genes resulting in effects on fetal and offspring development [94]. Gestational diabetes has been also associated with an increased rate of offspring childhood obesity [95], although the risks seem to be small [96]. Confounding factors like maternal obesity or increased insulin resistance susceptibility which favor the occurrence of both gestational diabetes and familial obesity may explain this association, all the more so as the relationship is statistically small. Gestational diabetes would therefore be a trait of the familial predisposition to obesity and not the cause of offspring overweight. It is, however, also possible that fetal exposure to maternal hyperglycemia results in fetal hyperinsulinemia, which in turn may alter the development of the body weight regulation center in the brain. Further obesity in offspring would therefore be the consequence of metabolic imprinting [97]. The role of several others postnatal factors are also discussed such as elevated birth weight [98], rapid weight gain early in life [99, 100], formal feeding [101], excess in $n-6$ polyunsaturated fatty acid [102, 103], or protein intake [104, 105]. They however seem to be more questionable than fetal programming and need further investigation.

The question is what are the mechanisms involved in the fetal programming. Since recently, an epigenetic origin is discussed [94]. Epigenetics is the modification of gene expression inherited by mechanisms of footprint but without changes in DNA sequence. It concerns particularly DNA methylation of cytosine bases in CpG islands located in the genome and implicated in gene expression. Methylation plays a key-role in the DNA modifications which help to make DNA accessible to factors involved in the gene expression regulation. For example, undernutrition during the first months of gestation generates an epigenetic modification (methylation) probably favoring a better metabolic efficiency to compensate for the energetic deficit [91].

In conclusion, some environmental factors seem to clearly induce a fetal programming towards obesity (undernutrition or overnutrition and maternal smoking during pregnancy, or gestational diabetes), but the putative role of postnatal factors in early obesity programming (formula feeding rather than breastfeeding, excess in $n-6$ polyunsaturated fatty acids or protein intakes) needs to be further explored. In any case, genetic programming towards childhood obesity appears to be much more relevant than early fetal or postnatal programming. It can, however, be assumed that early environmental factors may accelerate the phenotypic expression of a genetic predisposition, explaining in part why children seem to become obese earlier in their lifetime than their parents [106].

What Lessons for the Management of Common Obesity?

The main objective of genetic approach is to identify the pathophysiological pathways contributing to obesity in order to develop appropriate therapeutic targets and eventually predictive genetics. However, before considering this practice, it is necessary to answer to several questions.

Firstly, are we able to define the predictive risk of obesity in a subject carrying a mutation in a particular gene? While genetic prediction for monogenic forms is very high and can be calculated, the calculated predictive risk from large populations is extremely low and difficult to assess in a given individual with common obesity. Broadband approaches mentioned above reinforce this idea of a small risk brought by genetic polymorphisms and actually raises the question of the discrepancy between calculated risks in genetic epidemiology (e.g., heritability) and those calculated from concrete identification of susceptibility polymorphisms in populations. This gap is not currently explained. So researchers need to be careful not to confuse the probabilistic risk with a predictor risk.

Secondly, are we able to treat or prevent obesity in patients with genetic predisposition even in risk high individual? Until now, the usual management of obesity is based on nutrition, increased physical

activity, psychological supports and sometimes behavioral and social support. Excessive pressure—which could be exercised on diagnosed patients -may lead inexorably to weight gain. It will therefore be especially careful in the preventive management of obesity in targeted populations. For example, in families of patients with MC4R mutations, the frequency of mutations and the relative risk of developing obesity is not negligible (probably around 5–10), which could consider systematical screening of the MC4R gene. Indeed, MC4R agonists are being developed by industry and could be considered as therapeutic in patients with decreased melanocortinergic activity. However, as described above, the expression of obesity in subjects with MC4R mutations is variable (development of a more or less severe obesity) and penetrance is incomplete (the presence of the mutation does not lead always to obesity). In addition, the MC4R mutations have various functional consequences.

Conclusions

Considerable evidence is now available to support the existence of a programming towards childhood obesity, but the underlying mechanisms still remain to be explored. Clearly, the role of genetics dominates, but more research should be conducted to identify the genes involved. Fetal and perhaps early postnatal programming is also possible, but many studies are still required to confirm their contribution in obesity. If innovative methodologies and technologies lead to optimism, it seems that we are only at "the end of the beginning" of the search for genetic variants predisposing to obesity.

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Chapter 13
Epigenetics of Obesity

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Keywords DNA methylation • Epigenetics • Histone modifications • Hyperglycemia • Diabetes mellitus • Obesity • Metabolic syndrome • Inflammation

Key Points

- Epigenetic mechanisms contribute to obesity.
- Nutrients are major modulators of epigenetic plasticity.
- Early life events influence obesity risk through epigenetics.
- Epigenetic modulation of imprinted genes affects obesity.
- There are many challenges in understanding the role of epigenetics in obesity.

Introduction

Obesity is now considered a key concern for public health issues due to the increasing prevalence worldwide, especially in Western countries [83].

Besides dietary habits, genetic marks and many other acquired or predisposing factors, epigenetic mechanisms lately emerged as possible important players in the development of several diseases including obesity and, more in general, weight control. Epigenetics is indeed fundamental in regulating the way by which the different environmental/nutritional exposure brings each individual to develop a different phenotype [21].

Epigenetics refers to the branch of molecular biology pertaining to the study of features of chromatin and nucleic acids modifications that control gene expression without structural changes in the DNA sequence [2]. Epigenetics refers more specifically to DNA methylation, histone modifications

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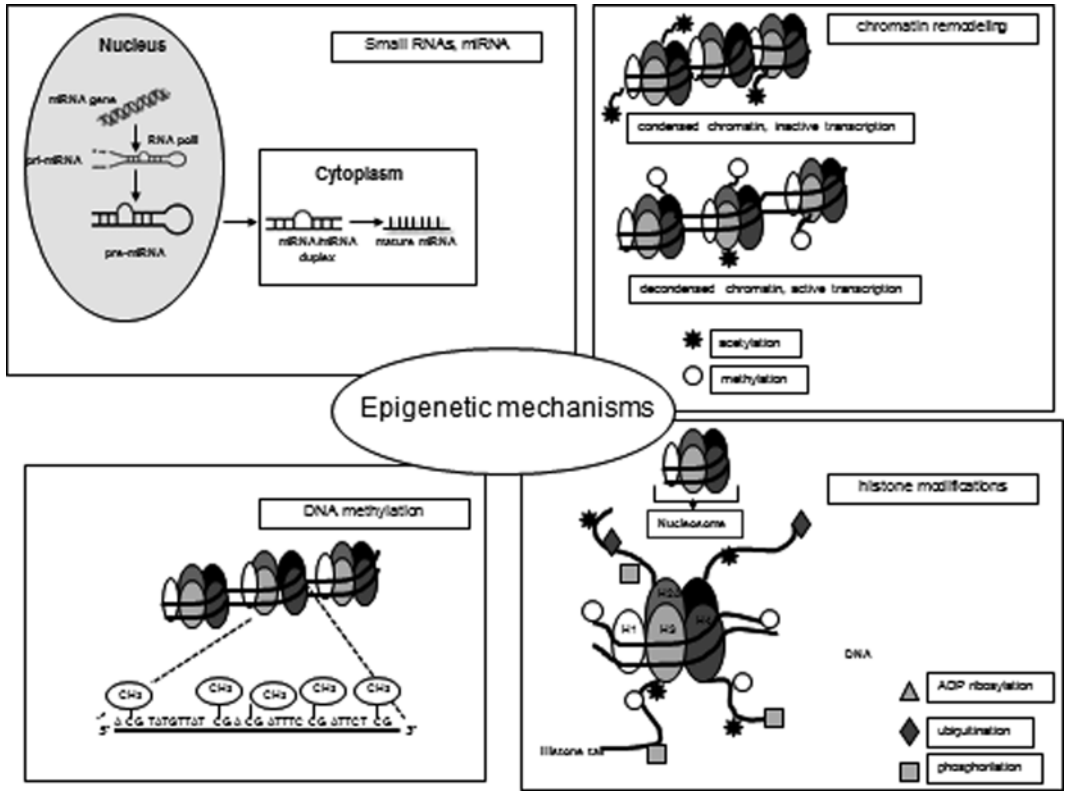

Fig. 13.1 Epigenetic mechanisms

and chromatin remodeling mechanisms controlling the expression of genes although its role has been mainly studied in cancer disease, so far [2, 50, 66]. The most studied epigenetic modification in mammalian cells is DNA methylation (Fig. 13.1) which relies on the transfer of a methyl group ($-CH_3$) to the 5′position of cytosines to form 5methylcytosine (5mC) in CpG dinucleotide sequences [3, 4, 45, 82]. Other major epigenetic phenomena are chromatin remodeling features that modify the chromatin conformation either versus a condensed or decondensed status that influence the transcriptional regulatory processes (Fig. 13.1). Posttranslational histone modifications occurring at histone tails such as histone acetylation, methylation, ADP ribosylation, and ubiquitination are also known to be epigenetic processes involved in gene transcriptional regulation (Fig. 13.1), and the recent implementation of more accurate methods to evaluate these processes allowed for a better understanding of those histone protein-related epigenetic processes [28].

In the scene of epigenetics the function of small noncoding RNAs including micro RNAs (miRNAs) as key mechanisms able to modulate and control the expression of genes has also emerged recently (Fig. 13.1). In a complex process, a several thousands primary-miRNAs (pri-miRNAs) are produced through the transcription of miRNA encoding regions by the action of RNA polymerase II [76]. The hairpin-shaped transcript possesses a 5'capped and a polyadenylated tail which is sequentially excised to a stem-loop structure called precursor miRNA (pre-miRNA) (Fig. 13.1). These first passages take place in the cell nucleus, then pre-miRNA is transported to the cytoplasm, where it is cleaved into a short double-stranded RNA fragment called miRNA:miRNA duplex subsequently split into two single mature miRNA strands (see scheme in Fig. 13.1) [74]. MiRNAs are usually present in multiple copies and lead to translational suppression of target messenger RNA (mRNA) [11].

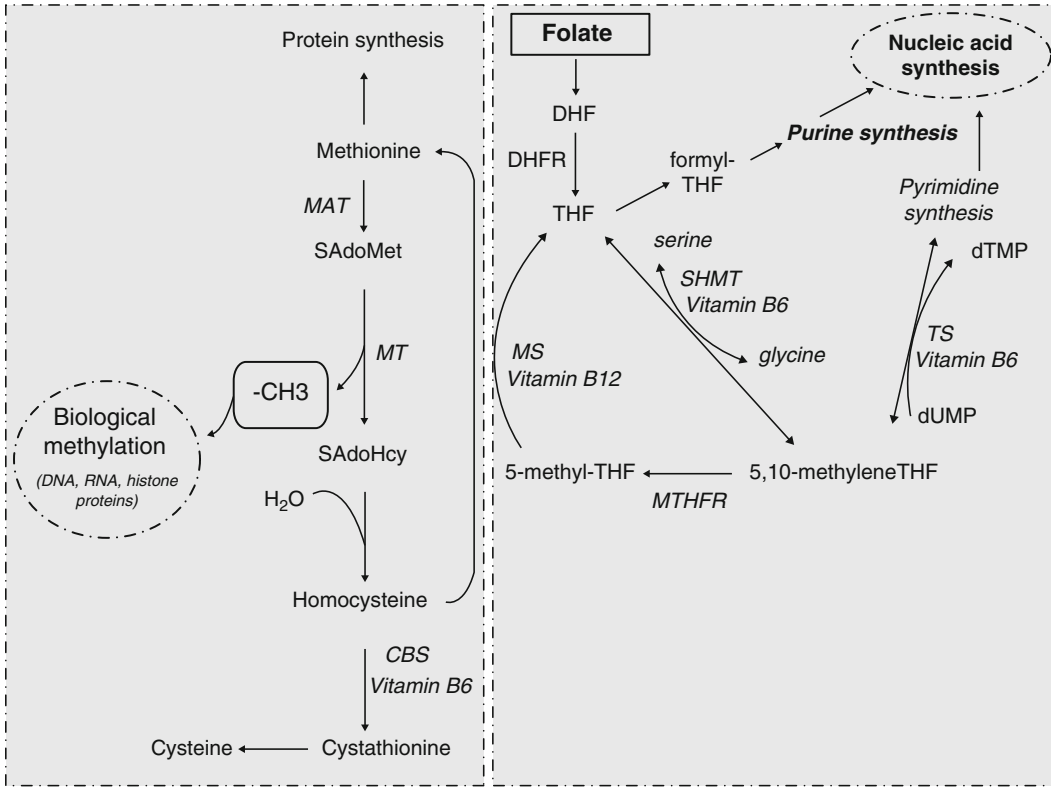

Fig. 13.2 DNA methylation

Several reports have recently highlighted the possible significant role of epigenetics in complex diseases other than cancer, including obesity [1, 10, 55] through its implication in several nutritional or metabolic pathways including those that are well known to link obesity to other major cardiovascular risk factors [76] and, in particular, to diabetes mellitus [29].

The role of epigenetics in weight control regulation and obesity seems to be important from several points of view. From the nutritional point of view it is to be highlighted the role of a number of nutritional factors in affecting epigenetic phenomena [12] and, more specifically the role of nutritional factors that serve as methyl group donors such as those taking part in folate-related one-carbon pathway for DNA methylation [23] (Fig. 13.2). From a different point of view, it is also important to consider that a number of bioactive food compounds may affect epigenetic phenomena and that genes involved in obesity development may be epigenetically regulated [55].

Epigenetic Mechanisms

The word "epi-genetics" refers to genome information that is "super"-imposed on the DNA sequence [2]. Epigenetics stands in fact for the complex of somatically heritable states of gene expression, resulting from modifications in chromatin structure that occurs without alterations in the DNA sequence [2, 66, 82] (Fig. 13.1) and affects development modalities of human diseases [35, 76].

Among the major epigenetic modifications (Fig. 13.1), DNA methylation is the most important and mainly studied epigenetic feature of DNA in mammals. It consists in the transfer of a methyl group to the 5′position of a cytosine base at the CpG dinucleotide residues, is catalyzed by DNA methyltransferases

(DNMTs) and regulates gene expression patterns by altering chromatin structures. Different forms of DNMTs are so far known, DNMT1, DNMT2, DNMT3a, DNMT 3b, and DNMTL. They can be classified according to their different functions, namely, that of maintenance DNA methyltransferase for DNMT1 and de novo DNA methyltransferases for DNMT3a, DNMT 3b, and DNMTL. The function of DNMT2 is yet not completely clear. By altering the function of these DNA methyltransferases, nutrients and bioactive food compounds can transform global DNA methylation status and posttranslational histone tail modifications and therefore modulate gene expression by altering chromosomal integrity as well as gene specific promoter DNA methylation. As compared to DNA methylation reactions, the process of DNA demethylation is currently highlighted because is important during embryonic development and cell differentiation progression. Most recently, 5-hydroxymethylcytosine (5hmC) whose formation is mediated by methylcytosine oxygenase TET1 [73] was described as an intermediate product in the DNA demethylation process [26] especially in aging and cancer but potentially in any tissue differentiating process [6, 71]. Further studies are certainly needed to delineate the role of 5hmC present in mammalian DNA in a tissue-specific manner [41], in mammalian DNA.

DNA is packaged in building blocks of proteins called nucleosomes that are formed by an octamer of two copies each of histone proteins (histone 2A, histone 2B, histone 3, and histone 4) around which a 146 bp sequence is wrapped [40] (Fig. 13.1). Short amino acid sequences attached to histones and called histone tails are the site of epigenetic histone modifications that may affect gene expression. Differently from DNA that is modified only by methylation, histone proteins can be modified also by acetylation, phosphorylation, biotinylation, ubiquitination, sumoylation, and ADP-ribosylation and other mechanisms that control the dynamics of chromatin to regulate gene expression [53]. Lysine residues in the histone tails can be either methylated (mono-, di-, and tri-) or acetylated, and arginine residues can be mono- or di-methylated. Histone acetylation status is balanced by histone acetyltransferase (HAT) and histone deacetylases (HDAC). Histone methylation is maintained by histone methyltransferases and histone demethylases [30] (Fig. 13.1).

The role of small non coding RNA and particularly that of microRNAs (miRNAs) emerged recently on the scene of epigenetics, as important mechanisms capable to modulate and control the expression of genes (Fig. 13.1). MiRNAs are short (19–25 bases) noncoding RNAs, which act as gene transcriptional repressors in animal and plant genomes [11]. More than a thousand different miRNAs have been described, so far in humans [20, 32].

Major epigenetic features of DNA have been mostly studied in embryonic development [46], aging [38] and cancer [43, 44] and include DNA methylation, histone modifications, and chromatin remodeling mechanisms [18, 30, 49]. Epigenetics has been presently highlighted in many other fields, such as chronic inflammatory diseases [48], obesity [10, 55], insulin resistance [72], type II diabetes mellitus [29], cardiovascular diseases [76], and immune diseases [67]. Because epigenetic modifications can be altered by environmental factors, epigenetics is now considered an important mechanism possibly regulating the unknown etiology of many diseases in which the environmental exposure to either nutritional or other factors may play an important role such as the case of obesity, thus providing a new framework for the understanding of etiological aspects in the ample complexity of environment-associated diseases.

Nutrition and Epigenetics

The role of nutritional-dietary factors is very important in affecting epigenetic phenomena. Nutrients and bioactive food compounds can, in effect, modify epigenetic phenomena and alter the expression of genes at the transcriptional level. Among the various nutrients a special role is to be given to folate-dependent one-carbon nutrients since they are fundamental in providing methyl groups ($-CH_3$) for biological methylation reactions including that of DNA [24, 25]. Folate-dependent one-carbon pathway regulates both nucleic acids synthesis and methylation and therefore plays a pivotal role in cell cycle regulation and cell differentiation (Fig. 13.2). Folate, betaine, choline, vitamin B-12 and

Table 13.1 Nutrients acting through epigenetics in metabolic pathways

Dietary factors/nutrients	Metabolic effect	Epigenetic feature
Methyl donors compounds		
Betaine	Liver steatosis, insulin resistance	Histone and DNA methylation
Choline	Liver steatosis	Histone and DNA methylation
Cobalamin	Insulin resistance, obesity	DNA methylation
Folate	Adiposity, insulin resistance	DNA and histone methylation, imprinting
Methionine	Insulin resistance, obesity	Histone and DNA methylation
Serine, Glycine, Histidine	Amino acid metabolism	Histone and DNA methylation
Vitamins		
Ascorbate	Antioxidant processes	DNA methylation
Retinol	Antioxidant processes	Histone acetylation
Tocopherols	Antioxidant processes	Histone acetylation
Fatty acids		
Arachidonic acid	$n-6$ Polyunsaturated fatty acid metabolism	DNA methylation
Butyrate	Inflammation	DNA methylation and histone acetylation
Docosahexaenoic acid	$n-3$ Polyunsaturated fatty acid metabolism	DNA methylation
Eicosapentaenoic acid	$n-3$ Polyunsaturated fatty acid metabolism	DNA methylation
Polyphenols and other compounds		
Epigallocatechin gallate	Weight reduction, insulin sensitivity, liver steatosis	Histone acetylation and DNA methylation
Genistein	Body weight control	DNA methylation, miRNAs
Soy isoflavones	Body weight, insulin sensitivity	DNA methylation
Curcumin	Inflammation, body weight	Histone acetylation, DNA methylation and miRNAs
Resveratrol	Body weight, liver steatosis	Histone acetylation
Alcohol	Liver steatosis, body weight	DNA methylation
Sulforaphane	Adipocyte differentiation	Histone acetylation

methionine can alter DNA methylation and histone methylation by modifying one-carbon metabolites (Table 13.1). The universal methyl-donor for methylation reactions is S-adenosylmethionine (SAdoMet) and it is produced within the folate-dependent one-carbon metabolism and it is one of the two major metabolites of one-carbon metabolism that can influence methylation of DNA and histones, the second being S-adenosylhomocysteine (SAdoHcy), the product reaction as well as the inhibitor of methyltransferases (Fig. 13.2). Thus, theoretically any nutrient, bioactive component or condition that can affect SAdoMet or SAdoHcy levels can alter the methylation of DNA or histones (Fig. 13.2). Besides the role of nutrients with a specific function as methyl donors, bioactive food components may also act by directly influencing enzymes involved in epigenetic mechanisms. For instance, genistein and tea catechin affects DNA methyltransferases. Resveratrol, butyrate, sulforaphane and diallyl sulfide inhibit histone deacetylases, while curcumin inhibits histone acetyltransferases (Table 13.1). Altered enzyme activity by these compounds may influence physiologic and pathologic processes during our lifetime by altering gene expression (Table 13.1).

Obesity, Nutrition, and Epigenetics

Among the different mechanisms that could lead to interindividual differences in human phenotypic expression including obesity, the epigenetic regulation of gene expression has emerged in the last years as a potentially very important contributor [31, 63]. Evidences on the role of epigenetics in

obesity are still incomplete but there are several finding showing a possible role for epigenetics in this disease. Among others, some mechanisms seem to have a crucial role, i.e., the dysregulation of known imprinted genes, the altered methylation profile at specific gene promoter sites as well as metastable epialleles and the altered regulation of histone methylation and acetylation.

An interesting evidence of the role of epigenetics in obesity comes for one of the first described imprinted-gene-linked diseases, the Prader-Willi syndrome, a complex, rare, genetic disease characterized by mental retardation with obesity being among the most significant health problems. Prader-Willi syndrome patients have a defect on the paternally inherited chromosome 15 [7] and demonstrate a maternal-only DNA methylation pattern despite the presence of both paternal alleles [8, 9, 34, 52]. This syndrome is the clearest indication that epigenetic mechanisms play an essential role in regulating energy balance in humans.

Calorie Restriction, Reduced-Protein Diet, and Epigenetics

As for the role of epigenetics in energy intake, several reports highlighted that both calorie restriction and low proteins diets induce metabolic alterations that leads towards obesity and other metabolic diseases. Suboptimal early nutrition and reduced growth in utero, for instance, are associated with increased risk of obesity, hypercholesterolemia and type 2 diabetes mellitus in adulthood [70] by epigenetic modifications such as a decreased insulin growth factor 2(IGF2)/H19 gene methylation [85]. Calorie restriction induces histone 4 acetylation in adipose tissue of mice fed a high-fat diet [79]. In humans, hypocaloric diet alters the methylation pattern of different genes in the adipose tissue [5] and in adult mice caloric restriction seems associated to a stress condition that induces binge-eating possibly by affecting epigenetic mechanisms [62].

Culturing of preimplantation mice embryos allowed the observation that epigenetic marks are susceptible to nutritional influences in the very early stages of development in mammals [57] and one of the most interesting opportunity of confirming such findings in humans was given by the evaluation of epigenetic signatures in people periconceptionally exposed to the severe energy restriction imposed by the Dutch Hunger period in the winter between 1944 and 1945 [33]. The insightful analysis of data obtained from people periconceptionally exposed to famine in that period around the end of World War II, showed persistent epigenetic differences as indicated by reduced DNA methylation of the imprinted insulin-like growth factor II (*IGF2*) gene compared with their unexposed, same-sex siblings [33]. *IGF2* is a key factor in human growth and development and is maternally imprinted. If altered by environmental conditions occurring in the early development, the aberrant *IGF2* methylation may therefore be detected even several years later and represent a crucial fingerprint for epigenetic phenomena occurred early in development and subsequently maintained throughout life [33]. The hypomethylation in *IGF2* is comparable to that observed for the nuclear receptor subfamily 3, group C, member 1 (glucocorticoid receptor) (*Nr3c1*) and peroxisome proliferator-activated receptor alpha (*Ppara*) genes in offspring of female rats fed an isocaloric protein-deficient diet starting before pregnancy. Similarly, prenatal under-nutrition induced by a maternal protein restricted diet with different amounts of folic acid, persistently altered the promoter methylation status of the hepatic *Ppara* in the offspring of rats [51].

Furthermore, it has been shown that maternal methyl dietary contents affect the coat color of the rodent offspring and alter the susceptibility of the animal to certain chronic diseases, obesity and cancer [58, 59]. This rodent model is, in fact, a paradigmatic example of the epigenetic regulation guided by dietary modulation of methyl nutrients intake of the coat color of mice via the epigenetic regulation of the agouti locus [59, 78]. In viable yellow (A(vy)/a) mice, transcription originating by a retrotransposon inserted upstream of the agouti gene causes ectopic expression of the agouti protein, resulting not only in yellow fur but also in higher risk of obesity, diabetes and increased susceptibility to cancer disease [16, 59].

Table 13.2 Genes involved in metabolic processes and whose expression is controlled by epigenetic mechanisms

Gene symbol	Gene full name	Epigenetic feature	Metabolic function
ADIPOQ	Adiponectin	Histone acetylation	Adipogenesis
CEBPA	CCAAT/enhancer binding protein (C/EBP), alpha	Histone acetylation and methylation	Body weight homeostasis
FASN	Fatty acid synthase	DNA methylation	Lipid storage
FTO	Fat mass and obesity associated	DNA methylation	Feeding and fasting regulation
GLUT4	Insulin-responsive glucose transporter 4	Histone acetylation	Adipogenesis, glucose transport
HIF1A	Hypoxia inducible factor 1	DNA methylation and histone modifications	Hypoxia
IFNG	Interferon, gamma	DNA methylation	Inflammatory response
IGF2	Insulin-like growth factor 2	DNA methylation and histone modifications	Homeostasis of glucose
INS	Insulin	DNA methylation and histone acetylation	Homeostasis of glucose
IRS1	Insulin receptor	DNA methylation	Homeostasis of glucose
LEP	Leptin	DNA methylation	Control of appetite
MC4R	Melanocortin 4 receptor	DNA methylation	Control of appetite
MTHFR	Methylenetetrahydrofolate reductase	DNA methylation	Vitamin metabolic process
NPY	Neuropeptide Y	DNA methylation	Control of appetite
NR3C1	Glucocorticoid receptor	Histone acetylation	Inflammatory response, Stress
POMC	Proopiomelanocortin	DNA methylation and histone modifications	Control of appetite
PPARA	Peroxisome proliferator-activated receptor-α	DNA methylation	Inflammatory response
SOD2	Superoxide dismutase 2, mitochondrial	DNA methylation	Oxidative stress
SOD3	Superoxide dismutase 3, extracellular	DNA methylation and histone acetylation	Oxidative stress
UCP1	Uncoupling protein 1	DNA methylation	Adipogenesis
TNF	Tumor necrosis factor alpha	DNA methylation	Insulin resistance

The coat color variation is correlated to epigenetic marks established early in development and it has been related to the impact of nutritional factors on the fetal epigenome [42, 46, 56]. The antagonizing effects of Agouti protein on melanocortin receptors may explain the brown coat color and a distinct neuroendocrine phenotype of obesity, hyperphagia, and hyperinsulinemia [59].

Another example of the role of epigenetic mechanisms in metabolic diseases comes from the observation that the H3K9-specific demethylase Jhdm2a is critical for the nuclear hormone receptor-mediated gene regulation and the activation of genes involved in metabolic processes, and that the disruption of the Jhdm2a gene results in obesity and hyperlipidemia in mice [75] (Table 13.2).

High-Calorie Diet and Epigenetics

Considering that high-calorie and high-fat diets are likely associated to an obese phenotype several studies considered to evaluate the epigenetic signatures in these conditions. It has been demonstrated that long term high-fat diet has an effect on methylation status of obesity related genes such as leptin in adipose tissue [54] or melanocortin receptor 4 in brain [81] which is known to play an important

role in body-weight regulation and as demonstrated in the obese Berlin fat mouse inbred line and the lean C57BL/6NCrl line of *Mus musculus* [81]. Moreover, high-fat diet during 4 weeks results in an increased expression of histone deacetylases HDAC5 and HDAC8 [27] and fasting decreased the number of acetylated histone H3- and acetylated histone H4-positive cells in the hypothalamus [27], thus showing that a hypercaloric diet may influence epigenetic mechanisms that regulate the expression of gene regulating appetite mechanisms and energy metabolism. Even in the case of hypercaloric diet there is a strong transgenerational influence in epigenetic marks as shown by maternal overfeeding that induce an obese phenotype in the offspring that appears not related to postnatal nutritional habits [37]. Moreover, changes in hypothalamic regulation of body weight and energy homeostasis has been demonstrated to occur after high-fat diet in mothers, and such modification led to the higher expression of leptin receptor, proopiomelanocortin, and neuropeptide Y in offspring at adulthood [61]. Interestingly, it has been hypothesized that the diet of the mother as well her degree of adiposity is more important than energy intake of the individual itself to determine increased body weight or insulin resistance in adulthood [80]. These metabolic abnormalities in the offspring seem to be related to epigenetic alterations that are maintained during later life and influence development of diabetes mellitus and obesity risk in later life [15]. For instance, neonatal overfeeding modulates the methylation status at promoter site of the most important anorexigenic neuropeptide gene, proopiomelanocortin (*POMC*) with a likely inhibitory effect via epigenetic mechanisms [64]. Maternal high-fat diet may also influence the expression of genes involved in the appetite regulation such as dopamine by inducing the preference for diets rich in sucrose and fat [77] and such alterations seem to be maintained at least across two generations [17]. These data taken altogether demonstrate that different epigenetic mechanisms are involved in the development of obesity even by influencing mechanisms that are maintained from one to the following generation. Further and larger studies especially to confirm in humans some observations so far evidenced only in animal models.

MicroRNAs and Obesity

MicroRNAs (miRNAs) have recently emerged as a class of small non coding RNAs with key regulatory function for gene expression including those pertaining to metabolic pathways as those regulating weight balance and therefore obesity development. Specific miRNAs have been associated to lipid metabolism regulation and their function may be of high interest both as endocrine signalling molecules and disease markers [68]. MiR-33a and miR-33b, for instance, are particularly important in controlling cholesterol [65] and lipid metabolism [13] and miR-103 and miR-107, regulate insulin and glucose homeostasis [68]. The miR-107 has been also demonstrated to be dysregulated in murine and rodent models of obesity and insulin resistance with miR-107 alteration contributing to both pathologic conditions [22]. Besides the key roles for miRNA-33 and for miRNA-122 in lipid metabolism regulation [19], further evidence implicates also miRNA-370 in the regulation of miRNA-122 [39, 69]. In addition, miRNA-378/378* and miRNA-27 [47] have been described to be involved in adipogenesis and miRNA-613 [60], miRNA-302a, and miRNA-168 [84] seem also to be involved in the regulation of lipid metabolism [69].

Several studies using high-throughput technologies identified differentially expressed miRNAs during adipogenesis with a specific link between molecular regulation through miRNAs and fat tissue pathologies [36]. MiRNAs have been also described in altering glucose homeostasis as well as adipocyte differentiation and insulin production, all of which are strictly related to obesity and therefore highlighting the possibility of using specific miRNAs as molecular biomarkers of obesity-linked metabolic diseases for either early diagnosis and potentially for preventive strategies [14]. In this regard, although the actual knowledge is still limited, there is growing evidence supporting the importance of miRNAs in regulating both cholesterol and fatty acid metabolism, leading therefore also to a

substantial interest in miRNAs as potential drug targets to modulate lipid and lipoprotein metabolism [69]. A possible use, in the near future, of specific miRNAs for therapeutic strategies seem quite promising [69].

Conclusions and Future Perspectives

Besides the known energy imbalance, namely, a dysregulation due to high energy intake versus lower expenditure, obesity is regulated by several other factors including environmental factors as well as genetic markers, epigenetics seems also to play a key role.

A number of studies, in fact, showed that epigenetics is involved in obesity development through different mechanisms. Some nutrients act by regulating the provision of methyl groups and their function as methyl donors to influence the main epigenetic feature of DNA in mammals, DNA methylation. Other nutrients or bioactive food compounds also serve as possible regulator of epigenetic mechanisms including posttranslational histone modifications. A more clear identification of the function of specific nutrients in epigenetic regulation may be useful to define novel therapeutic strategies, especially if one considers that epigenetic features are potentially reversible. Moreover, differential energy intake, both high-fat and -protein diets as well as calorie restriction may influence through epigenetics different mechanisms ultimately leading towards obese phenotype and diseases known to be associated to obesity such as insulin resistance and diabetes mellitus or metabolic syndrome. In this regard, the epigenetic signatures may help to design personalized features able to define the risk of development of metabolic diseases including obesity itself or obesity-associated pathologies. It should be also taken into account the effect of diet in transgenerational epigenetic modifications therefore by influencing the mother diet it is possible to hypothesize that an appropriate diet may prevent obesity in future generations.

Further and more extensive human studies addressing the role of epigenetics in obesity are indeed warranted.

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Chapter 14
Consequences of Rapid Weight Loss

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Keywords Bariatric surgery • Comorbidity • Diabetes mellitus • Hypertension • Sleep apnea • Weight loss • Gastric bypass • Adjustable gastric banding

Key Points

- Weight loss can be induced by both nonsurgical methods and bariatric Surgery. Rapid weight loss may be associated with negative consequences.
- Weight loss is associated with significant positive impacts including resolution of obesity related comorbidities and improvement of overall health and quality of life.
- A healthy rate of weight loss is about 1–2 lb a week. Rapid weight loss may cause life-threatening repercussions, which may include water, electrolyte and nutritional problems, biliary complications, skeletal muscle loss, and cardiac dysfunction.

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Introduction

Consequences of Rapid Weight Loss

Obesity has become an epidemic in the USA and the Western world. During the past 20 years, there has been a dramatic increase in obesity in the USA and rates remain high. In 2009–2010, over 78 million US adults and about 12.5 million US children and adolescents were obese and more than one-third of adults and almost 17 % of youth were obese in 2009–2010 [1].

Obesity has a far-ranging negative effect on health and morbidly obese patients have a high prevalence of obesity-related comorbidities, such as infertility, hypertension, metabolic syndrome, type 2 diabetes, coronary heart disease, stroke, gastroesophageal reflux disease (GERD), osteoarthritis, sleep apnea, and certain cancers [2].

There are many options for weight loss, including diet, exercise, medications and bariatric surgery. All those can lead to either slow or rapid weight loss. Rapid weight loss results can motivate patients to stick to their weight loss plan. When they see themselves shedding off pounds they experience more energy, new-found pride in appearance, enhanced self-image and improvement in intimate relationships that enthuses them to stick to a healthy diet and a regular exercise routine. This weight loss can resolve obesity-related comorbidities, which improve the overall health, quality and length of life.

The purpose of this chapter is to review both positive and negative consequences associated with rapid weight loss.

Methods of Inducing Weight Loss

Nonsurgical Weight Loss

The nonsurgical weight loss strategies include diet, pharmacological and behavioral treatment. Dietary restriction is an effective strategy for weight loss in obese individuals. The most common form of dietary restriction is daily calorie restriction (CR), which involves reducing energy by 15–60 % of caloric intake every day. Another form of dietary restriction employed is intermittent CR, which involves 24 h of ad libitum food consumption alternated with 24 h of complete or partial food restriction. Both dietary restrictions appear to be equally as effective in decreasing body weight, fat mass, and potentially, visceral fat mass. However, intermittent restriction regimens may be superior to daily restriction regimens in that they help conserve lean mass at the expense of fat mass [3].

Regular exercise is an important part of effective weight loss. It helps to control weight by burning excess calories that would otherwise be stored as fat, it may prevent many diseases and improves the overall health. Regular exercise, combined with healthy eating, is one of the most efficient and healthy way to control the weight. However, a problem with lifestyle modification is weight regain after treatment termination.

According to National Institutes of Health (NIH) guidelines, pharmacotherapy for the treatment of obesity can be considered if a patient has a body mass index (BMI) $\geq$30 kg/m^2, or has a BMI $\geq$ 27 kg/m^2 if weight-related comorbidities, including hypertension, type 2 diabetes mellitus, dyslipidemia, and/or obstructive sleep apnea are present [4]. With the recent removal of sibutramine from the US market, orlistat is still approved by the US Food and Drug Administration (FDA) for the long-term treatment of obesity [5].

Medical weight loss is physician-directed weight management based on medical scientific principles that target the root causes of obesity and weight gain to achieve and sustain a healthy weight for the long-term. The number of successful patients in physician supervised weight loss programs is

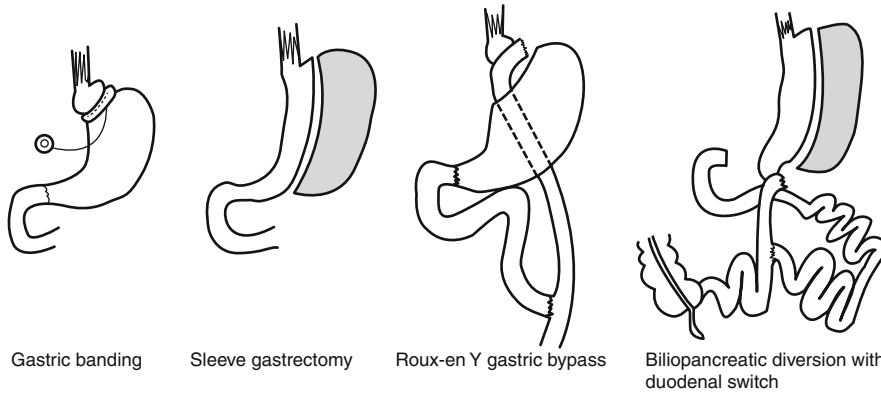

Fig. 14.1 Gastric banding, sleeve gastrectomy, Roux-en-Y gastric bypass, and biliopancreatic diversion

increasing. An effective medical weight loss program requires five basic elements: (1) an effective means of caloric restriction, either a balance of fuel sources in patients who are less than 30 % above ideal body weight, or a very-low-calorie diet in patients with obesity that is a significant threat to their health; (2) extensive nutritional instruction, to enable the patient to make wise food choices that are varied and palatable; (3) an individual exercise program sufficient to maintain the patient's goal weight on maintenance food; (4) behavioral modification, to allow patients to control their food consumption; and (5) continuing support. Finally, medical weight loss doctors have the ability to view weight loss and maintenance within the context of total health profile [6, 7]. A recent study showed that primary care physicians can successfully manage and treat obese patients using behavioral modification techniques coupled with meal replacement diets. Moreover, patients seen in primary care clinics had greater declines in percent body fat than those seen at weight loss clinics [8].

Bariatric Surgery

Bariatric surgery is a reliable method to obtain significant and sustained weight loss, which in turn results in improved comorbidities and survival. Obese patients lose more weight with bariatric surgery than with medical weight loss treatment. Patients typically lose more than 50 % of their excess weight after bariatric surgery. Meanwhile obesity-related diseases markedly improve, reducing cardiovascular risk and improving life expectancy [9].

Ongoing research and technological innovations have contributed greatly to the evolution and advancement of bariatric surgery. Currently, the two most popular procedures performed worldwide are gastric bypass and gastric banding (Fig. 14.1), accounting for 44 % and 46 % of the total case volume, respectively [10]. Sleeve gastrectomy, initially considered as the first component of a two-stage procedure in high-risk patients, has been shown to be effective as a stand-alone bariatric procedure and is gaining popularity [11, 12].

Roux-en Y Gastric Bypass achieves excellent results in terms of weight loss—often as high as 75 % of excess body weight [13]. It also boasts exceptional results in the reduction or elimination of related diseases—reducing the chances of diabetes by 84 % in most patients [14]. The risk of dehydration, nutritional deficiencies, marginal ulcer and dumping syndrome should be considered after gastric bypass.

Adjustable Gastric Banding produces a slower and less overall weight loss, at around 35–50 % excess body weight. Strong patient commitment and frequent follow-up is required. Best results occur for patients with BMIs between 40 and 50 [15].

In Sleeve Gastrectomy the volume of stomach is reduced by removing 85 % or more of the stomach along the greater curvature without any intestinal bypass. The procedure significantly reduces the size of the stomach and decreases levels of Ghrelin (a hormone that stimulates hunger). By avoiding any intestinal bypass, the chance of intestinal obstruction, anemia, osteoporosis, protein deficiency, and vitamin deficiency are decreased [16]. On average, sleeve gastrectomy produces a 60–80 % loss of excess weight. It is currently indicated as an alternative to the adjustable gastric banding procedure for lower weight patients and as a safe option for patients with a higher BMI.

Biliopancreatic diversion (BPD), with or without duodenal switch, reduces gastric volume and bypasses part of the small intestine, so that fewer calories are absorbed [17]. BPD is the most effective weight loss procedure, as the total excess body weight loss is as high as 80 %. However, BPD/DS reduces the absorption of essential vitamins and minerals and may result in serious, long-term complications.

Although bariatric surgery is an effective weight loss method, it is important to understand that following a lifelong commitment to a healthy lifestyle including healthy diet and regular exercise is critical in maintaining weight loss after surgery.

Positive Consequence of Rapid Weight Loss

Metabolic Syndrome

Obesity results in increased visceral fat accumulation, insulin resistance and risk for progression to a broad spectrum of metabolic disorders including type II diabetes mellitus (T2DM), hypertension (HTN), cardiovascular disease (CVD), and nonalcoholic fatty liver disease (NAFLD) [18, 19]. Weight loss is a key component in improving all aspects of metabolic syndrome [20]. Even a modest weight loss of 5–10 % of total weight can reduce central obesity, positively affect blood pressure, increase sensitivity to insulin, increase HDL cholesterol, and decrease LDL cholesterol and triglycerides [21]. Weight loss can also reduce the risk of developing type 2 diabetes. Samaha et al. [22] randomized 132 obese patients with a mean BMI of 43 to a low-carbohydrate versus a low-fat calorie restricted diet. At 6 months, the metabolic profile of the low carbohydrate group showed greater weight loss and reduced triglycerides than the low fat group. In addition, the fasting glucose of diabetics decreased in the low carbohydrate group and insulin sensitivity improved in the nondiabetic group.

Nonalcoholic fatty liver disease (NAFLD), the hepatic manifestation of metabolic syndrome, is one of the most prevalent liver diseases worldwide. Studies have shown improvement in liver enzymes and evidence of hepatic steatosis by ultrasonography after weight loss, but long-term histological improvements have not been fully established. Bariatric surgery may improve conditions associated with metabolic syndrome and NAFLD in morbidly obese patients [23]. Xourafas et al. [24] studied the impact of bariatric interventions on alanine aminotransferase (ALT) levels in patients with or without T2DM in patients and found that ALT levels decreased and remained at the new low level up to year 3 after surgery.

Obstructive Sleep Apnea and Respiratory Problems

In 1999 Camargo et al. [25] reported a strong positive association between body mass index (BMI) and risk of adult onset asthma in the Nurses' Health Study. Increased BMI has also been associated with increased severity of asthma symptoms and use of health services [26]. A cross-sectional survey

study by Chen et al. [27] reported that nonallergic individuals had a higher risk of asthma in the setting of obesity than allergic individuals. Sideleva et al. [28] found that in obese asthmatics there is decreased neutrophilia and increased lymphocyte function in airways following bariatric surgery. Airway hyperreactivity in obese patients with late onset disease and low IgE also improved after weight loss.

The prevalence of the obstructive sleep apnea (OSA) among obese people is as high as 40 % [29], in morbidly obese patients (BMI $\geq$ 45 kg/m^2), the prevalence ranges from 50 to 77 % and in those with BMI of 60 kg/m^2 or more, the disorder may occur in 90 % [30, 31]. Schafer et al. [32] reported that OSA severity is significantly correlated with intra-abdominal fat accumulation. Weight loss was associated with almost complete resolution of sleep apnea [33] in patients with OSA in whom the upper airway critical pressure fell below <4 cm H_2O. Weight loss changes pharyngeal anatomy and decreases airway collapsibility by increasing the pharyngeal closing pressure [34]. Bariatric surgery is an effective treatment for obstructive sleep apnea, causing remission in high percentage of cases.

Cardiovascular Disease

Hypertension is a common comorbidity in obese individuals, and patients with obesity and hypertension are at an increased risk of developing cardiac events and death. Though the exact mechanism of how obesity causes hypertension is unknown, a lot can be attributed to the neuroendocrine mechanism such as the renin–angiotensin–aldosterone system [35]. Adipose tissue deposition can lead to irregular functioning of the kidney, which can subsequently lead to alteration of blood pressure [36]. Critical weight loss is an effective way to control obesity related hypertension. Bariatric surgery leads to a significant lowering of blood pressure and a systemic review regarding the remission of hypertension after sleeve gastrectomy demonstrated that 75 % of patients had resolution or improvement of their hypertension and 58 % experienced complete resolution of their hypertension [35].

Overweight and obesity contribute to the development, acceleration, and exacerbation of Coronary heart disease (CHD) [37]. High heart rate has been associated with increased mortality in obese [38]. Studies have demonstrated that sustained moderate (5–10 %) weight loss, when undertaken in conjunction with increased physical activity, can improve cardiovascular disease risk factors and reduce associated mortality [39]. Multiple studies have reported prolongation of corrected QT interval (QTc) and/or increased QT or QTc dispersion in obese patients, suggesting an association between obesity and delayed ventricular repolarization. Ventricular repolarization improves after weight loss in obese patients [40, 41]. Mukerji et al. [42] assessed the effect of weight loss on ventricular repolarization in 39 morbidly obese patients. Mean QTc and QTc dispersion decreased significantly with weight loss in patients with LV hypertrophy but not in subjects without LV hypertrophy.

Excessive myocardial triglyceride (MTG) content accumulated in the heart of obese patients is associated with left ventricular hypertrophy, adverse cardiac remodeling and impaired cardiac function [43]. Hammer et al. [44] reported that prolonged caloric restriction with a very low caloric diet, decreases MTG levels in obese diabetic patients with favorable influences on diastolic function. Utz et al. [45] observed that magnetic resonance parameters of myocardial diastolic longitudinal function (PLV) and left ventricular filling (PFRE) were reduced after weight loss. Moderate dietary weight loss significantly reduced MTG content in women with uncomplicated overweight or obesity.

In all, weight loss has many beneficial effects on the cardiovascular system including reductions in left ventricular mass, heart rate, cardiac output and blood pressure. Changes in cardiac structure and function with weight loss may result from intrinsic changes in myocardial metabolism, changes in cardiac loading conditions, altered autonomic nervous system (ANS) and renin–angiotensin system (RAS) activities or combination of these mechanisms.

Diabetes

Obesity, a potent risk factor for type 2 diabetes, contributes to its development by inducing insulin resistance and inflammation, which in turn impair glucose regulation [46]. Fat deposits in the abdomen, muscles, and liver contribute to elevations of circulating free fatty acids and adipocyte-derived cytokines that mediate insulin resistance and inflammatory pathways [47].

In the Diabetes Prevention Program [48], modest weight loss (5–10 % of body weight) through diet and exercise reduced the incidence of type 2 diabetes, and in the Action for Health in Diabetes (Look AHEAD) study of the National Institutes of Health, it improved glucose homeostasis [49, 50].

Bariatric surgery results in significant weight loss and remission of diabetes in most patients. Schauer et al. [51] observed studied 1,160 morbidly obese patients, of whom 240 (21 %) had type 2 diabetes or impaired fasting glucose. After laparoscopic Roux-en-Y gastric bypass surgery, fasting glucose and hemoglobin A1c levels returned to normal levels in 83 % of cases and were markedly improved in the remaining 17 %. Significantly fewer patients needed oral antidiabetic medicines (80 % fewer) or insulin (79 % fewer). Patients most likely to achieve complete remission of diabetes were those with the shortest duration (<5 years), the mildest severity (diet-controlled), and greatest weight loss after surgery. The rate of diabetes remission in patients who had been diabetic for 5 years or less was 95 %, compared with 75 % in those who had been diabetic for 6–10 years and 54 % in those who had been diabetic for more than 10 years ($P < 0.001$).

Three major mechanisms have been proposed to explain how bariatric surgery reverses diabetes including increased insulin sensitivity. The enforced caloric restriction, negative energy balance, and weight loss after bariatric surgery reduce insulin resistance. Another theory is that bariatric surgery lessens insulin resistance by reducing "lipotoxicity" a condition related to dysregulated fatty acid flux, lipid metabolites in tissues, and direct and indirect effects of hormones secreted by adipocytes. The third theory is likely the most relevant and relates to various hormones secreted by the gut in response to food. The immediate weight loss-independent of T2DM resolution after LRYGB suggests that surgery modifies the enteroinsular axis. Changes in gut hormones, such as ghrelin, peptide YY (PYY), and glucagon-like peptide-1 (GLP-1) after LRYGB are well documented. The increasing level of GLP-1 appears to be critical for improving the response to insulin. The primary function of GLP-1 includes the potentiation of glucose-stimulated insulin secretion, enhancement of β-cell growth and survival, inhibition of glucagon release, and control of food intake [52].

Immunology

Obesity causes a state of low-grade chronic inflammation and higher circulating levels of inflammatory proteins are found in obese individuals. Adipose tissue is not metabolically inert but synthesizes and secretes proinflammatory mediators, including interleukin 6 (IL-6), tumor necrosis factor α (TNFα), IL-8, and monocyte chemo attractant [protein 1] (MCP-1) [53]. High sensitivity C-reactive protein (hsCRP) levels, which are a more sensitive and specific marker of inflammation, are also elevated in overweight and obese patients [54].

Studies have shown that caloric restriction improves parameters of immunity such as T cell responses to mitogens, NK cell activity, and the ability of mononuclear cells to produce proinflammatory cytokines. Subjects with weight loss, induced by 14-days fast, showed improvement in serum immunoglobulin levels, delayed-type hypersensitivity (DTH) response, bactericidal capacity of blood monocytes and NK cell cytotoxic activity, although there was a decrease in mitogen-stimulated lymphocyte proliferation. Tanaka et al. [55] showed that mitogen-stimulated lymphocyte proliferation, which was suppressed in obese patients, was restored after weight loss induced by very low calorie diet (VLCD). In contrast, some studies report that weight loss is associated with reduction in some aspects of immune function [56].

Several studies reported a reduction in serum hsCRP Levels, after Roux-en-Y gastric bypass with greater decrease in patients who lost more weight after surgery. Existing evidence suggests that lifestyle measures, anti obesity agents, and bariatric surgery reduce serum levels of inflammatory markers in obese patients [54]. It appears that the reduction in inflammation is primarily driven by weight loss. In a recent meta-analysis, a linear relation was observed between weight loss following lifestyle changes or bariatric surgery and the fall in hsCRP levels, which declined by 0.13 mg/L for each 1 kg of weight loss [57].

Infertility

Obese people were significantly more likely to be infertile than normal-weight people [58]. Obesity may adversely affect male fertility by endocrinologic, thermal, genetic, and sexual mechanisms [59]. Other factors may include aspects of lifestyle and increased accumulation of reproductive toxins in fatty tissue. Obese men have hypogonadotropic hyperestrogenic hypoandrogenemia, characterized by decreased total and free testosterone, decreased gonadotropins, and increased circulating estrogen (E2) levels. Studies suggest that physical activity and leanness is associated with reduced risk of sexual dysfunction [60].

Obese men showed increases in sex hormone-binding globulin (SHBG) and testosterone (free and total) after a very-low-energy diet [61]. Obesity can exert effects upon the hypothalamic–pituitary–ovarian (HPO) axis and as such disturb menstrual cyclicity and ovulation and there are beneficial effects of weight reduction on ovulatory function in overweight anovulatory women [62, 63].

Obese women are more likely to experience pregnancy loss and elevated miscarriage rates [64]. As obesity is a known factor in infertility [65], weight reduction is a cornerstone of the treatment of obesity-related infertility and has been observed to restore normal menstrual cycle [66].

In women, bariatric surgery is associated with significant weight loss, improvement in menstrual pattern, and a reduction in obstetric complications [67]. However, it should be recognized that severe ketosis associated with the initial weight loss may induce fetal anomalies. It is recommended that patients wait for a period of at least 1 year before attempting pregnancy after bariatric surgery.

Joint Problems Including Osteoarthritis (OA)

Obesity can affect the knees and hips by numerous pathways including mechanical stress, systemic inflammation, and relative loss of muscle mass and strength over time. Excessive adipose tissue compresses load-bearing joints and creates an inflammatory environment within tissues and joints [68]. For every 5 kg of weight gain, there is a commensurate 36 % increased risk that OA will develop. Several studies suggest that weight loss may prevent the development or worsening of knee and hip osteoarthritis. In an observational study, Abu-Abeid et al. [69] found that when BMI was reduced by an average of 6.3 kg/m^2 after bariatric surgery, joint space widened from 4.6 to 5.25 mm.

Weight loss with medications, exercise (with or without diet), and bariatric surgery can favorably alter the mechanical and biochemical profiles of obese adults with OA. Mechanical stress can be reduced, as shown by a lowering of maximal knee compressive forces relative to magnitude of weight loss. Weight loss also can substantially lower joint compressive forces, which may increase the joint space width [69]. Reductions in the central deposition of fat on the abdomen and in the girths of lower limb segments may facilitate normalization of joint alignment. The collective benefits of lower joint loading and joint realignment would attenuate cartilage stress and silence one trigger of local joint inflammation [68].

Gastroesophageal Reflux Disease (GERD)

Gastroesophageal reflux disease (GERD) currently affects between 8 and 26 % of western population with markedly higher prevalence in overweight and obese individuals as compared to those with normal BMI [70]. The prevalence of GERD, however, even in the setting of severe obesity, is <50 % [71]. This suggests that severe obesity itself is not sufficient to cause GERD, and that in the majority of severely obese individuals, at least some of the physiological mechanisms that prevent GERD remain reasonably intact.

Fundamental to the development of GERD is a failure of the anti-reflux barrier. In a large cohort of patients with foregut symptoms, the prevalence of a mechanically defective LES (based on hypotensive lower esophageal sphincter (LES), total length, or abdominal length) increased as BMI increased, with 55 % of obese patients demonstrating a defective LES [72]. Several factors might contribute to the increased gastroesophageal gradient seen with obesity, including increased intra-abdominal pressure, increased intra-gastric pressure, increased negative inspiratory intra-thoracic pressure, and a mechanical separation between the LES and the extrinsic compression provided by the diaphragmatic crura [73].

Nissen fundoplication is a standard surgical treatment for GERD. However, there is substantial controversy regarding the long-term efficacy and durability of fundoplication in the setting of obesity. Several studies have demonstrated a significant reduction in GERD symptoms after bariatric surgery and it is increasingly being seen as a more appropriate surgical treatment for GERD in morbidly obese [72, 73]. Gastric bypass appears to have a more favorable impact on GERD compared to gastric banding and sleeve gastrectomy.

Cancer

Lew and Garfinkel in 1979 in a long-term prospective study of 750,000 men and women reported an increased incidence of mortality from cancer in the obese [74]. Obesity has been shown to increase incidence of cancer of colon and rectum, endometrium, kidney, pancreas, postmenopausal breast, and adenocarcinoma of the esophagus (the so-called obesity-related cancers). Obese subjects have an approximately 1.5- to 3.5-fold increased risk of developing these cancers compared with non-obese people and current estimates suggest that 15–45 % of these cancers can be attributed to excess body fat [75].

Birks et al. reported the association between weight loss and cancer in systematic review of the literature. Of the 34 studies considered, 16 found a significant reduction in the risk of incident cancer in those who experienced weight loss. Intentional weight loss (including surgical weight loss) almost universally resulted in a significant reduction in the overall incidence of cancer, predominantly noted for postmenopausal breast cancer, colorectal cancer, prostate cancer, and endometrial cancer [76].

Obesity-associated dys-regulation of adipokines is likely to contribute not only to tumor genesis and tumor progression, but also to metastatic potential. Obesity affects the insulin and insulin-like growth factor (IGF) axis and adipokines. This results in an increase in the levels of free or "bioactive" IGF-I, and concomitant changes in the cellular environment favoring tumor development [77–79]. There is both epidemiological and preclinical evidence that demonstrates a link between increased IGF-I levels and risk of various cancers. Leptin is another potent pro-inflammatory agent that is elevated in obese individuals. It is mitogenic for various cell types such as hematopoietic progenitor cells, normal and transformed epithelial cells, colonic epithelial cells, and vascular endothelial cells [80, 81]. Weight loss reduces the level of leptin and adiponectin and potentially decreases cancer risk as well.

Urinary Incontinence (UI)

Obesity has been shown to be a strong risk factor for incontinence [82] and several studies have suggested that weight loss may reduce the frequency of urinary incontinence [83]. It is theorized that excess body weight increases abdominal pressure, which in turn increases bladder pressure and urethral mobility, leading to stress UI and also exacerbating detrusor instability and overactive bladder. Weight loss is associated with changes in urodynamic measures, leading to decreased incontinence. After large, surgically induced weight loss statistically significant changes were reported in urodynamic measures, including decreased intravesical pressure, greater bladder pressure increases with coughing, bladder-to-urethra pressure transmission with cough and urethral axial mobility [84]. Subak et al. showed that overweight and obese women with urinary incontinence randomized to lifestyle intervention had greater improvements in urinary incontinence than women in the control group at 6 and 12 months, especially for stress incontinence [85]. They also found that modest weight losses of 5–10 % were associated with statistically and clinically significant reductions in urinary incontinent episodes and with satisfaction with improvements in continence. Women who lost 5–10 % of their body weight were two to four times more likely to achieve at least a 70 % reduction in total and urge incontinent episode frequency compared with women who gained weight at 6, 12, and 18 months.

In observational studies severely obese women (greater than 45 kg above ideal weight) with incontinence who had dramatic weight loss after bariatric surgery (45–50 kg) had significant improvement in UI [86, 87].

Renal Function

Severe obesity is associated in with increased systemic arterial pressure, high renal plasma flow, increased GFR, and enhanced albumin excretion rate. Studies show that in severe obesity the glomerular capillary bed is subjected to an elevated transcapillary hydrostatic pressure gradient resulting in hyperfiltration. Obesity is associated with the occurrence of nephrotic syndrome and renal failure. Obesity-related glomerulopathy was recently defined morphologically as glomerulomegaly with or without focal segmental glomerulosclerosis. Obesity may also accelerate the course of idiopathic glomerular disease, such as IgA glomerulopathy. The prevalence of obesity-related glomerulopathy, which may lead to end-stage renal disease, has increased tenfold over the last 15 year as a consequence of "the spread of the obesity epidemic" [88]. Although a cause-and-effect relationship between the obesity associated glomerular hyperfunction and the development of nephrotic syndrome and renal failure has not been demonstrated, experimental and clinical data suggest that hyperfiltration and glomerulomegaly may lead to glomerular damage. Therefore, reducing glomerular hyperfiltration may provide a way to prevent or delay the development of renal disease in the obese. A study by Chagnac et al. [89] demonstrated that obesity-related glomerular hyperfiltration ameliorates after weight loss. The improvement in hyperfiltration may prevent the development of overt obesity-related glomerulopathy. Another systematic review showed that in patients with chronic kidney disease (CKD), weight loss that was attained through nonsurgical interventions was not associated with a change in GFR. Conversely, weight loss that was attained through bariatric surgery was associated with a normalization of glomerular hyper filtration [90].

In summary, weight loss improves the glomerular hemodynamic abnormalities associated with severe obesity. These findings suggest that weight loss may delay the progression of renal insufficiency in obese patients with glomerular disease.

Psychosocial Consequences

In a culture where often the ideal of physical attractiveness is to be overly thin, people who are overweight or obese frequently suffer disadvantages. Many reports during the past 25 years have shown positive mood changes in persons treated in group behavioral weight-loss programs [91]. These studies revealed improvements in depression and anxiety or, at a minimum, no worsening in affect.

A clinical study by Wadden et al. [92] reviewed the psychosocial consequences of weight reduction and concluded that weight loss is usually associated with improvements in mood in significantly obese individuals (≥20 % overweight) who are treated by diet and lifestyle modification. Most people with a dramatic change in their weight and physical fitness report sweeping changes in self-image and a boost in their confidence. Body image improves after LSG and this improvement might reflect changes to patients attitudes, beliefs, and thoughts rather than real weight lost [93].

Negative Consequences of Rapid Weight Loss

Biliary Complications

Obesity predisposes to gallstone formation especially cholesterol stones due to production of bile supersaturated with cholesterol and less contractile gallbladder and rapid weight loss raises bile lithogenicity increasing odds of gallstone formation [94]. Within 6 months after bariatric surgery, biliary sludge develops in as many as 13 % and new gallstones in as many as 36 % of patients [95]. About a third of these patients will develop symptomatic gallstone disease. A long-term follow-up study found that after gastric bypass the observed number of cholecystectomies exceeded the expected number by over fivefold [96]. In a follow-up of the Nurses Health Study cohort, women who lost 4–10 kg had 44 % increase in the risk of gallstone disease and women who lost more than 10 kg had a 94 % increased risk for gallstone disease compared to those women who lost less than 4 kg [97]. Bariatric surgery with associated rapid weight loss also increases risk of developing gallstones. In the Nurses Health study, 12.1 % of patients developed gallstones after 8–16 weeks of very low calorie diets, while 37.8 % of patients developed gallstones after gastric bypass surgery [97]. During rapid weight loss, ursodesoxycholic acid should be given and is recommended that patients stay on it postoperatively for 6 months [98].

Malnutrition

Rapid weight loss increases risk of vitamin and mineral deficiencies and these deficiencies are more prevalent after bariatric surgery.

Iron absorption is facilitated by reduction of the ferric iron in foods to the ferrous state by hydrochloric acid in stomach [99]. After Roux-en-Y gastric bypass surgery, iron deficiency is common, with an incidence as high as 49 % [100]. Oral supplementation may be needed to prevent iron deficiency especially after malabsorptive procedures particularly in menstruating women. As vitamin C supplementation increases iron absorption, consider vitamin C supplementation in recalcitrant iron deficiency.

Decreased chloride production, after Roux-en-Y gastric bypass can prevent the cleavage of vitamin B12 from food products leading to vitamin B12 deficiency [99]. Many patients may be unable to consume meats and dairy products. Also, intrinsic factor may be inadequately secreted after surgery, which impacts vitamin B12 absorption [99]. Vitamin B12 deficiency can lead to clinically significant consequences, such as megaloblastic anemia, thrombocytopenia, leukopenia, and glossitis.

Folic acid deficiency can also occur with Roux-en-Y gastric bypass surgery, affecting as many as 35 % of patients [101]. In order for folate to be absorbed, hydrochloric acid and vitamin B12 are needed. Both are decreased after bariatric surgery. However, the predominant mechanism for folate deficiency is decreased folate consumption from dietary sources [102]. Folate deficiency can result in megaloblastic anemia, thrombocytopenia, leukopenia, and glossitis.

The absorption of fat-soluble vitamins can also be affected by Roux-en-Y gastric bypass. With bypass of the duodenum, there can be delayed mixing of dietary fat with pancreatic enzymes and bile salts, leading to malabsorption of both fat and fat-soluble vitamins [99]. Vitamin A deficiency occurs in 10 % of gastric bypass patients and can result in visual difficulties [100]. Vitamin D and calcium deficiencies may also occur. As thiamine absorption primarily occurs in the proximal small bowel, thiamine (vitamin B1) deficiency can also occur especially in patients with persistent vomiting. Thiamine deficiency can lead to Wernicke–Korsakoff syndrome.

Cardiac Dysfunction/Arrhythmias

Rapid weight loss in patients on very low caloric diet (less than 500 kcal/day) is associated with ventricular tachydysrhythmias leading to sudden death. Protein depletion due to rapid weight loss may be the underlying cause. EKG changes seen may include reduction in QRS voltage by the seventh week and prolongation of the QT interval. These may lead to ventricular arrhythmias and even death [103]. LV hypertrophy is a key determinant of QTc and QTc dispersion in normotensive morbidly obese patients. Regression of LV hypertrophy associated with weight loss decreases QTc and QTc dispersion [42].

Immunosuppression

Massive weight loss leads to catabolic state which may suppress immune response and lead to infections or worsening of infections [94]. Weight reduction results in a decrease in the mRNA expression of IL-1beta, IL-1 receptor antagonist, and tumor necrosis factor alpha and an increase in expression of IL-6 and IL-8 [104].

Water and Electrolyte Problems

Rapid weight loss may cause significant diuresis leading to a loss of essential electrolytes, (like sodium, potassium, magnesium, calcium and phosphorous) leading to potential adverse effects on the heart and other important cellular functions [94].

When carbohydrate intake is restricted, two metabolic processes occur, both of which simultaneously reduce total body water content. The first process is mobilization of glycogen stores in liver and muscle. Each gram of glycogen is mobilized with approximately 2 g of water. The liver stores approximately 100 g of glycogen and muscle has 400 g of glycogen. Mobilization glycogen stores result in a weight loss of approximately 1 kg. The second process is generation of ketone bodies from catabolism of dietary and endogenous fat. Ketone bodies are filtered by the kidney as non reabsorbable anions. Their presence in renal luminal fluids increase distal sodium delivery to the lumen, and therefore increase renal sodium and water loss [105].

These electrolytes should be monitored and appropriately supplemented during periods of rapid weight loss.

Liver Dysfunction

Obese individuals also commonly have elevations in serum transaminase levels due to pressure on hepatocytes and bile canaliculi created by or secondary to a fatty liver [94]. Unfortunately, when patients consume a very low calorie diet and undergo rapid weight loss, these elevations in serum transaminases may be exacerbated. This appears to occur because of the rapid mobilization of stored fat to the liver. Weight loss by very-low-calorie diets (VLCD) reduces fatty change but may induce slight portal inflammation and fibrosis [106].

Increased Uric Acid Levels

Rapid weight loss can also lead to elevated uric acid levels. This elevation is partly due to cell breakdown as well as urate and ketone competition for tubular reabsorption [94]. Although elevation of uric acid can occur, the elevation is seldom high enough to become clinically significant. Elevated uric acid can contribute to gout exacerbations as well as kidney stone formation.

Metabolic syndrome, commonly associated with obesity, alters renal acid–base metabolism, resulting in a lower urine pH and increased risk of uric acid stone disease. The low urine pH is caused by deficient ammonia production, which appears to be related to insulin resistance. Even weight-loss programs to combat obesity can influence stone risk. Contemporary bariatric surgery has been shown to frequently cause hyperoxaluria with associated stone formation and even oxalate nephropathy. Commonly used low-carbohydrate diets increase the risk of both calcium and uric acid stones. Certainly, the many health risks of obesity, including urolithiasis, necessitate weight loss, but recognition of the potential complications of such therapies is required to prevent induction of new and equally severe medical problems [107].

After RYGB surgery, 7.65 % of patients were diagnosed with urolithiasis compared with 4.63 % of obese patients [108]. Urinary oxalate level is higher and urinary citrate level and urinary volume lower after bariatric surgery [109]. It is possible that hyperoxaluria associated with bariatric surgery is linked to fat malabsorption leading to steatorrhea. In normal individuals, calcium and oxalate within the lumen of the intestine combine to form insoluble calcium oxalate complexes that are excreted in the faces. After bariatric surgery, excessive intraluminal fatty acids bind to calcium and leads to inhibition of the formation of calcium oxalate. Therefore, this leads to excess reabsorption of oxalate by intestinal mucosa and increases the risk of hyperoxaluria with subsequent increased risk of renal calculi formation [110]. Oxalate nephropathy may occur and is characterized by tubular crystalline deposition of calcium oxalate leading to renal failure. Strategies to prevent it include restricting oral oxalate and fat ingestion and increasing fluid and calcium intake [110].

Constipation/Diarrhea

Rapid weight loss can also lead to increased gastrointestinal disturbances, such as constipation and diarrhea [94]. Constipation occurs due to combination of factors including decreased water and fiber intake, associated use of constipating agents especially iron, dehydrating action of glycogen store mobilization and increased urinary ketone bodies filtration. Increasing oral fluid and dietary fiber intake while avoiding drinks like coffee, cola and alcohol may prevent constipation.

Diarrhea, which may be due to dumping syndrome, infection, altered ileocecal value dysfunction, lactose intolerance, or pelvic floor disorders, can occur after bariatric surgery. As with any other cases of diarrhea, structural and physiologic causes of diarrhea need to be looked for [111].

Bariatric Surgery Related Complications

Rapid weight loss due to bariatric surgery may be associated with complications including stomal and marginal ulceration, stomal stenosis, band erosion, staple line dehiscence, internal hernias, bile reflux, dumping syndrome, gastroesophageal reflux disease, and functional symptoms, like abdominal distension, abdominal pain, flatus, and diarrhea [101].

Skeletal Loss

Rapid weight loss has also been associated with clinically significant skeletal loss, both osteopenia and osteoporosis. In a study of patients with anorexia nervosa, significant under nutrition and subsequent neuroendocrine dysfunction were found to be associated with rapid and severe bone loss [112]. Specifically, in women with active anorexia nervosa, there was a mean annual rate of Bone mineral density decline of 2.6 % at the spine and 2.4 % at the hip. Investigators found that it was the loss of lean body mass that greatly contributed to loss of bone mineral density. Interestingly, when these women improved their body weight and subsequently had return of menses, they were noted to have an increase in their bone mineral density [112]. Among individuals who lose weight without surgery, epidemiological evidence supports increased rates of hip bone loss in older individuals, irrespective of body mass index (BMI) and an increased risk for hip fracture in middle-aged and older women [113]. Bariatric surgery, in particular gastric bypass surgery, has also been found to negatively impact bone mineral density. In particular, patients who undergo gastric bypass surgery suffer impairment of calcium intake and absorption in the duodenum and proximal jejunum. As a result, they are at an increased risk of bone mass loss and potential fractures. A study of gastric bypass patients showed there was significant bone mineral density loss at the femoral neck and lumbar spine after gastric bypass, with 16.1 % of women having osteopenia at the femoral neck and 19.3 % at the lumbar spine [114]. A literature review, however, found that there is no appreciable difference in areal bone mineral density at the hip region but greater aBMD at the spine and radius in post-surgical bariatric patients compared to obese or overweight populations [113]. Calcium and vitamin D supplementation along with exercise are recommended to prevent any bone mineral density loss during active weight loss.

Infertility

Although there has been significant investigation into the relationship between obesity and male fertility, there have been few studies looking into the effect of weight loss on sperm parameters. A recent case study evaluated male patients who underwent rapid weight loss after undergoing bariatric surgery and found that these patients suffered issues with infertility [115]. Specifically, these male patients were found to have a severe worsening of sperm parameters, such as extreme oligoasthenoteratozoospermia. Investigators hypothesized that this effect was likely the result of the negative impact of both nutritional deficiencies and the release of toxic substances.

Superior Mesenteric Artery Syndrome

Rapid weight loss has been implicated as a cause for Superior mesenteric artery (SMA) syndrome [116]. SMA syndrome is characterized by abdominal pain, postprandial fullness, nausea, vomiting, and weight loss that results from compression of the third part of the duodenum between the

superior mesenteric artery and aorta. Many different medical and psychiatric disorders, such as eating disorders, neuropathies, and cardiac diseases, have been thought to lead to loss of the mesenteric periduodenal fat pad and increase superior mesenteric artery compression of the duodenum [117]. The resulting vomiting and obstructive syndrome leads to further significant weight loss, which only serves to exacerbate the condition.

Neurological Complications

Neurological complications have also been associated with the rapid weight. These neurological complications include peripheral neuropathy, myotonic syndrome, myelopathy, burning feet syndrome, meralgia paresthetica, lumbosacral plexopathy, and Wernicke–Korsakoff encephalopathy [118]. Peripheral neuropathies are the most frequent neurological complications encountered after bariatric surgery, affecting up to 16 % of post-operative patients [118]. In this study, investigators described three different patterns of peripheral neuropathy that occur after bariatric surgery, which include sensory-predominant polyneuropathy, mononeuropathy, and radiculoplexus neuropathy [118].

Nutritional deficiencies are thought to play a very important role in the development of these neurological complications. Patients who undergo bariatric surgery are at an increased risk for vitamin deficiencies due to decreased intake and absorption post-operatively. Thiamine deficiency and vitamin B12 are thought to be the main culprit behind the pathogenesis of polyneuropathies after bariatric surgery [119]. Without vitamin supplementation after bariatric surgery, patients who sustain rapid weight loss are, subsequently, at an increased risk for neurological complications.

Other

There are many other negative consequences associated with rapid and significant weight loss. These include hypotension, hair loss, and cold intolerance [94].

Conclusion

Weight loss resolves or improves multiple comorbidities associated with morbid obesity and evidence suggests that earlier intervention is better in resolution of these comorbidities. Patients with metabolic syndrome and obesity related comorbidities should be promptly evaluated and referred for weight loss and if appropriate be considered for bariatric surgery.

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Part IV
Adipose Tissue and Disease

Chapter 15
Human Lipodystrophy: An Update in Molecular Genetics and Possible Mechanisms of Fat Loss

Anil K. Agarwal

Keywords Lipodystrophy • Molecular genetics • Adipose tissue • Lipid droplets • HIV-associated lipodystrophy • Immunoproteasome • Autoinflammation associated lipodystrophy

Key Points

- Lipodystrophy is a disease characterized by lack of adipose tissue which is either due to genetic defects or acquired.
- Genetic lipodystrophies are inherited both in an autosomal dominant or recessive fashion. Several genetic loci have now been identified affecting differentiation of adipose tissue or lipid storage.
- Although it appears counterintuitive, lack of adipose tissue results in similar clinical burden seen in subjects with obesity, chiefly, insulin resistance, hypertriglyceridemia, fatty liver, and diabetes. This implies a significant role for adipose tissue at the center of energy homeostasis.
- Study of human lipodystrophies has provided a rich trove of genetic loci which affect adipogenesis and/or lipid storage as illustrated by mutations in *AGPAT2*, BSCL2, *LMNA*, or *ZMPSTE24* genes. Thus, identifying additional genetic loci in patients with lipodystrophies will continue to be a rich source of biological material to study the loss of both white and brown adipose tissues.
- In this review I discuss some of the recent findings which have occurred over the last 5 years.

Introduction

The role of adipose tissue in animal physiology is for maintaining energy balance, storing excess energy in the form of triacylglycerol (TAG) mainly in adipocytes, and releasing energy when needed as free fatty acid to be utilized by other organs. This is a finely tuned feature of an animal energy physiology and any dysregulation can result in metabolic diseases. This is illustrated by the fact that excess energy storage results in obesity, which has gained epidemic proportions in the modern era, and is associated with hyperinsulinemia, insulin resistance, diabetes and hypertriglycemia. So it

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would be anticipated that loss of adipose tissue would be beneficial, especially in humans, although this is not always the case. Selective loss of adipose tissue in humans has been described as "lipodystrophy" [1]. Lipodystrophy has a wide spectrum and pattern of adipose tissue loss. Generalized lipodystrophy manifests with near total loss of adipose tissue which can be congenital (since birth) or acquired later in life. Partial lipodystrophy manifests with loss of adipose tissue from the upper and lower extremities, torso, or from other regions of the human body such as retro-orbital, soles of the palms and feet, and those of the skull [2]. Similarly, partial loss of adipose tissue could also be familial or acquired. The classification of lipodystrophy has been discussed in several recent reviews to which readers are referred [3, 4]. Contrary to what would be expected, patients with lipodystrophies also carry similar clinical burden as those observed with obesity. Thus, this observation: both obesity and lipodystrophy place adipose tissue at the center of energy homeostasis. In this review, I discuss the recent advances in the study of lipodystrophy.

Generalized Lipodystrophy

Since we last summed up the studies on lipodystrophy in this series in 2007 [1], new genetic loci and mouse models have been developed identifying additional genes associated with fat loss and helping to understand the pathophysiology of lipodystrophy.

Mutation in Genes Associated with Lipid Droplets

Proteins like Caveolin-1 (Cav1), Polymerase I and transcript release factor (PTRF1 or cavin-1), and Peripilin1 and Cell death-inducing DNA fragmentation factor 45-like effector (CIDE) protein family member C (CIDE-C) have been found to be mutated in patients with either generalized or partial lipodystrophy (see Table 15.1 for specific gene mutations and general clinical features). Before discussing the mutations in these proteins, a primer in the formation of cellular lipid droplets (LDs) is essential (Fig. 15.1). LDs are mainly found in mature adipocytes but can also be found in additional cell types under certain pathological conditions such as hepatocytes (hepatic steatosis) or myocytes (myosteatosis), although the biochemical nature of LDs in hepatocytes and myocytes is unclear and is still not widely studied [5, 6]. Almost all of the LD biogenesis initiates in the lumen of the endoplasmic reticulum (ER) where it is protected from the hydrophilic nature of cytoplasm [5, 6]. All the enzymes of TAG synthesis are primarily located in the ER and their substrates are either generated in the ER or are transported to the ER. As the LD grows it bulges into the cytoplasm covered by the lipid monolayer consisting mostly of phospholipids. It is during this increase in LD size that numerous proteins are deposited onto the LD surface mostly via their hydrophobic domain to protect the growing LD from degradation. Upon external cues, the lipases, including hormone sensitive lipase (HSL) or adipose triglyceride lipase (ATGL), hydrolyze the TAG in the LD to release the free fatty acids (FFAs) into circulation [7]. Any perturbations of this pathway will have profound effects in the formation and degradation of LDs and thus affect the adipose tissue.

Table 15.1 Patients with lipodystrophies carrying the mutations found in genes associated with lipid droplets

Gene	Mutation	Phenotype	References
CAV1	c.112G>T pGlu38X (homozygous)	Insulin resistance, diabetes, hypertriglyceridemia Generalized lipodystrophy (near total absence of AT)	[12]
	c.410delAI134fsX137 (heterozygous)	Hypocalcemia Partial lipodystrophy (loss of AT in upper part of body, sparing lower half—legs, gluteal region, and visceral) Neuropathy	[13]
	-88delC (heterozygous) 5′-untranslated region	Partial lipodystrophy (pattern of AT loss different than those of I134fsX137. Affected were arms, legs, gluteal region but spared were face, neck, and viscera)	
PTRF	c.696_697insC (p.Lys233fsX191) c.512C>A (p.Ser171X) (compound heterozygous)	Generalized loss of AT High serum creatine kinase Muscle hypertrophy (myopathy)	[16]
	c.696_697insC (p.Lys233fsX191) 525delG (p.Glu176fsX98) (compound heterozygous)		[17]
	c.696_697insC (p.Lys233fsX191) (homozygous)		[17]
	c.135delG (p.Lys45fsX5) (homozygous)		[19]
	c.481_482insGTGA (p.Lys161fsX41) (homozygous)		[19]
	c.518_521delAAGA (p.Lys173fsX101) c.IVS1 + 1G>T (p.Asp158fsX49) (compound heterozygous)		[19]
	c.160G (homozygous)		[18]
	c.362dupT (homozygous)		[18]
CIEDC	c.556G>T (p.Glu186X) (homozygous)	Partial lipodystrophy (loss of fat from lower extremities, gluteal fat, striking hepatomegaly, hepatic steatosis, hyperglyceridemia, diabetic ketoacidosis, developed microalbuminuria, hypertension)	[24]
PLIN1	c.1210-1G>T (p.Leu404fsX158) (heterozygous) c.1191_1192delAG (p.Val398fsX166) (heterozygous)	Partial lipodystrophy (even though BMI indistinguishable from those unaffected, total fat (DEXA) in lower. Reduced AT in upper and lower extremities and truncal fat, hypertriglyceridemia, insulin resistance, and diabetic. Inherited as autosomal dominant)	[28]

For gene abbreviations see the text

Caveolin-1 (Cav1) and Polymerase I and Transcript Release Factor (PTRF1 or Cavin-1)

The caveolin family of proteins (isoforms 1–3) was first identified in caveolae, small invaginations of the plasma membrane found in many cells including adipocytes [8]. In addition to Cav1, additional proteins have also been identified in caveolae such as the cavin protein family member cavin-1 [9]. Since caveolae are plasma membrane structures, it is difficult to envision how Cav1 and/or cavin-1 will

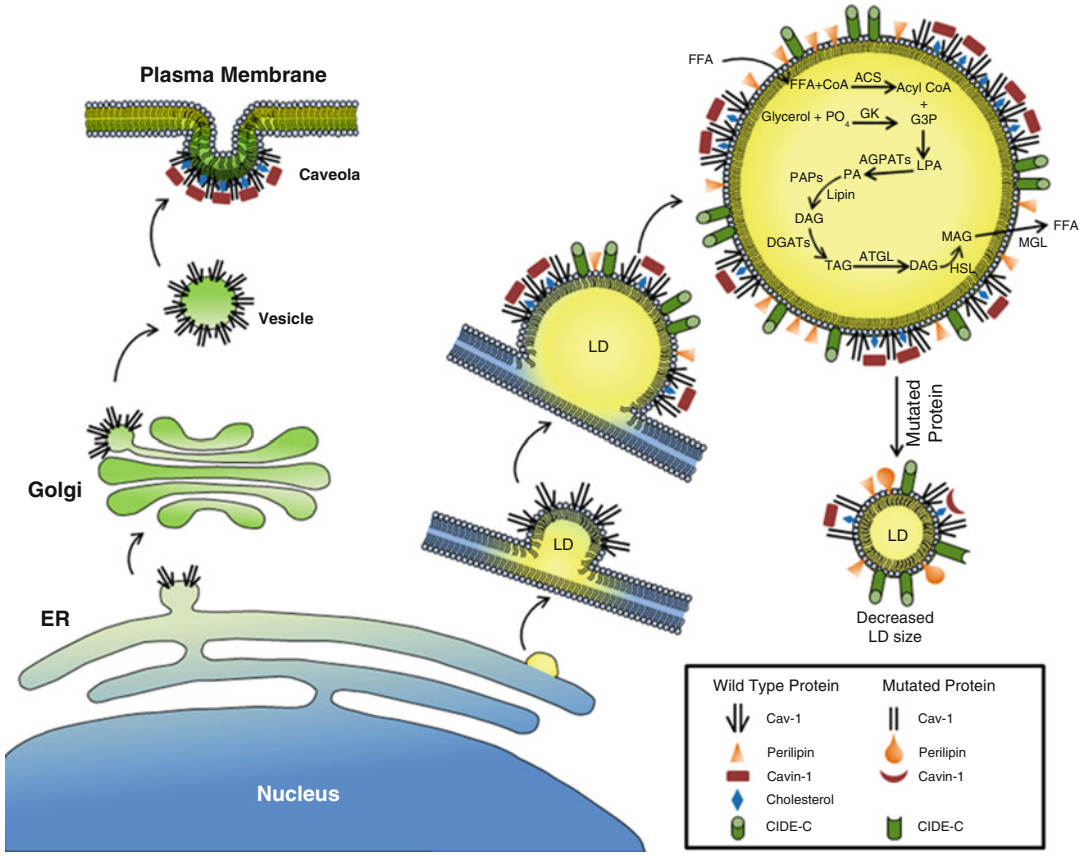

Fig. 15.1 Schematics of the biogenesis of caveolae and lipid droplet. The assembly of caveolae at the plasma membrane has recently been elucidated in cultured cell expression of caveolin-green fluorescence protein and real-time cell imaging techniques. The process initiates by synthesis of caveolin (cav-1) in the endoplasmic reticulum (ER) followed by its exit to Golgi where it buds as a vesicle and ultimately fuses with the plasma membrane, where it can acquire additional proteins including cavin-1 (encoded by *PTRF*). In adipocytes, the biogenesis of a lipid droplet (LD) initiates in the lumen of the ER. As the LD grows, it is covered by a monolayer of phospholipids/lipids. It can be envisioned that the cav-1 does not exit the ER but is instead retained in the ER and attracts additional proteins. Mutant proteins will interfere with the appropriate functioning of LD. On the LD, cav-1 might not form the caveolae as observed on the plasma membrane and remain as a flattened layer. Shown within the LD are the pathways for triacylglycerol synthesis and lipolysis. Shown also are the recently identified proteins whose mutations affect the LD and thus cause lipodystrophy. The caveolae pathway has been partially adapted from Hayer et al. [10]

affect the LD formation. Recently, using fluorescently labeled Cav1 protein and live cell imaging, it was nicely shown how the biogenesis of Cav1 in ER follows the maturation route from ER to Golgi apparatus and to the plasma membrane where it can attract additional proteins [10]. From this model it can also be envisioned, at least in adipocytes (and possibly hepatocyte and myocyte LDs), when the LD which is formed in the lumen of ER begins to enlarge the Cav1 protein remains associated with the ER, thus allowing the binding of Cav1 associated protein such as cavin-1 (Fig. 15.1). Caveolin-1 is an integral membrane protein with a 33 amino acid hydrophobic domain (caveolin scaffolding domain, CSD) which anchors to the lipid layer and provides a scaffold to bind additional hydrophobic protein or lipids [8]. Several proteins have been proposed to interact with CSD including *src*-family kinase, Ras, PPARγ, and β-catenin [11]. A null mutation in Cav1 has been located in the N-terminus (Glu38X) (see Table 15.1) [12] which will render this protein ineffective in localizing either to the

plasma membrane or onto the LD and thus cause loss of all its function. However, it remains unclear how the lack of a single protein, Cav1, in adipocytes will result in the loss of adipose tissue in human subjects. More perplexing is the finding that lipodystrophy occurs even when the mutations are heterozygous (I134fsX137 and -88delC) (Table 15.1) [13]. It is interesting to note that the frame shift mutation I134fsX137 retains the CSD which suggests that the functional domain of Cav1 is located at the carboxy-end of the protein. It is also possible that additional mutant alleles are still to be identified in these patients. Could the caveolae assume a different configuration on the LD surface than those found at the plasma membrane? It is possible that the caveolae on the LD could assume a planer structure, which seems logical due to the continuous increase of LD size and stretching of the lipid layer [14, 15]. It remains to be seen how this feature will change the biogenesis and morphology of associated proteins on LDs, signal transduction to the lumen of ER and the transport of substrate for the synthesis of TAG. It is also interesting to note that the caveolae-associated protein cavin-1 has also been found to be mutated in several patients with congenital generalized lipodystrophy (see Table 15.1) [16–19] but the molecular mechanism as to how mutations in *PTRF-1* will result in loss of adipose tissue is still lacking.

Cell Death-Inducing DNA Fragmentation Factor 45-Like Effector-C (CIDE-C)

The cell death-inducing DNA fragmentation factor 45-like effector (CIDE) protein family which includes three members, CIDE-A, CIDE-B, and CIDE-C (Fsp27 is an ortholog of CIDE-C in mouse) were initially identified as apoptosis factors [20]. These proteins contain two domains: (a) a 40 kDa domain possessing the DNA fragmentation activity (DFF40; aka caspase-activated DNase (CAD)) and (b) a 45 kDa domain (DFF45) possessing the inhibitor activity of CAD (ICAD). Normally, DFF45 binds to DFF40 and prevents DNA fragmentation. Upon apoptotic signaling, DFF45 is cleaved by caspase-3, releasing DFF40 which then fragments DNA [20]. However, these proteins have additional functions as well [21]. CIDE-C (Fsp27) is very highly expressed in white adipose tissue and was found to be localized to LDs, protecting them from lipases. *Fsp27* (CIDE-C) deficient mice have a lean phenotype (decreased white adipose tissue) with associated mild clinical features of lipodystrophy as observed in humans [22, 23]. This is consistent with the null mutation observed in a female patient, Glu186X, in exon 6 (Table 15.1) [24]. In vitro studies in COS-7 or differentiated 3T3-L1 cells showed that the mutant failed to localize to the LDs which resulted in multi-locular LDs [25]. It is interesting to note that incubation of HeLa cells with an excess of oleic acid offsets the apoptotic function of CIDE-C (Fsp27) [26]. Expression of Fsp27 protein (amino acids 174–192), which is critical for LD localization, is also required for apoptosis [26]. The CIDE-C mutation Glu186X lies in this critical region [24].

Perilipin 1 (PLIN1)

The perilipin family of proteins [27], the most widely studied LD associated proteins, was also shown to harbor mutations in patients with lipodystrophy [28]. Most mutations were frame shifts, were inherited as autosomal dominant and were predicted to generate a truncated protein (Table 15.1). *PLIN1* mutants Leu404fsX158 and Val398fsX166, when overexpressed in preadipocytes, failed to bind to abhydrolase domain containing 5 (ABHD5), a co-activator of ATGL [29], thus failing to suppress basal lipolysis.

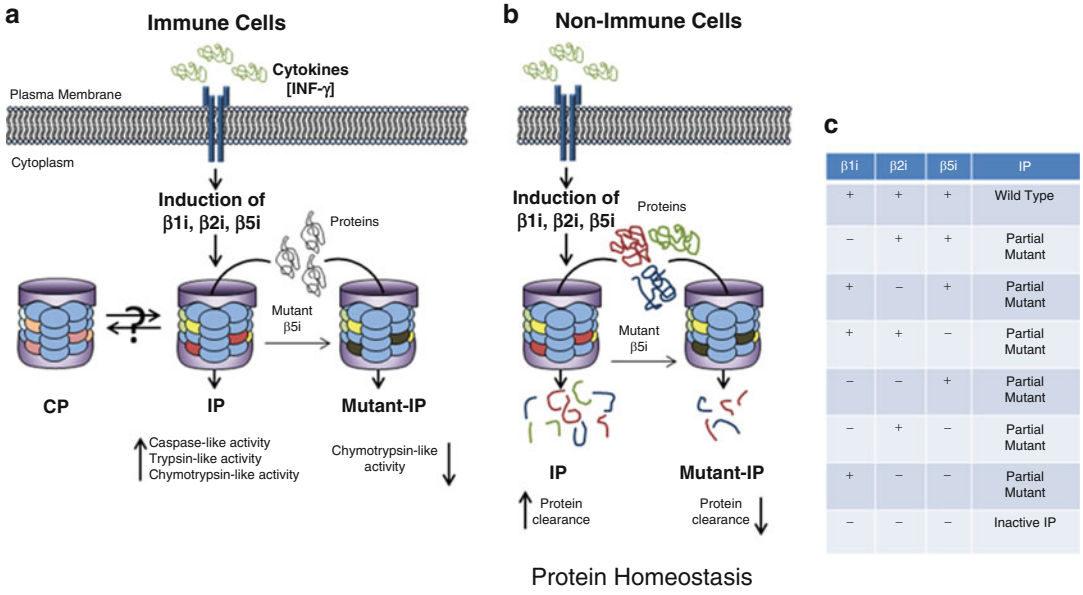

Fig. 15.2 Schematics of constitutive proteasome (CP) and immuno-proteasome (IP) function in immune and non-immune cells. (**a**) CP, in addition to various other subunits, includes subunits β1, β2, and β5. Stimulation by cytokines such as interferon gamma (INFγ) induces the transcriptional activation of subunits β1i (LMP2 or PSMB9), β2i (MECL1 or PSMB10), and β5i (LMP7 or PSMB8) which are assembled into proteasomes known as IP. These subunits have caspase-like, tryptic-like and chymotryptic-like protease activities. Upon mutation of a subunit, such as β5i (PSMB8), the mutant-IP will reduce the chymotryptic-like protease activity resulting in inefficient presentation of MHC class I epitopes. The assembly of CP and IP appears to be independent of each other, although the possibility does exist that the exchange of subunits can occur as shown by the question mark. (**b**) Recent evidence suggests that IP in non-immune cells are involved in the clearance of oxidized proteins, the mutant-IP will result in its inability to maintain the intracellular protein homeostasis in these cells. It is not clear what the ratio of CP to IP is in non-immune cells and how this will affect the function of the cells. (**c**) Theoretically, several possibilities exist in the variation of subunits in mutant-IPs. Either the mutant-IPs exist with individually mutated subunits, as described in panel **b**, or by having double or triple subunits mutant-IPs. A recently generated triple knockout mouse for subunits *Psmb8*, *9* and *10* revealed that these subunits do have some redundant function that was not obvious when these were deleted individually. However, it is highly unlikely that in humans there would be double or triple mutations of these subunits

Proteasome Subunit, Beta-Type, 8 (PSMB8)

Mutations in this gene have also been associated with human lipodystrophy. We initially identified a homozygous missense mutation (Thr75Met) in patients with an autosomal recessive autoinflammatory disorder with lipodystrophy characterized by Joint contractures, muscle atrophy, Microcytic anemia, Panniculitis (JMP) [30]. Since then, two additional groups have reported mutations in *PSMB8*: patients with Nakajo-Nishimura Syndrome (NNS) [31] and those with Chronic Atypical Neutrophilic Dermatosis with Lipodystrophy and Elevated temperature (CANDLE syndrome) [32]. Common to all these patients is loss of adipose tissue and features of autoinflammation, a relatively new word coined to distinguish unexplained recurrent attacks of inflammation from autoimmune diseases. The defining features of autoinflammation are (a) increased inflammation mediated by cytokines of the innate immune system, (b) without the presence of autoantibodies or autoreactive T lymphocytes, and (c) with host predisposition [33].

PSMB8 encodes β5i, a catalytic subunit of the immunoproteasome. Immunoproteasome-mediated proteolysis generates immunogenic epitopes presented by major histocompatibility complex (MHC) class I molecules (Fig. 15.2). This degradation is followed by covalent modification of the proteins

with polyubiquitin [34, 35] which targets them to the 26S proteasome. The 26S proteasome is composed of two subcomplexes: the 20S proteasome that contributes protease function and PA700 (19S regulator) that mediates translocation of the attached substrates to the 20S proteasome. The 20S proteasome is a 700 kDa complex composed of four axially stacked heptameric rings. Each of the two identical outer rings contains seven different α type subunits and each of the two identical inner rings contains seven different β type subunits. The β1, β2, and β5 subunits display different specificities for peptide bond hydrolysis and have predominantly caspase-like, trypsin-like, and chymotrypsin-like protease activities, respectively. Higher eukaryotes contain isoforms of β1, β2, and β5, termed β1i, β2i, and β5i, respectively, which are selectively incorporated into immunoproteasomes. Immunoproteasomes are constitutively expressed in cells of hematopoietic origin, particularly lymphocytes and monocytes, but can be induced in nonhematopoietic cells after exposure to inflammatory cytokines such as interferon-γ (INF-γ). Proteasomes and immunoproteasomes are functionally very similar but not identical. Immunoproteasomes stimulate cleavage after hydrophobic, basic and branched chain residues, while suppressing cleavage after acidic residues and thus potentially enhancing the generation of some antigenic epitopes differently from the constitutive proteasomes. However, a clear distinction of the role of constitutive proteasomes and immunoproteasomes in immune response has not yet emerged [34]. How mutations in *PSMB8* result in decreased adipose tissue in these patients is not entirely clear. It can be speculated that since INF-γ is increased during the autoinflammatory state, expression of mutated *PSMB8* will also increase, generating an increased number of defective immunoproteasomes. Although not shown for adipose tissue, it is likely that such compromised immunoproteasomes will result in defective or reduced protein clearance resulting in dysregulated adipocytes [35]. As shown previously, in vitro proteosomal activity in lymphoblasts from affected patients did show decreased chymotrypsin-like protease activity [30]. It is also possible that these defective immunoproteasomes in adipocytes might generate peptide fragments (antigenic-epitope), inducing tissue specific self-destruction of adipose tissue.

Fibrillin I (FBN1)

Mutations in the fibrillin (*FBN1*) gene were first reported in a female patient who presented with a generalized lipodystrophy and also had features of Marfan syndrome (MFS; fulfilling the Ghent criteria) which includes dilated aortic bulb and severe myopia [36]. However, this patient, even at age 25, did not have metabolic complications, i.e., insulin resistance, hypertriglyceridemia, hepatic steatosis and diabetes. The clinical features of this patient compare well to those noted in patients with Wiedemann–Rautenstrauch syndrome which is characterized by accelerated aging with lipodystrophy [37]. Upon mutational analysis for several genes, a de novo heterozygous dinucleotide deletion was noted in *FBN1* (c. 8155–8156 delAA in exon 64; p.Lys2719fsX18). Subsequently, a 20-year-old man with clinical features as noted above also carried a de novo 20-nucleotide heterozygous deletion resulting in a frame shift mutation (c. 8156–8175 del, p.Lys2719fsX12) in the *FBN1* gene [38]. This was followed by a report which again identified a de novo heterozygous splice site mutation in a 35-year-old female subject with clinical features of accelerated aging and lipodystrophy [39]. This splice site mutation (c.8226 + 1G>T) is predicted to retain 11-nucleotides of intron 64 and will have an aberrant carboxyl-terminus fibrillin protein. It is interesting to note that all the FBN1 mutations in these patients occurred de novo and the patients were heterozygous for these mutations.

Fibrillin is a rather large protein with a MW of ~350 kDa, is glycosylated and has several domains, 47 of which are epidermal-growth-factor like (EGF) domains. Most of these EGF domains are calcium binding EGFs [40] and are interspersed with transforming growth factor-beta (TGF-β) binding protein-like (TB) domains. Fibrillin protein is part of the extracellular matrix (ECM) which forms microfibrils anchoring several proteins and binding to the cellular membrane via integrins which are proposed to be sites for cross talk between various growth factors, integrins and the cell [41, 42].

Several fibrillin microfibril models have been suggested [43] but the one which most closely relates to the mutations found in patients with lipodystrophy corresponds to the carboxy-terminus end of the fibrillin protein. Mutations most likely result in mis- or inappropriate formation of amino-terminus to carboxy-terminus interaction between fibrillin proteins. This is reminiscent of the inappropriate assembly of lamin A/C found in the nucleus in patients with familial partial lipodystrophy of the Dunnigan variety.

New Murine Models of CGL

The last few years have seen the development of two new mouse models of CGL: *Agpat2*$^{-/-}$ and *Bscl2*$^{-/-}$.

AGPAT2 Null Mice (Agpat2$^{-/-}$*)*

1-Acylglycerol-3-phosphate-*O*-acyltransferase 2 (AGPAT2) enzyme converts 1-acylglycerol-3-phosphate (aka lysophosphatidic acid, LPA) to 1,2-diacylglycerol-3-phosphate (aka phosphatidic acid, PA). There are 11 known isoforms of AGPAT, each encoded by a different gene. The bioinformatics, biochemical characterization and tissue distribution for each of these AGPAT isoforms have been described before [44]. The role of human AGPAT2 became apparent when we discovered a variety of mutations in patients with autosomal recessive CGL1 [45, 46]. Patients with CGL1 have a generalized lack of body fat from birth and develop severe metabolic complications. These patients are hyperinsulinemic, insulin resistant and, in extreme cases, develop acanthosis nigricans (an extreme manifestation of insulin resistance), hypertriglyceridemia, early onset of diabetes (mostly during pubertal years), and hepatic steatosis. Some affected women develop polycystic ovaries (PCOS) [2, 45–47]. Women with PCOS, either congenital generalized or partial or acquired, showed significant improvements in clinical features of PCOS e.g., decreased plasma level of testosterone but increased serum hormone binding globulin (SHBG) and increased insulin sensitivity when placed on leptin replacement therapy. Thus, this human model of PCOS supports the role of insulin resistance in the development of PCOS [48]. To further understand the physiological role of AGPAT2, we developed and phenotyped the *Agpat2* knockout mouse [49]. Like human CGL1, *Agpat2*$^{-/-}$ mice develop lipodystrophy and metabolic complications. The *Agpat2*$^{-/-}$ mice lose both the white and brown adipose tissue (Fig. 15.3a), become highly insulin resistant and develop diabetes and hepatic steatosis. In analyzing the livers of these *Agpat2*$^{-/-}$ mice we discovered that the de novo lipogenesis was independent of sterol regulatory element-binding protein-1c (*Srebp-1c*), a transcription factor associated with almost all forms of hepatic steatosis. We also discovered a very robust upregulation of monoacylglycerol acyltransferase 1 (*Mgat1*). As shown in Fig. 15.3b, in the absence of AGPAT2 in the livers of *Agpat2*$^{-/-}$ mice, the alternate MGAT pathway to synthesize TAG is activated, which is mainly found to be active in enterocytes. Surprisingly, the total glycerophospholipid (GPL) levels in the livers of *Agpat2*$^{-/-}$ mice were not very different from those of wild-type mice. Similar observations were made where inhibiting *Agpat2* using siRNA in cultured cells (OP9, a cellular model for adipogenesis) indicated additional pathway(s) to generate PA [50]. Lack of any adipose tissue in humans and *Agpat2*$^{-/-}$ mice precludes any study with adipose tissue per se. However, a recent study using muscle-derived multipotent cells (MDMCs) isolated from vastus lateralis biopsies obtained from CGL1 patients failed to differentiate into the preadipocyte lineage, whereas differentiation to myocytes remained unaffected. It appears that AGPAT2 generates a specific ligand for PPARγ which is lacking in MDMCs

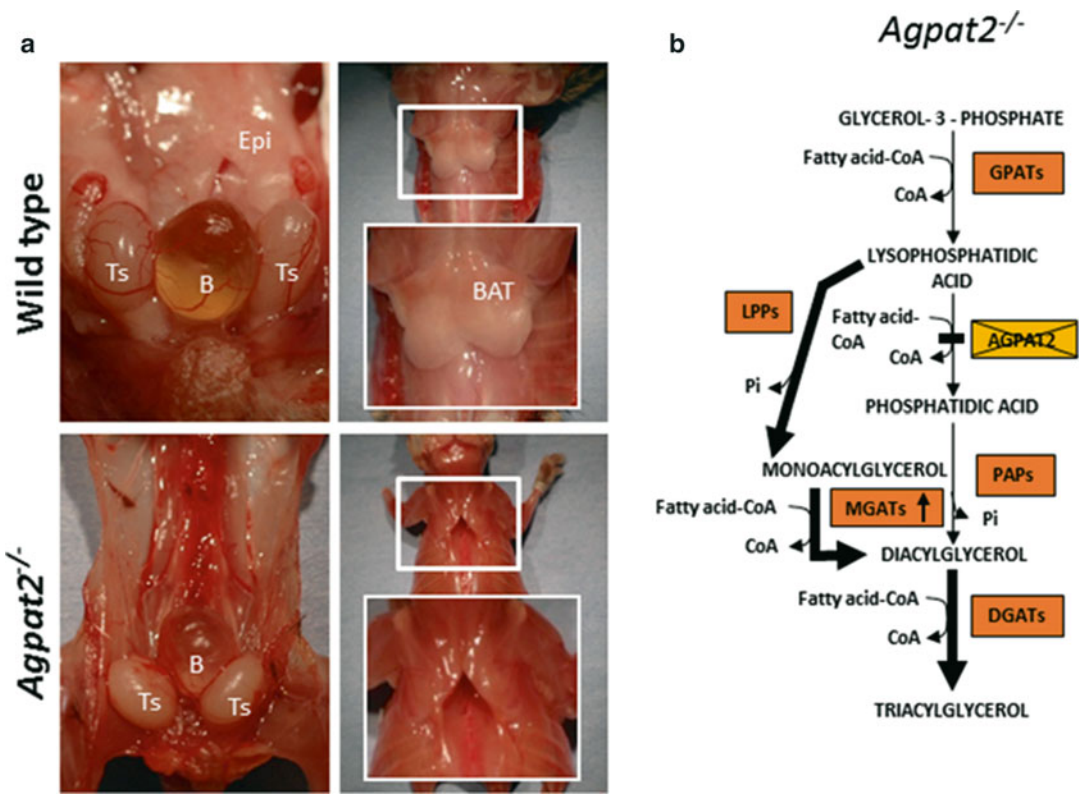

Fig. 15.3 Images of lipodystrophic (fatless) mice and schematics for the activation of monoacylglycerol acyltransferase (*Mgat*) pathway for triacylglycerol synthesis: (**a**) *upper panel* shows the white and brown adipose tissue in wild-type mouse and *lower panel* shows the loss of both white and brown adipose tissue as seen in the *Agpat2*$^{-/-}$ male mice. (**b**) Shows the likely pathway for the synthesis of DAG and PA in the livers of *Agpat2*$^{-/-}$ mice. In the absence of *AGPAT2* in the livers of the *Agpat2*$^{-/-}$ mice, *Mgat1* is robustly upregulated (25–50-fold) which then generates DAG, serving as a precursor for both TAG and PA synthesis. *Epi* epididiymal adipose tissue, *Ts* testis, *B* bladder, *BAT* brown adipose tissue. Reproduced, with permission, from Wolters Kluwer Health. Agarwal, A., Lysophospholipid acytransferases: 1-acyglycerol-3-phosphate O-acytransferases. From discover to disease. *Current Opinion in Lipidology*, 23, 290–302, 2012

obtained from CGL1 patients. However, this experiment should be interpreted with caution as the in vitro differentiation protocol of MDMCs was carried out in the absence of any known PPARγ agonists [51]. Thus, it is unclear if these MDMCs will differentiate to preadipocyte/adipocyte lineage upon incubation with PPARγ agonists which could substantiate the lack of generation of PPARγ ligand(s) in CGL1 derived MDMCs. This observation further corroborates our previous observation in *Agpat2*$^{-/-}$ mice that AGPAT2 plays a significant role in forcing cells toward the adipocyte lineage. More intriguing is the fact that upon overexpressing AGPAT2 using recombinant adenovirus in the *Agpat2*$^{-/-}$ mice and despite the normalization of AGPAT enzymatic activity to that of wild-type mice, the *Agpat2*$^{-/-}$ mice did not show any amelioration of insulin resistance, diabetes or hypertriglyceridemia [52]. Furthermore, overexpressing AGPAT1, which has very similar biochemical properties to AGPAT2, failed to ameliorate the metabolic features of *Agpat2*$^{-/-}$ mice, indicating non-redundant functions of either AGPATs [52]. This observation suggests the specific role of AGPAT2 is in the generation of adipose tissue in humans and mice and illustrates the importance of adipose tissue in maintaining the lipid homeostasis.

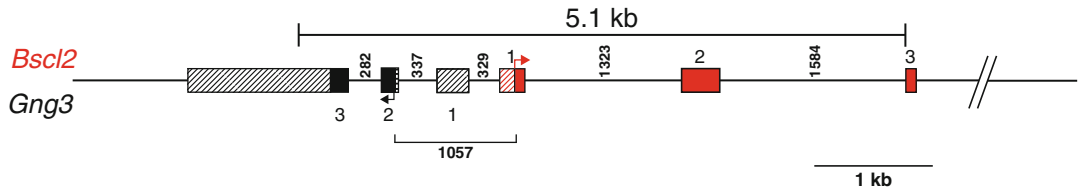

Fig. 15.4 Schematic of mouse *Bscl2* and *Gng3* genes. The mouse *Bscl2* and *Gng3* genes are localized on chromosome 19A and share the promoter region. The *Bscl2* gene, marked in *red*, is transcribed from the upper strand with the *arrow* indicating the direction of the putative translational start site. The *Gng3* gene, shown in *black*, is transcribed from the lower strand in the opposite direction to that of the *Bscl2* gene. The *arrow* indicates the translational start site. As shown, there is an extensive overlap between the proximal promoter regions of these genes. The targeting construct for generating the floxed allele for *Bscl2* in order to remove exon 3 of *Bscl2* will require the construction of at least a 5–10 kb arm, which overlaps the *Gng3* sequence. It is likely that such a targeting strategy might disrupt the expression of *Gng3* as well. Exons are shown as *filled boxes*; *striped boxes* represent untranslated regions. Shown only is the overlapping portion for *Bscl2* and *Gng3*. The number of intronic nucleotides is shown between exons

Berardinelli–Seip Congenital Lipodystrophy Type 2 Null Mice (Bscl2$^{-/-}$)

In mice, homozygous deletion of *BSCL2* (exon 3 deletion), which encodes for the protein seipin, provides an interesting lipodystrophic model [53]. As opposed to the *Agpat2*$^{-/-}$ mouse [49], the presence of adipose tissue was detectable in adult *Bscl2*$^{-/-}$ mice, albeit with considerably reduced fat pads. Nevertheless, these mice did develop hepatic steatosis, glucose intolerance, and hyperinsulinemia, but interestingly not hypertriglyceridemia as observed with CGL2 patients. *Bscl2*$^{-/-}$ mice also had a different phenotype regarding the presence of adipose tissue to those of human subjects [54] where a more severe and significant loss of all adipose depots, including retro-orbital, palm, sole, and skull, are observed. The mechanism proposed for the reduced adipose tissue in *Bscl2*$^{-/-}$ mice is due to increased lipolysis which in turn interferes with adipocyte development [53]. Whether seipin in adipocytes contributes towards adipocyte differentiation is unclear, but based on the presence of reduced adipose tissue it appears unlikely. Alternatively, given that adipose tissue is a heterogeneous tissue (several different cell populations), only some specific cell types are lost, resulting in reduced adipose tissue. Another *Bscl2*$^{-/-}$ model (also generated by deleting exon 3) showed a very similar phenotype, further illustrating that the increased lipolysis is due to the activation of cyclic AMP (cAMP) dependent protein kinase A [55]. If the role of seipin in adipocytes is to protect it from increased lipolysis, then overexpression of seipin in adipocytes should decrease the lipolysis. However, adipose tissues obtained from Tg-aP2-hseipin-398aa (short-form of seipin protein) mice are not protected from elevated lipolysis [56] as seen in the *Bscl2*$^{-/-}$ mice. These transgenic mice also have reduced adipose tissue, but not undetectable as observed in *Agpat2*$^{-/-}$ mice. However, the Tg-aP2-hseipin-398aa mice are resistant to obesity on a high fat diet but remain glucose intolerant [56]. Another possibility for the varied phenotype between CGL2 patients and *Bscl2*$^{-/-}$ mice could be the very nature of *Bscl2* gene deletion. The *Bscl2* and adjacent G protein gamma 3 subunit (*Gng3*) genes have a shared proximal promoter region and exon 3 of *Bscl2* lies very close to this promoter region (Fig. 15.4). It can be speculated that the expression of *Gng3* might be affected which in turn affects the cAMP induced lipolysis. Neither work reported whether the expression of *Gng3* remained unaffected in adipose tissue or hepatocytes. While the role of AGPAT2 in the synthesis of GPLs and TAG has been well documented, the biochemical properties of seipin protein still remain unclear. Seipin lacks any known functional domain which might provide clues to its function [57]. Given the phenotypic differences observed between *Bscl2*$^{-/-}$ mice, transgenic-seipin mice and the patients with mutations in *BSCL2* [54], it is now important to determine and understand the function of endogenously expressed seipin in adipocytes as well as in other tissues and its interacting protein partners to define its cellular function.

Human Immunodeficiency Virus (HIV) Associated Lipodystrophy

In addition to the patients described above who have lipodystrophy mainly associated with germline or de novo mutation, there is a large group of subjects who are infected with HIV-1 virus and develop lipodystrophy when put on a drug regimen consisting of viral reverse transcriptase inhibitor, nucleoside analogues (NRTIs) or non-nucleoside analogues (NNRTIs), and viral protease inhibitors (PIs) collectively referred to as highly active antiretroviral therapy (HAART) [58]. Soon after the introduction of PIs in the HAART regimen, changes in the distribution of body adipose tissue were observed characteristic of those seen in patients with familial partial lipodystrophy, albeit with one additional feature. In some patients there was excessive accumulation of fat in the dorso-cervical region referred to as "buffalo hump".

There is yet no consensus as to how the PIs affect the redistribution of adipose tissue, but it is clear that loss of adipose tissue carries the same clinical burden as observed in severe cases of lipodystrophy (insulin resistance, hypertriglyceridemia, steatosis of liver, muscle, and pancreas, and diabetes). Mutations in zinc metalloproteinase (*ZMPSTE24*) have been found in human subjects who have lipodystrophy [59]. Therefore it is reasonable to assume that inhibition of ZMPSTE24 enzymatic activity might be involved in fat loss or its re-distribution in HIV-1 patients receiving PIs. Recent studies suggest that PIs inhibit the protease activity of ZMPSTE24, thereby affecting the maturation of lamin A protein [60], the only known substrate for ZMPSTE24. The generation of mature lamin A from prelamin A involves farnesylation of cysteine in the conserved CAAX motif at the carboxy-terminal. The AAX tripeptide is then proteolytically removed by ZMPSTE24, followed by methylation of the prenylated cysteine. Finally, a second proteolytic cleavage by ZMPSTE24 or other protease removes 15 amino acids from the carboxy-terminal [61]. Thus, the generation of prelamin A in the absence of ZMPSTE24, either unfarnesylated or farnesylated, will affect the cellular function. We have previously reported that prelamin A interacts with nup53 (a nucleoporin) when overexpressed in cultured human embryonic kidney 293 cells [62]. In fact, images obtained from mouse embryonic fibroblasts treated with PIs do show a nucleoplasmic reticulum (NR) complex with prelamin A affecting the nuclear pore [63]. Thus, one potential mechanism by which PIs dysregulate the adipose tissue would relate to inappropriate communication between the nucleus and the cytoplasm. As an example, SREBP-1c is a master regulator of lipogenesis which was found to be mislocalized in cells treated with PIs [64]. Most PIs, including indinavir, nelfinavir, tipranavir, lopinavir, and atazanvir [63], but not darunavir [65], cause accumulation of unfarnesylated or farnesylated prelamin A. It is still unclear if this is due to the defective processing of ZMPSTE24 either by proteolytic cleavage PIs or by the PIs binding the ZMPSTE24 protein itself and thus making it catalytically inactive.

Several studies were performed in vitro using cultured 3T3-L1 cells and various PIs and reporting the usual set of adipogenesis factor (C/EBP-α, PPARγ, aP2), these studies still did not provide a molecular mechanism as to how PIs will inhibit these adipogenesis factors. Recently, attention has shifted to an unbiased approach using global transcriptional analysis of gene sets in adipose tissue obtained from naïve or HAART treated HIV-1 subjects [66]. Through this approach a 417 amino-acid protein containing high levels of hydrophobic residues named FAP48 has been identified. This protein, which binds with FK506-binding proteins (FKBPs), belongs to the family of immunophilins and inhibits adipocyte differentiation when overexpressed [67]. Other culprit(s) are the proteins encoded by the HIV-1 virus itself. Although it is still debated whether HIV-1 virus directly infects the adipocytes, recent experiments suggest that tumor necrosis factor-alpha (TNF-α) stimulates the HIV-1 production in human primary adipocytes. The HIV-1 proteins, mainly viral protein R (Vpr) and Trans-Activator of Transcription (Tat) modulate the glucocorticoid receptors in target organs like adipose tissue and liver, resulting in insulin resistance. How each of these viral proteins affects the adipose tissue is not entirely clear, but recent experiments indicate that Vpr dysregulates the mitochondrial membrane potential releasing apoptotic factors like cytochrome c [68]. The overexpression

of Tat protein in human Simpson-Golabi-Behmel syndrome (SGBS) adipocytes [69] inhibited the progression of adipogenesis gene expression but enhanced the secretion of proinflammatory cytokines such as TNF-α. However, additional experiments are required to fully appreciate the molecular mechanism behind the action of Vpr and Tat proteins in adipogenesis.

Possible Molecular Mechanisms for Loss of Adipose Tissue

While recognition of adipose tissue has been around for some time, understanding the mechanism of committed preadipocyte recruitment and differentiation to mature adipocytes is a relatively young enterprise. A recent survey of PubMed for entries relating to "adipogenesis" showed relative inactivity until the 1990s. The spurt in references relating to adipogenesis was only noted around 2000, correlating well with the onset of obesity worldwide. As shown in Fig. 15.5, the process of adipogenesis undergoes at least two well-defined processes: (a) recruitment of mesenchymal stem cells (MSC) to committed preadipocytes followed by (b) external cues triggering differentiation into mature adipocytes acquiring the ability to synthesize and store TAG and secrete adipokines such as leptin and adiponectin. Study of human lipodystrophy has revealed a number of genetic loci which upon mutation lead to loss of adipose tissue and thus derangements in the adipogenesis program. A loss-of-function mutation in *Agpat2* in mice leads to loss of both the white adipose tissue (WAT) and brown adipose tissue (BAT) [49] which indicates the molecular defects at the "preadipocyte commitment" stage while residual adipose tissue could still be observed in adult mice with loss of functional *Bscl2* [53, 55], suggesting that seipin's role is downstream of the differentiation step; that is, after the "pre-adipocyte commitment stage". Studies in mouse models suggest increased lipolysis in adipocytes. How the increased lipolysis will kill the cells is still unclear.

At least four different genes related to LD synthesis and maintenance have also been found to be mutated in patients with lipodystrophy as alluded above. All are related to the maintenance/lipolysis of LD in the adipocytes. It is to be noted that while these mutated proteins result in defective adipocytes, these same proteins have little or no effect on the LD found in the liver. In fact, lipodystrophic patients commonly have liver hepatocytes [2, 3, 49] engorged with lipids normally found in LDs. This raises an important question: is the formation of LDs in adipocytes and hepatocytes different? If so, what are those unique features associated with the LD in the adipocyte and the liver? Another important factor to reconcile relates to the clinical features of patients with CGL and those of the murine models of CGL. Patients with mutations in *BSCL2* gene (CGL2) are significantly more lipodystrophic than those with mutations in *AGPAT2* gene (CGL1), yet the mouse models have an inverse relationship. *Agpat2*$^{-/-}$ mice have more severe loss of adipose tissue than those of *Bscl2*$^{-/-}$ mice. Do mice have proteins which compensate for the loss of seipin protein while humans do not? From this perspective, as mentioned above, the study of the biochemistry of the seipin protein from both human and mice should receive priority. Seipin protein neither contains any known functional domains found in other proteins nor belongs to a family of proteins with known function(s) that might indicate its possible functional role in adipocytes.

The molecular mechanism for loss of adipose tissue in partial lipodystrophy still eludes us. However, as we see, this study is hampered by the fact that, in partial lipodystrophy, obtaining adipose tissue before the onset of lipodystrophy is not always possible, making it difficult to compare adipose tissue before and after the onset of lipodystrophy. As an approach towards this goal, a pediatrics population can be screened to identify mutations in gene(s) associated with partial lipodystrophy, biopsy the adipose tissue and follow the subject later in life to obtain additional samples of adipose tissue to compare and contrast the differences before and after loss of adipose tissue. This approach will require substantial personnel and financial commitment. Another approach might be to generate a regulated adipose tissue specific knock-in transgenic mouse to follow changes in the expression of transcripts affected by the expressed mutant protein(s). Proteomics, an unbiased global protein analysis, is the

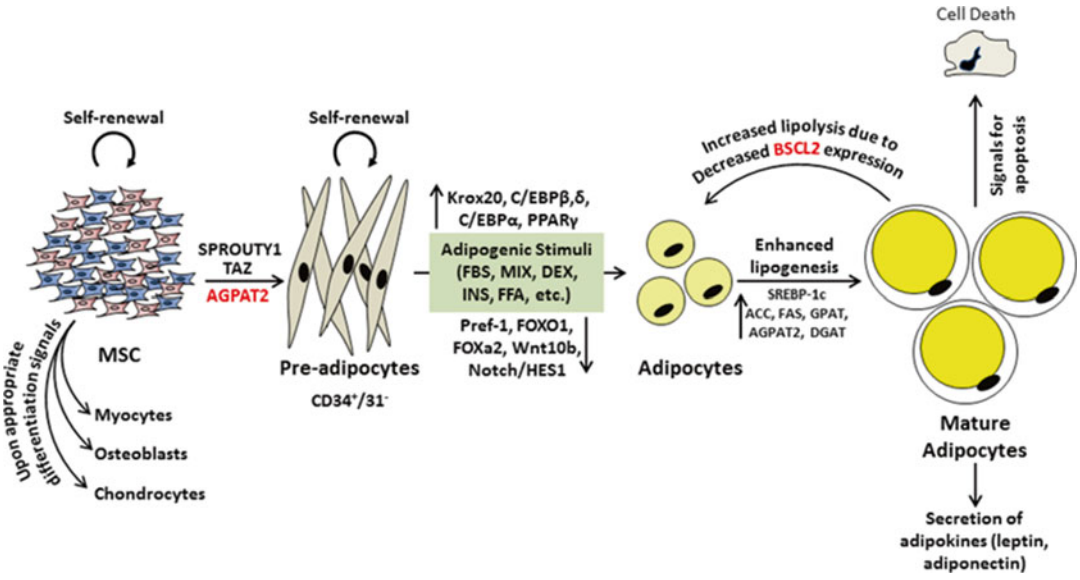

Fig. 15.5 Schematics for adipocyte differentiation and maturation. The mesenchymal stem cells (MSC), upon cues from such factors as sprouty1 [70], transcriptional co-activator with PDZ binding motif (TAZ) [71] and still unidentified factor(s), help commit the MSC to pre-adipocyte cell lineages. Based on our studies of congenital generalized lipodystrophy type 1 (CGL1) patients carrying the *AGPAT2* gene mutations and *Agpat2*$^{-/-}$ mice, we can now consider AGPAT2 as a necessary factor to commit MSC toward pre-adipocyte lineage. Both the MSC and pre-adipocyte can self-renew themselves. The differentiation of pre-adipocytes to mature adipocytes is well studied in cultured cell systems, mainly using 3T3-L1 cells. The transcription factor Krox20 is upregulated, increasing the expression level of C/EBP-β and -δ. C/EBP-β and -δ in turn activate C/EBP-α and PPARγ. Simultaneously, the differentiating adipocytes down-regulate factors including, but not limited to, Pref-1, FOXO1, FOXA2, members of the Wnt family of proteins (Wnt10b), and notch (Notch/HES-1) signaling pathway. These adipocytes undergo a step shown as enhanced lipogenesis when expression of genes involved in fatty acid synthesis (*Srebp-1c*, *Acc*, *Fas*) and triacylglycerol synthesis (*Gpat*, *Agpat2* and *Dgat*) is increased. Adipocytes engorged with lipids secrete several adipokines including leptin and adiponectin or upon apoptotic signals may result in cell death. Based on the *Bscl2*$^{-/-}$ mouse model, we have shown seipin, encoded by the *Bscl2* gene, as enhancing lipolysis in adipocytes and thus affecting the size of adipose tissue but not the differentiation. Increase in the intracellular level of fatty acid resulting from increased lipolysis might result in increased cell death. However, sufficient data are lacking to corroborate this step

next logical approach, comparing the differential proteins found in the serum of patients with or without partial lipodystrophy. However, detecting small levels of serum/plasma proteins is still a challenge and requires significant advances in this proteomic approach.

Conclusion

Studies relating to human lipodystrophies have provided previously unsuspected genetic loci for the development and physiological function of adipose tissue. In the future, continuing efforts by investigators worldwide will provide additional genetic loci and insights into the function of adipose tissue in energy homeostasis and its development in humans.

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Chapter 16
Adipose Tissue and Type 2 Diabetes Mellitus

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Keywords Obesity • Insulin resistance • Type 2 diabetes mellitus • Beta-cell failure • Adipokines/cytokines

Key Points

- Increased production of adipokines/cytokines by enlarged adipose tissue
- Nutrient excess
- Ectopic fat deposition
- Mitochondrial dysfunction
- Impairment of neurocircuits(hypothalamic) regulation of energy homeostasis and insulin sensitivity in the liver and other peripheral areas
- Inflammation in brain area
- Beta-cell failure and development of diabetes.

Abbreviations

AMPK	Adenosine monophosphate kinase
ApN	Adiponectin
BMI	Body mass index
CV	Cardiovascular
CVD	Cardiovascular disease
CHD	Coronary heart disease
CRP	C-reactive protein

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FA(s)	Fatty acid(s)
FFA(s)	Free fatty acid(s)
FABP(s)	Fatty acid binding protein(s)
IL	Interleukin
IR	Insulin resistance
LXR	Liver X receptor
Mc	Microbiota
MCP-1	Monocyte chemoattractant protein-1
MS	Metabolic syndrome
NHANES	National Health and Nutrition Examination Survey
PAI-1	Plasminogen activator inhibitor-1
PPAR	Peroxisome proliferator-activated receptor
ROS	Reactive oxygen species
SAT	Subcutaneous adipose tissue
TAG	Triacylglycerol
Tg	Triglycerides
TNF-α	Tumor necrosis factor-α
T2DM	Type 2 diabetes mellitus
VAT	Visceral adipose tissue

Epidemiological Links Between Obesity and Diabetes

The risk of T2DM increases exponentially as body mass index (BMI) increases above 25 kg/m^2 [1, 2]. By comparison with individuals with a normal BMI of 22 kg/m^2, the risk of T2DM is increased 2–8-fold in those with a BMI of 25, 10–40-fold in those with a BMI >30 and >40-fold in those with a BMI >35, depending on age, gender, duration, distribution of adiposity, and ethnicity [3]. The rates of diabetes and obesity have been rising in recent years in many countries, mostly within Europe and North America, and particularly in the USA [4].

In the USA, the rates of obesity (BMI ≥30) and morbid obesity (BMI >40) have undergone a rapid and sharp increase since 1982 [5]. This rapid increase in the rates of obesity in the USA was followed about 10 years later by a rapid increase in the prevalence of diabetes [5].

In the USA, both African American and Hispanic populations are more affected by obesity and diabetes than obese people of European origin [6, 7]. The prevalence of obesity is elevated in Black and Hispanic females, while a minority of males has rates of obesity similar to those in European American females and males (Centers of Disease Control and Prevention, National Health and Nutrition Examination Survey (NHANES) data, as cited by Garvey [5]).

The rate of diabetes is increasing in all groups of obese subjects, including European American men and women. Minority groups show about double the prevalence rate seen in European American men and women, with African-American females showing the highest prevalence followed by African-American males and then Hispanic males and females (Centers for Disease Control and Prevention, NHANES data [5]). According to Garvey [5], the higher rates of diabetes in both African-American and Hispanic females can be explained by increased obesity, but the higher rates in males cannot. Therefore, gender and racial factors affect the relationship between obesity and T2DM, with African-Americans and Hispanic-Americans showing strong epidemiological associations between obesity and T2DM. This will be discussed later in this chapter.

Similarly in people of South Asian origin, obesity confers a substantially higher risk of T2DM. A BMI of 27.5 in South Asians is associated to similar morbidity to a BMI of 30.0 in caucasians, leading some authorities to suggest that a BMI of 22 or 23 should be considered overweight for

South Asians [3]. Worldwide, the greatest percent increase in the rate of diabetes has been seen in China (31 % increase), followed by India (19 % increase), the USA (17 % increase), and Asia (15 % increase) [5]. In the USA, the region with the highest prevalence of both diabetes and obesity is the southern tier of states, Appalachia and the Indian reservations in the southeast [5].

Relationship Between Obesity and Diabetes

It is well known that there is a rapid rise in the risk of diabetes parallel to a progressive weight gain in adults [8]. It is generally true that an accumulation of abdominal fat (central obesity), as indicated by an expanding waist circumference or an increased waist/hip ratio, is an independent risk factor for T2DM, irrespective of the extent of obesity [9]. This is mainly attributed to increased intra-abdominal (visceral) adiposity. Excessive deposition of lipid in muscle and liver also enhances the risk of T2DM through mechanisms of intracellular "lipotoxicity."

In a cross-section of patients in the NHANES database, among diabetics with progressive obesity and glucose control similar to that in normal-weight T2DM patients, the presence of obesity increased insulin resistance (increased levels of fasting insulin and C-peptide for any given level of ambient glucose) [10]. On the other hand, modest weight loss of up to about 10 % greatly decreased fasting plasma glucose levels, improved comorbidities, and decreased mortality [11]. To prevent or delay diabetes, a loss of 5–10 % of body weight is recommended by the American Diabetes Association, while the National Heart, Lung, Blood Institute recommends a 10 % weight loss.

Pathophysiological Role of Obesity in the Development of T2DM

As previously indicated, there are strong and consistent epidemiological associations between obesity and T2DM in African-Americans and Hispanics. However, the pathophysiological role of obesity in the development of T2DM is complex; determination of this role will require analysis of the mechanisms underlying obesity-associated insulin resistance and its cardiometabolic consequences.

The clustering of dyslipidemia, hypertension and glucose intolerance, predominantly in overweight individuals at risk of heart disease, has received many names, including metabolic syndrome (MS). In Reaven's original description, a central etiological role was attributed to insulin resistance (IR) and/or hyperinsulinemia, which are in part, determined by obesity [12]; his assumption has become the dominant paradigm for MS [13]. However, there is substantial controversy in such association, in part because of measurement problems and the fact that, although "insulin resistance" may be important, it provides an insecure foundation for MS to a level that is no longer considered a useful (or at least measurable) criterion. A number of associated clinical features congregate in individuals at increased risk of heart disease. They cluster together for a reason, and it is important to seek an explanation for this at a pathophysiological level [14].

Yudkin and colleagues [15] showed in a population of healthy adults, a good and consistent correlation between a score for low-grade inflammation derived from circulating concentrations of cytokines TNF-α and IL-6 and the acute-phase markers C-reactive protein (CRP) and fibrinogen, and anthropometric measures of obesity and central fat distribution; approximately 20 % of the variance in the levels of acute-phase markers could be explained statistically on the basis of adiposity.

Many prospective studies have shown that of body fat in the upper (central or abdominal) part of the body is frequently correlated with the features of MS. In contrast, individuals with fat stored in gluteal, femoral, or peripheral depots (lower-body obesity) or with female-type fat distribution have a lower risk of morbidity from these metabolic disturbances [16].

As reviewed by Karelis and colleagues [17], approximately 20 % of the general population can be categorized as obese but metabolically healthy, having low visceral fat levels with a high BMI and high insulin sensitivity. However, 18 % of the population had a normal body weight or was slightly overweight (metabolically obese normal weight), with high visceral fat, low BMI, low insulin sensitivity (insulin resistant) and high liver fat, displaying severe metabolic abnormalities.

The prevalence of these two phenotypes among US adults (≥20 years of age) in the NHANES population (1999–2004) indicated that 23.5 % of normal-weight adults were metabolically abnormal, whereas 51.3 % of overweight adults and 31.7 % of obese adults were metabolically healthy. The independent findings of clustering of cardiometabolic abnormalities among normal-weight individuals were older age, lower physical activity levels and large waist circumference. The conditions related to the abscence of cardiometabolic abnormalities among overweight and obese individuals were younger age, non-Hispanic ethnicity, higher physical activity levels, and smaller waist circumference [18]. The results of this study clearly indicate that obesity may or may not be associated with IR, which was assessed using homeostasis model assessment.

Stefan et al. [19] found that the measurement of visceral fat (with magnetic resonance imaging) provides a powerful tool to discriminate between insulin-sensitive and insulin-resistant (estimated from oral glucose tolerance test results) individuals. In obese subjects, the predictive effect of visceral fat was relatively weak. The amount of visceral fat was lower in the obese-sensitive group than in the obese-insulin-resistant group, but the difference was not significant. In contrast, ectopic fat in skeletal muscle and particularly in the liver (measured with proton magnetic resonance spectroscopy) and the intima–media thickness of the common carotid artery were lower and insulin sensitivity was higher in obese, insulin-sensitive subjects compared with those in the obese, insulin-resistant group. The authors concluded that metabolically benign obesity is not accompanied by IR and early atherosclerosis in humans. Furthermore, ectopic fat in the liver might be more important than visceral fat in the determination of such a beneficial phenotype in obese individuals.

Regarding the relationship of obesity to progression from prediabetes to overt T2DM, IR appears very early, with plasma glucose being kept normal for many years secondary to an exaggerated insulin secretory response. After years of "metabolic stress," beta-cells begin to fail, glucose levels rise, diabetes becomes overt, and the IR worsens [5]. By evaluating glucose disposal rates using hyperinsulinemic clamps and measuring insulin sensitivity in a large number of individuals, Garvey et al. (unpublished data, [5]) noted that about 44 % of individuals without diabetes overlap with diabetics in terms of their degree of IR. On the other hand, 3.1 % of diabetics overlap with nondiabetics in terms of their insulin sensitivity.

Generally there is a positive association between BMI and IR, but with a great deal of variability. Among individuals with a BMI <25, 54 % are in the most insulin-sensitive tertile; 24 % of those with a BMI of 25–29 are insulin sensitive, as are 11 % of those with a BMI of 30–34.9. Conversely, in the most obese group, with a BMI ≥35, 60 % are in the lowest insulin sensitivity tertile. Obese individuals with the greatest degree of IR had higher blood pressure, triglyceride levels, fasting and 2-h glucose levels (oral glucose tolerance test) and lower HDL-cholesterol levels [20], corresponding to the MS trait cluster discussed previously.

While BMI explains 11 % of the individual variability in insulin sensitivity, in the study by Garvey [5], the association between central fat (intra-abdominal plus abdominal subcutaneous fat measured by dual-energy X-ray absorptiometry) and insulin sensitivity assessed by euglycemic/hyperinsulinemic clamp in 23 healthy women with differing risk factors for T2DM showed a strong negative correlation ($r = 0.89$, $p < 0.001$), independent of total adiposity, family history of T2DM and past gestational diabetes. There was a large variation in insulin sensitivity, with a similar variation in the amount of central fat whether the individuals had a BMI <25 or were overweight [21]. Although excess fat in any region of the body is associated with increased risk of T2DM and cardiovascular disease [22], the accumulation of abdominal visceral fat, as indicated by an increased waist/hip ratio

or better, by evaluating fat accumulation in the visceral component (computed tomography or magnetic resonance imaging scan at the L4–L5 level) is an independent risk for T2DM, irrespective of the extent of obesity.

Several distinct mechanisms have been proposed to link obesity to IR and predispose individuals to T2DM [23]:

- Increased production of adipokines/cytokines, including tumor necrosis factor-α (TNF-α), resistin, and retinol-binding protein 4, all of which contribute to IR, as well as reduced levels of adiponectin [24].
- Ectopic fat deposition, particularly in the liver and perhaps also in skeletal muscle, and dysmetabolic sequelae [25].
- Mitochondrial dysfunction, as evidenced by decreased mitochondrial mass and/or function [26]; mitochondrial dysfunction could be one of the many important underlying defects linking obesity to diabetes, both by decreasing insulin sensitivity and by compromising beta-cell function.
- Because obesity is associated with an impairment of the neurocircuits (hypothalamic) regulating both energy homeostasis and insulin sensitivity in the liver and perhaps other peripheral tissues [27], and inflammation similar to that induced by obesity in peripheral insulin-sensitive tissues also occurs in these areas of the brain [28, 29]. Obesity induced IR may arise not only as a direct consequence of excessive adipose mass but also via neuronal mechanisms [23]; this mechanism linking obesity to IR will be discussed later.

Increased Production of Adipokines/Cytokines

As suggested by Yudkin and colleagues [13, 15] a more likely paradigm for MS seems to be an adipose tissue-generated molecules initiating a state of low-grade inflammation, with the known actions of these pro-inflammatory cytokines resulting in the combined metabolic, hemodynamic, and vascular consequences of this state. In this paradigm, IR merely becomes another consequence of this low-grade inflammatory state. Furthermore, IR (MS) was a more important contributor than obesity to cardiovascular (CV) risk in a study of CV risk in women referred for coronary angiography. It was shown that patients with a low risk of CVD events are metabolically normal or insulin sensitive regardless of whether they are overweight, obese or of normal weight. On the other hand, patients that showed IR had greater rates of CVD events independent of their BMI [5, 30].

As indicated below, adipose tissue secretes a number of bioactive peptides or proteins, collectively named adipokines, which are markedly dysregulated in those with obesity, T2DM, or "MS" [31, 32].

1. Overproduction of potentially deleterious adipokines in obesity
   (a) Pro-inflammatory cytokines and chemokines
      - TNF-α levels are increased in individuals with IR, who also show decreased adiponectin levels and increased lipolysis (VAT > SAT).
      - IL-6 level is increased in individuals with IR (VAT > SAT).
      - The level of monocyte chemoattractant protein-1 (MCP-1), also known as C–C motif chemokine ligand 2 (CCL2), which is involved in recruitment of monocytes/macrophages into the adipose tissue, is increased in individuals with IR (VAT ≈ SAT).
      - The level of resistin (whose role in humans is not clear) is possibly also increased in those with IR, directly or indirectly, via inflammatory pathways related to CVD.

(b) Adipokines directly involved in thrombosis and hypertension

- Plasminogen activator inhibitor-1 (PAI-1) level is decreased in those with fibrinolysis and increased in those with IR (VAT > SAT).
- The level of angiotensinogen, a precursor of the vasoactive peptide angiotensinogen II and associated with blood pressure and proinflammatory effects is increased in individuals with angiogenesis (VAT > SAT).

(c) Adipokines linked to vitamin A metabolism

- Retinol-binding protein-4 (RBP-4) is potentially involved in the pathogenesis of IR and T2DM.

2. Dysregulated secretion/action of potentially beneficial adipokines.

(a) Leptin is a satiety signal with endocrine, angiogenic. and atherogenic effects; its level is increased in insulin sensitivity and decreased in osteogenesis (SAT > VAT).
(b) Adiponectin (ApN) (SAT > VAT): Circulating ApN is negatively correlated with BMI and decreased in obese individuals with T2DM and CVD.

Receptors

ApN R1—activation of AMPK.
ApN R2—activation of PPAR-α (increase in FA oxidation and decrease in gluconeogenesis: increase in insulin sensitivity).

After binding, ApN exhibits insulin-sensitizing and fat burning effects reminiscent of those of leptin, but it also possesses anti-atherogenic, anti-inflammatory, and antioxidant properties, thereby simultaneously preventing several facets of MS.

(c) Apelin (VAT = SAT) is an endogenous ligand of the orphan G-protein-coupled receptor AJP, which is the closest homolog to the angiotensin II receptor. Beneficial effects of this adipokine on metabolism (insulin sensitivity) and CV function have been shown.
(d) Visfatin (VAT > SAT) has nicotinamide adenine dinucleotide (NAD) biosynthetic activity and is essential for pancreatic beta-cell function [33].

The primary mechanisms of action of adipose tissue-produced inflammatory adipokines can be related to the anatomic location of the fat depot in which the adipokines are produced. Thus, adipokines released by the visceral depot (VAT) would exert a greater effect on hepatic carbohydrate and lipid metabolism, stimulating hepatic release of acute-phase response proteins in the liver (such as CRP, a nonspecific acute-phase reactant that serves as an indicator of systemic inflammation) as well as having autocrine/paracrine effects on the depot.

Adipokines produced by the subcutaneous depot (SAT) would mainly affect adipose cell development and function locally (autocrine/paracrine effects) and exert systemic effects on, the skeletal muscle. The former effect type may represent the mechanism by which inflammatory adipokines induce hepatic IR and chronic systemic inflammation, as the latter type would diminish adipose tissue storage of lipids leading to ectopic fat accumulation in the liver and skeletal muscle [34].

Hotamisligil and colleagues [35] were the first to describe the molecular connection between inflammation and obesity when TNF-α, an inflammatory cytokine, was found to be expressed in adipose tissue in obese animal models, contributing to the inhibition of insulin signaling pathways. This has led to the exploration and characterization of several other adipokines with similar metabolic effects [36]. The fact that these cytokines inhibit the effects of insulin on endothelial cells [13] may additionally contribute to IR by limiting the nutrient-induced increase in nutritive capillary flow to muscle as well as muscle glucose uptake.

Growing evidence from human and animal research has established that a chronically activated inflammatory state of adipose tissue has a crucial role in the development of obesity-related IR [37]. This inflammation is characterized by an increase in macrophage markers, such as CD86 [38].

As previously indicated, CCL2 and its receptor CCR-2 (C–C motif chemokine receptor-2), expressed on adipocytes, regulate monocyte and macrophage recruitment and are necessary for macrophage-dependent inflammatory responses and the development of atherosclerosis [39]. Under normal conditions, adipocytes store lipids and regulate metabolic homeostasis, while macrophages function in the inflammatory response, although each cell type has the capacity to perform both functions. In obesity, adipose tissue becomes inflamed, both via infiltration of adipose tissue by macrophages and as a result of the adipocytes themselves becoming producers of inflammatory cytokines.

Inflammation of adipose tissue is a crucial step in the development of peripheral IR. In addition, in proatherosclerotic conditions such as obesity and dyslipidemia, macrophages accumulate lipid to turn into foam cells. Adipocytes and macrophages share common features such as expression of cytokines, FABPs (fatty-acid binding proteins), nuclear hormone receptors and many other factors. As evidenced by genetic loss-of-function models, adipocyte/macrophage FABPs modulate both lipid accumulation in adipocytes and cholesterol accumulation in macrophages, as well as the development of IR and atherosclerosis. PPAR-γ and LXR pathways oppose inflammation and promote cholesterol afflux from macrophages and lipid storage in adipocytes [40].

Inflammatory pathways in adipocytes or macrophages can be initiated by extracellular mediators such as cytokines and lifestyle changes (lipids) or by intracellular stresses such as endoplasmic reticulum (ER) stress or production of reactive oxygen species (ROS) by mitochondria. Signals from all these mediators converge on inflammatory signaling pathways, including the kinases JNK (c-Jun N terminal kinase) and IKK (inhibitor NFκB kinase). These pathways lead to the production of additional inflammatory mediators through transcriptional regulation as well as to the direct inhibition of insulin signaling. Other pathways are also involved in inflammation-mediated inhibition of insulin's action. Recently, it has been found that different inflammatory pathways occur in different adipose tissue depots; as the omental tissue showed increased levels of NF-κB, while increased JNK activity was seen in subcutaneous abdominal adipose tissue. Additionally, NF-κB was shown to be influenced by obesity and diabetes status. These data suggest that NF-κB may play a more predominant central role in inflammatory-related metabolic disease in comparison to JNK [41].

Opposing the inflammatory pathways are transcription factors from the PPAR and LXR families, which promote nutrient transport and metabolism. More proximal regulation is provided by FABPs, which likely sequester ligands of these transcription factors, thus promoting a more inflammatory environment. The absence of FABPs results in an anti-inflammatory state. The cell must strike a balance between metabolism and inflammation. In conditions of overnutrition, this becomes a particular challenge, as the very processes required for the response to nutrients and nutrient utilization, such as mitochondrial oxidative metabolism and increased levels of protein synthesis in the ER, can induce an inflammatory response [40].

Adipokines are involved in every step of the atheromatous process, which begins with inflammatory changes in the vascular wall. The vascular insult is first caused by cytokines like TNF-α and IL-6, leading to increased expression of adhesion molecules such as intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1), which enhance monocyte adhesion to the endothelium. At the same time, MCP-1 (CCL2), which is secreted by adipocytes and injured endothelial cells, not only increases the migration of monocytes but also favors their transformation into foam cells. These foam cells secrete metalloproteinases that may lead to plaque rupture. Because of its low levels in obese individuals, the protective effects of adiponectin against plaque formation are decreased. Adiponectin is a specific white adipose tissue-derived protein, with, as indicated previously, anti-inflammatory/antiatherogenic properties, such as decreasing the expression of adhesion molecules, decreasing monocyte adhesion to endothelial cells, decreasing uptake of oxidized low-density lipoprotein (LDL), decreasing foam cell formation, and decreasing the proliferation and migration of vascular smooth muscle [36].

Furthermore, obesity is also linked to a hypercoagulable state caused by an increase in circulating levels of procoagulants such as tissue factor, fibrinogen, von Willebrand factor and factor VII. In addition, many circulating cytokines with elevated levels in obese individuals cause endothelial activation, leading to platelet activation and plug formation [42].

The inhibition of fibrinolysis caused by an increased level of PAI-1 (which is particularly high in individuals with obesity and diabetes) is another component of the hypercoagulable process, favoring thrombus formation upon rupture of atherosclerotic plaques [43].

Nutrient Excess

The recent major increase in the global incidence of T2DM suggests that most cases of the disease are caused by changes in environment and lifestyle factors. All major risk factors for T2DM (overnutrition, low dietary fiber, sedentary lifestyle, sleep deprivation, and depression) have been found to induce local or systemic low-grade inflammation that is usually transient or milder in individuals not at risk forT2DM. By contrast, inflammatory responses to lifestyle factors are more pronounced and prolonged in individuals at risk for T2DM and appear to also occur in the pancreatic islets. Chronic low-grade inflammation will eventually lead to diabetes if counter-regulatory circuits to inflammation and metabolic stress are compromised because of a genetic and/or epigenetic predisposition (for example, in individuals with a dominance of activation signals via NFκ-B, JNK, and transcription factor activator protein-1). Hence, it is not the lifestyle change per se but a deficient counter-regulatory response in predisposed individuals that is crucial to disease pathogenesis. Novel approaches to intervention may target these deficient defense mechanisms [44]. In susceptible individuals, therefore, obesity-induced metabolic impairment can favor IR on the one hand and progressive β-cell failure on the other. Reduced insulin secretion can in turn worsen the nutrient excess problem by raising circulating concentrations of glucose, FFAs, and other nutrients. In this way, a vicious circle arises whereby an obesity-induced nutrient excess triggers inflammatory responses that cause IR, placing a greater demand on β-cells and, as β-cell function declines, the cellular toll taken by nutrient excess increases [23]. The subject of nutrient excess will be further discussed when mitochondrial dysfunction in obesity is analyzed.

Ectopic Fat Deposition

The other mechanism that could link obesity to IR and predispose individuals to T2DM, according to Eckel and colleagues [23] is ectopic fat deposition, particularly in the liver and skeletal muscle. Lipotoxicity describes the detrimental cellular effects of chronically elevated concentrations of FAs and excess lipid accumulation in tissues other than adipose tissue, accepting that excess adipose tissue is often a source of increased FA supply in obese individuals. Lipotoxicity constitutes an important pathogenic link among obesity, IR and T2DM [3]. Excess circulating FAs that are taken up by muscle (intramyocellular) are often stored as Tg in this tissue or used for extra-β-oxidation. However, T2DM is associated with mitochondrial dysfunction, as previously discussed [26], requiring excess FAs to be diverted into non-oxidative pathways such as the production of diacylglycerol and ceramide. Both of these metabolites of FAs activate isoforms of protein kinase C (PKC) that promote serine phosphorylation of insulin signaling proteins such as insulin receptor substrates (for example, IRS-1, IRS-2), thereby reducing their signaling activity [45, 46]. This partly accounts for the effect of lipotoxicity in impeding the translocation of GLUT 4 (glucose transporter 4) into the plasma membrane, reducing glucose transport into the cell. Because skeletal muscle accounts for >70 % of glucose disposal compared with about 10 % into adipose tissue, the development of IR in muscle is a major feature of obesity-induced IR [47, 48].

Deposition of excess fat in the liver (hepatic steatosis) or nonalcoholic fatty liver disease (NAFLD) also impairs insulin signaling. This may involve a similar interruption to early insulin signaling to that noted with intramyocellular lipid, but resulting principally in a failure to inhibit excessive glucose production as well as causing lipotoxic hepatocyte death [49].

The results of epidemiological studies suggest that fatty liver, as measured by ultrasonography or estimated by elevated blood markers of fatty liver and hepatic inflammation, is not only cross-sectionally associated with IR independent of measures of adiposity in adults and children, but also predicts incident T2DM and CVD [50, 51]. A strong negative relationship was identified between liver fat content measured by magnetic resonance spectroscopy and insulin sensitivity estimated from the oral glucose tolerance test (as proposed by Matsuda and DeFronzo [52]), after adjustment for sex, age and total body and visceral fat mass, in 327 individuals without and 10 with newly diagnosed T2DM ($r=-0.39$; $p<0.0001$). However, individuals with a very similar liver fat content could be identified who were relatively insulin sensitive and insulin resistant [53]. These findings indicate that fatty liver is directly involved in the pathogenesis of T2DM and CVD. However, it may be that it was not possible to precisely account for the exceptionally strong relationship of fatty liver with visceral adiposity in these studies. Hence, the true pathogenic factor in subjects with fatty liver may be visceral obesity. Furthermore, fatty liver may simply be a consequence of elevated levels of insulin and glucose, which induce "de novo" hepatic lipogenesis, and of circulating FFAs, all of which are found in individuals with IR. If this was the only explanation for the observed relationships then, according to the AA [53], fatty liver could serve as a very good marker of the elevated risk, for example, in the prediabetic state, but specifically targeting fat accumulation in the liver is not a promising approach when it comes to the prevention or treatment of T2DM or CVD.

Animal studies provided the first evidence that fatty liver may develop independently of IR of adipose tissue and skeletal muscle. Fabbrini and colleagues [54] showed that excessive intrahepatic triglyceride content in obese persons is a robust marker of metabolic abnormalities (IR in liver, muscle, and adipose tissue, as well as alterations in FFA metabolism and increased VLDL-Tg secretion rate), independently of BMI, percent body fat and visceral fat mass. Furthermore, there are also human data showing that fatty liver may even have a primary role in the pathophysiology of skeletal muscle IR; when the effects of the PPARγ agonist rosiglitazone and metformin were compared, only the latter produced increased hepatic insulin sensitivity via activation of AMPK. However, a decrease in liver fat was only seen in patients receiving rosiglitazone and, more importantly, the insulin sensitivity of glucose disposal increased only in the rosiglitazone group [55]. Because skeletal muscle is not a major target of PPARγ action [56], these data support the notion that the increase in skeletal muscle insulin sensitivity in the rosiglitazone group may be mediated by the decrease in liver fat [57].

Hepatic IR can be attributed to impaired insulin-stimulated insulin receptor substrate (IRS)-1 and IRS-2 tyrosine phosphorylation, as described for skeletal muscle; however, such phosphorylation events result in increased gluconeogenesis. Euglycemic/hyperinsulinemic clamp studies using tracer methods to measure the suppression of endogenous glucose production and provide an estimate of hepatic insulin sensitivity showed that liver fat is particularly strongly correlated with hepatic insulin sensitivity [57].

Considering these findings together, there is strong support to show that fatty liver produces humoral factors (hepatokines) affecting insulin signaling in insulin-responsive tissues. Fat accumulation in the liver induces hyperglycemia, subclinical inflammation, dyslipidemia, and the possible secretion of the so-called hepatokines (for example, fetuin-A), thereby inducing IR, atherosclerosis and possibly β-cell dysfunction and apoptosis. The degree of these conditions may be moderate (benign fatty liver). Mechanisms explaining these findings are effective hepatic TAG synthesis (as an adaptive process in situations when TAG precursors are abundant that allows storage of lipids in these less toxic forms), lipid desaturation, and inhibition of lipid-induced inflammatory signaling. However, the same amount of hepatic fat accumulation may, by mechanisms that are yet not fully understood, be strongly associated with hepatic lipotoxicity, resulting in aggravation of hyperglycemia, dyslipidemia, and an imbalance in hepatokine production as well as subsequent metabolic consequences (malignant fatty liver) [53].

Although IR, overweight/obesity and fatty liver are strongly correlated as risk factors for T2DM, there is clear evidence of dissociation among these three risk factors. The dissociation among these risk factors suggests that different pathogenetic mechanisms may underlie their contributions to T2DM [58].

Affected individuals who develop T2DM may have any one, two or all three of these risk factors. Overweight/obesity had the weakest association with incident diabetes in a population of 12,853 subjects without diabetes from a South Korean occupational cohort at 5-year follow-up (OR 1.62); IR had strongest association (OR 3.92), followed by fatty liver (2.42). The OR for the presence of all three factors was 14.13. The AA suggested that treatment for each factor is needed to decrease the risk of T2DM.

The third mechanism that was proposed to link obesity to IR and a predisposition to T2DM by Eckel and colleagues [23] was mitochondrial dysfunction, which will be now evaluated.

Mitochondrial Dysfunction

It is well known that mitochondria play a central role in ATP production, energy expenditure and disposal of ROS. Mitochondrial oxidative dysfunction is correlated with IR in the skeletal muscle of obese and diabetic subjects [59, 60]. IR in skeletal muscle in obese individuals and those with T2DM is associated with reduced muscle oxidative capacity, reduced expression of nuclear genes responsible for oxidative metabolism, and reduced activity of the mitochondrial electron transport chain compared with lean control groups [60]. This dysfunction correlates with reductions in mitochondrial numbers and size [61]. Thus, adipocytes respond to metabolic challenges by altering their number, morphology and/or the distribution of mitochondria within the cell, as well as by changing metabolite, enzyme and/or mitochondrial DNA (mtDNA) content [26].

Excessive caloric intake, which increases the mitochondrial substrate load leading to mitochondrial dysfunction that precludes effective dissipation of the proton gradient, can induce increased ROS production and subsequent oxidative stress, which in turn has significant consequences for mitochondrial function and energy substrate metabolism. The reduction in the level of mitochondrial biogenesis and gene expression after excessive energy substrate and increased ROS production causes IR. IR is mitigated by mitochondrial antioxidants or overexpression of mitochondrial scavengers.

Hypothalamic Dysfunction

It is well known that T2DM results from the complex association of IR and β-cell failure. Obesity is the main risk factor for T2DM, and recent studies have shown that, in diet-induced obesity in rats, the hypothalamus becomes inflamed and dysfunctional, resulting in the loss of the perfect coupling between caloric intake and energy expenditure. Because β-cell function is, in part, under the control of the autonomic nervous system, loss of the first phase of insulin secretion, accompanied by increased expression of markers of apoptosis, is present together with the earliest markers of hypothalamic inflammation, which are already observed at 8 weeks after the beginning of the high fat diet [29].

During the onset and progression of obesity in humans, the levels of insulin rise in direct proportion to body mass. Although pancreatic β-cells can cope with the peripheral needs for insulin, glucose homeostasis will prevail [62]. However, depending on genetic and environmental factors, insulin production and secretion may decline, and the onset of T2DM becomes inevitable [63]. At diagnosis, T2DM is associated with a significant decrease in β-cell function, which can be further compromised during the progression of the disease [63]. Several mechanisms have been shown to play a role in this process, such as glucotoxicity, lipotoxicity, the damaging effect of increased leptin levels, the deposition of amylin and the activation of inflammation, all contributing to accelerated apoptosis, which results in the reduction of up to 60 % of pancreatic islet mass in the pancreata of individuals with T2DM [64]. Obese patients present distinct functional activity patterns in selected brain regions, including the hypothalamus, compared with lean subjects. After bariatric surgery, increases in

cerebrospinal fluid (CSF) levels of IL-10 and IL-6 are accompanied by changes in functional magnetic resonance imaging (fMRI) patterns, particularly in the hypothalamus. In conclusion, the findings indicated that reduction of body mass in obese humans increased anti-inflammatory activity in the cerebrospinal fluid and partially corrected the dysfunctional activity in response to glucose in select brain areas. These data suggest that obesity and body mass loss affect the human brain in a manner similar to that seem in animal models for this disease [65].

The twin epidemics of obesity and T2DM might as already indicated, not only be explained by increased energy intake and/or reduction of energy expenditure. On the other hand, as presented below, geneticists have mainly focused on the human genome in their attempts to unravel the risk factors for T2DM. Nevertheless, there is an increasing body of literature focused on a possible third culprit, the gut Mc (ref. cit in [66, 67]). These microorganisms and, thus, their bacterial genomes, are increasingly being considered important pathogenic factors in various diseases ranging from gastrointestinal tract diseases to obesity. Intestinal Mc may play a pivotal role in converting nutrients into energy. Variations in the composition of Mc are found in obese humans and mice. Increased energy yield from diet in obese mice and humans could be a contributing factor to obesity [68] but further mechanisms linking gut Mc to obesity have been proposed, including chronic inflammation induced by low-grade endotoxinemia, regulation of adipose tissue and liver FA composition by gut microbes, modulation by gut-derived peptide secretion (such as peptide YY and glucagon-like peptides 1 and 2), and activation of the lipopolysaccharide toll-like receptor-4 axis [67]. The role of the Western diet in promoting an obesogenic gut Mc is being confirmed in humans. Following encouraging results in animals, several short-term randomized controlled trials have already showed the benefits of prebiotics and probiotics in terms of insulin sensitivity, inflammatory markers, postprandial incretins, and glucose tolerance [67].

In summary, there is no a single mechanism that can explain the links between obesity, IR and T2DM [48]. A defect in insulin release by β-cells is crucial, resulting in disordered regulation of glucose levels by decreasing suppression of hepatic glucose levels and reducing the efficiency of glucose uptake in insulin-sensitive tissues. Decreased insulin output could also impair adipocyte metabolism, resulting in increased lipolysis and elevated levels of FFAs. Elevations in the levels of both FFAs and glucose can occur simultaneously, and together are more deleterious to islet health and insulin action than either alone. Thus, the process may slowly feed forward, in keeping with other observations that the onset of T2DM is usually a slow process that takes many years. Even mild impairments of insulin release may have central effects on metabolic homeostasis. Insulin acts in the hypothalamus to regulate body weight, and impaired insulin signaling is associated with changes in food intake and body weight [69]. Thus, β-cell dysfunction resulting in a relative reduction in insulin release would be expected to result in decreased insulin action in this crucial brain region and be associated with weight gain and an aggravation of IR. Besides, IR at the level of β-cell might have a role in the pathogenesis of defective insulin release [48]. Regarding the mechanisms underlying progressive β-cell dysfunction in obese individuals, the link between obesity and hyperinsulinism reflects a compensation by insulin-secreting β-cells to systemic IR, with obese normoglycemic individuals having both increased β-cell mass and function (ref. cit in [23]). Thus, obesity-induced glucose intolerance reflects a failure to mount one of more of the compensatory responses [48]. A growing understanding of the genetics and cellular function of β-cells could help identify potential mediators predisposing obese individuals to T2DM.

Genome-wide association scans (GWAS) and candidate gene approaches have now identified ~40 genes associated with T2DM [70] and a similar number, albeit largely different, with obesity. Most T2DM genes appear to be related to β-cell dysfunction, with only a few involved in pathways related to IR independent of obesity (ref. cit in [23]). Although numerous diabetes- and obesity-associated genes have been identified, the known genes are estimated to predict only 15 % of the T2DM risk and 5 % of the obesity risk [71]. Although additional genes with important roles will undoubtedly be discovered, this low predictive power may reflect the importance of environmental factors, less frequent genetic variants with stronger effects, or gene–environment, gene–gene, and epigenetic interactions that can not readily be identified using methods based on population genetics [23].

Conclusions

Although the link between obesity and T2DM is widely held to involve two lesions—obesity-induced insulin resistance and β-cell failure—both disorders may share an underlying defect, raising questions about whether defects favoring progressive weight gain and metabolic impairment also contribute to β-cell decompensation [23]. One potential link could be sustained cell exposure to nutrient concentrations exceeding energy requirements. However, because not all obese individuals develop hyperglycemia, an underlying abnormality of the β-cell must coexist with nutrient excess to promote T2DM [48].

Another question linking obesity to T2DM, put forward by Eckel et al. [23] was "What mechanisms do obesity and IR contribute to β-cell decompensation and if/when obesity prevention ensues, how much reduction in T2DM incidence will follow?"

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Chapter 17
Adipokines in Nonalcoholic Fatty Liver Disease

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Keywords NAFLD • NASH • TNF-alpha • Adiponectin • Leptin • Secretion

Key Points

- The pathogenesis of NAFLD and NASH is hastened by a disturbance of adipokine production.
- Decreased production of adiponectin and increased production of TNF-α, characteristic of obesity, directly contribute to NASH.
- NASH suppressive effects of leptin are diminished by the widespread leptin resistance, while its pro-oxidant and fibrogenic properties augment the progression of NAFLD.
- Resistin's involvement in NASH is documented in rodent models, which may not be applicable to the human disease of NAFLD.
- Effects of vaspin, visfatin, apelin, nesfatin, omentin, and chemerin require further study in patients with NAFLD and NASH.

The Spectrum of Nonalcoholic Fatty Liver Disease (NAFLD)

Nonalcoholic fatty liver disease (NAFLD) represents a spectrum of clinicopathologic conditions characterized by significant lipid deposition in the liver parenchyma of patients who do not consume excessive amounts of alcohol [1, 2]. At one end of the NAFLD spectrum is steatosis alone ("simple steatosis"), and at the other end are nonalcoholic steatohepatitis (NASH), NASH-related cirrhosis and hepatocellular carcinoma. NASH is characterized by hepatic steatosis and by evidence for hepatocyte

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ballooning degeneration, lobular inflammation, and occasionally, Mallory hyaline or sinusoidal fibrosis [3]. Differential diagnosis between NASH and steatosis alone is important because of their differential risk for progression [3].

The impact of NAFLD relates to its prevalence and potential for progression. Estimates of the prevalence of NAFLD are high and are expected to increase with the global epidemic of obesity. According to the National Health and Nutrition Examination Surveys (NHANES) conducted between 1988 and 2008, the prevalence of nonalcoholic fatty liver disease (NAFLD) increased from 5.51 % to 9.84–11.01 % [4]. From 1988 to 1994, NAFLD accounted for 46.8 % of Chronic Liver Disease (CLD) cases; from 1994 to 2004 its prevalence increased to 62.84 %, and then to 75.1 % from 2005 to 2008 [4]. The prevalence of NAFLD is highest in populations with preexisting metabolic conditions such as obesity and type II diabetes (up to 50–90 %) [2, 5–7]. The prevalence of histologically confirmed NASH is estimated as 1.2–4 %. However, in patients with risk factors such as morbid obesity, prevalence of NASH is higher and is estimated as 20–47 % [5, 8, 9].

The progression of NAFLD and its subtypes can be estimated from historical cohort studies, population-based studies and studies reporting sequential liver biopsies. Importantly, patients with steatosis alone rarely progress to cirrhosis, while 10–25 % of those with biopsy proven NASH can progress to cirrhosis [1, 3, 8]. In fact, most patients with cryptogenic cirrhosis seem to have had "burned-out NASH" [11]. NASH-related cirrhosis can cause hepatocellular carcinoma (HCC) [10, 11]. The major risk factors for progression or hepatic fibrosis in NASH are the presence of type 2 diabetes, obesity, metabolic syndrome, as well as elevated aminotransferase and histologic features of ballooning degeneration of hepatocytes and Mallory's hyaline [8, 12]. Additionally, patients with NAFLD have higher mortality rates than the general population, in particular, due to associated cardiovascular risks [13].

Definitive diagnosis of NASH can be made only by liver biopsy evaluated by strict pathologic criteria. Although many noninvasive biomarkers for NAFLD assessment have been developed [14–17], liver biopsy remains "the imperfect gold standard" for diagnosing NASH and staging the extent of fibrosis. Nevertheless, liver biopsy is expensive, associated with small but definite medical risks and can be flawed by sampling errors [18, 19]. Studies of the involvement of adipokines in the pathogenesis of NAFLD and NASH hold particular promise for the development of noninvasive diagnostic biomarkers for these conditions.

Several treatment strategies are currently in use, but no therapy has proven to be effective for NASH [9, 20]. Treatment strategies include modification of the clinical conditions associated with NASH such as type II diabetes mellitus, hyperlipidemia, and obesity [2, 9]. Specific therapeutic interventions that have been evaluated so far include weight reduction, the use of ursodeoxycholic acid (UDCA), clofibrate, betaine, *N*-acetylcysteine, gemfibrozil, atorvastatin, thiazolidinediones, nitroaspirin, pentoxifylline, and vitamin E [2, 9, 20, 21]. Although some of the results are encouraging, none of these interventions have been approved by the FDA for preventing NASH progression. Importantly, commonly used bariatric procedures have an impact on liver histology; a number of studies have shown post-surgical improvement of steatosis with a few studies suggesting an increase in portal fibrosis [22].

Pathogenesis of NASH

In the past few years, a substantial body of knowledge on the pathogenesis of NASH has been accumulated. However, the factors involved in its progression from steatohepatitis to fibrosis and cirrhosis remain to be elucidated. The pathogenesis of NASH appears to be multifactorial. To acquire an insight into the relationship between triglyceride accumulation within the hepatocyte and the necroinflammation and fibrosis, the following hypotheses are being investigated: the influences of abnormal lipid metabolism and the production of reactive oxygen species, increased

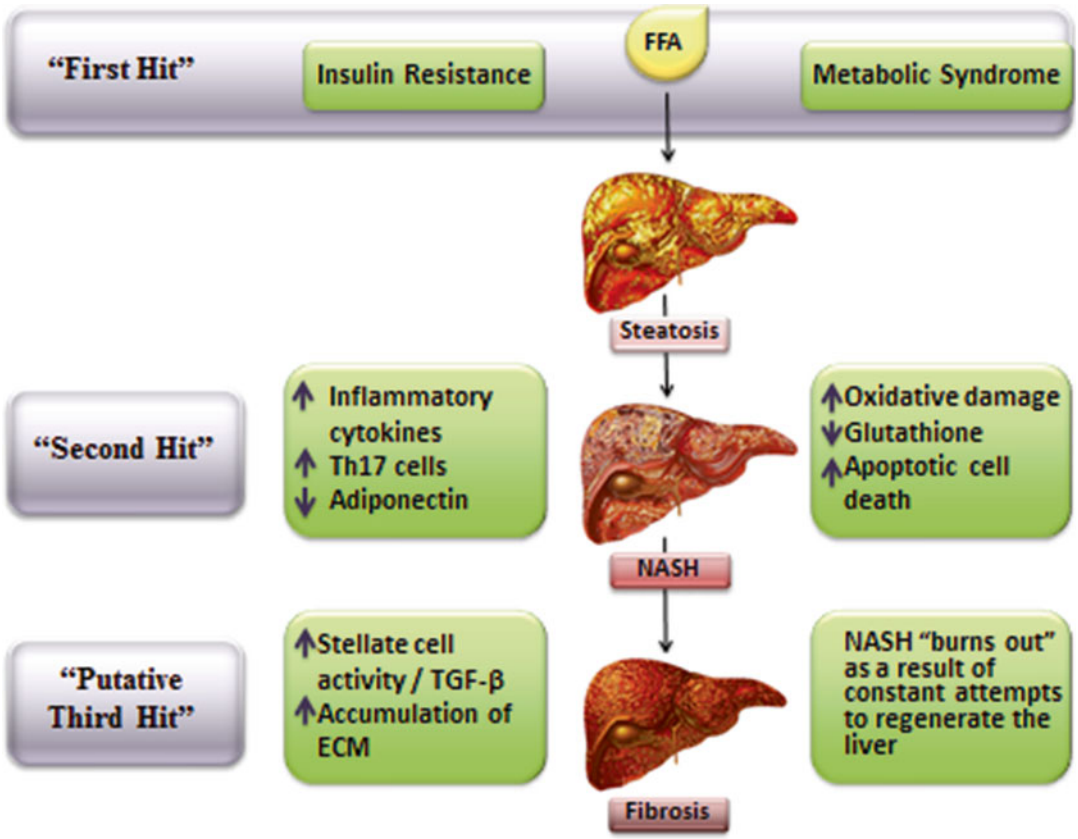

Fig. 17.1 The "multi-hit" hypothesis of the pathogenesis of NASH

hepatic lipid peroxidation, activated fibrocytes, and abnormal patterns of cytokine production leading to liver cell injury and fibrosis [23].

The "two-hit hypothesis" of NASH suggests that the first "hit" is the accumulation of excessive fat in the hepatic parenchyma [2, 8, 24]. This first step has been linked to insulin resistance (IR), which is consistently observed in patients with NASH [23]. The evidence for IR in NAFLD comes from both animal and human studies. Animal models of NASH show insulin resistance [25] and the use of the insulin-sensitizing agent, metformin reverses hepatic steatosis [23]. Clinical features of the metabolic syndrome (obesity, diabetes mellitus, or hypertriglyceridemia) are commonly observed in NAFLD [1, 8]. Additionally, polycystic ovary syndrome (PCOS), an insulin resistance-associated condition, was identified as a risk factor for developing NAFLD [26]. Growing evidence suggests that patients with more "severe" forms of insulin resistance are at an even greater risk for progressive liver disease [8]. Importantly, insulin resistance in the adipose tissue plays a larger role in the severity of NAFLD as compared to liver or muscle IR [27].

The second "hit" in the development of NASH could be multifactorial, involving fatty acid beta oxidation, oxidative stress, gut-derived endotoxins, pro-inflammatory cytokines, and adipokines. In fact, due to the multifactorial nature of the second "hit," the "two-hit hypothesis" of NASH now is being expanded to "multi-hit model" (Fig. 17.1). Oxidative stress implies an imbalance between pro-oxidant and antioxidant processes. In the setting of NAFLD, oxidative stress can result from the induction of microsomal CYP2E1, H_2O_2 release from peroxisomal β-oxidation of fatty acids, cytokines released from activated inflammatory cells, or other unknown factors [23, 28].

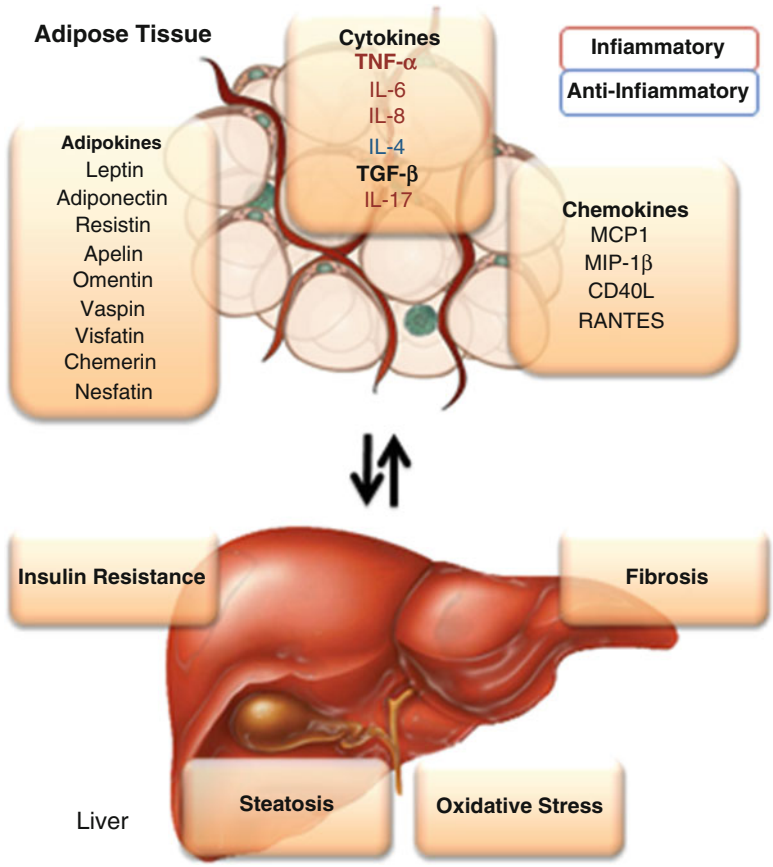

Fig. 17.2 Molecules involved in complex interplay between cells composing the adipose tissue and the liver

This oxidative stress could potentially lead to peroxidation of membrane lipids resulting in the production of malondialdehyde and 4-hydroxynonenol, which in turn can induce the production of proinflammatory cytokines, stellate cell activation, and fibrogenesis, as well as direct hepatocyte damage [8, 23, 29]. The increase in oxidative stress observed in NAFLD hepatocytes may be linked to mitochondrial dysfunction, as mitochondria are a major potential source of reactive oxygen species (ROS) in living cells [29].

As noted, both the first and second hits in NASH may involve changes in circulating levels of various pro- and anti-inflammatory cytokines, including adipokines (Fig. 17.2). Although a role for cytokines such as TNF-α and IL-6 has been suggested for quite some time [30, 31], recent works center on the particular roles for adipokines in the pathogenesis of NASH [31].

Adipose Tissue, Adipokines and NAFLD

White adipose tissue produces and releases a variety of proinflammatory and anti-inflammatory factors, including adipokines (leptin, adiponectin, resistin, apelin, omentin, vaspin, visfatin, chemerin, nesfatin, and others), cytokines (such as TNF-α, IL-6), and chemokines (such as monocyte chemoattractant protein 1, and others). In addition to adipocytes, white adipose tissue contains several other

cell types including macrophages and monocytes. It is likely that macrophages are attracted into the adipose tissue by macrophage migration inhibitory factor (MIF), which is released by both preadipocytes and mature adipocytes in the amounts proportional to body mass index [32]. By producing several cytokines and adipokines, both adipocytes and other pro-inflammatory cells found within adipose tissue contribute to the increased systemic inflammation associated with obesity [33]. The exact contribution of each component of white adipose tissue in the "pro-inflammatory" state of obesity is not entirely clear. Over 90 % of the adipokines released from adipose tissue, except for adiponectin and leptin, originate from nonfat cells embedded in the extracellular matrix [34]. Additionally, most adipokines are also produced elsewhere in the body [35]. These data suggest that serum adipokine measurements in obese patients most likely reflect secretions by various cells, including, but not limited to, adipocytes.

Biologically active white adipose tissue plays an important role in metabolic syndrome and associated NAFLD. Although the role of proinflammatory cytokines (TNF-α, IL-6) in the pathogenesis of NASH is well documented, the role of adipokines in the pathogenesis of NAFLD is a recent observation. The exact source of adipokines in patients with NAFLD (e.g., adipocytes, macrophages, monocytes) remains unclear, because serum levels of adipokine probably reflect a "net" effect of the secretion from various cells in the body. Determining the exact role of adipokines in the pathogenesis of NAFLD is complicated by the interaction of adipokines with insulin resistance and obesity. Most studies have not controlled for these important confounders. This issue is critical for future research focused on elucidating pathways involved in the pathogenesis of NAFLD, NASH and its progression.

Adipokines in Experimental Models of NAFLD

Common experimental models of NAFLD include mice or rats that are fed high fat or high carbohydrate or methionine/choline deficient diets, or mice that exhibit one or another genetic deficiency, for example, leptin-deficient mice and rats. Additionally, many other animal models spontaneously develop steatosis, and some progress to steatohepatitis and cirrhosis.

Leptin-deficient ob/ob mice are obese, insulin resistant, hyperglycemic, and hyperlipidemic. Similar animal models such as db/db mice and fa/fa rats have homozygous loss-of-function due to a mutation in a leptin receptor encoding gene. All phenotypes (fa/fa rats, ob/ob and db/db mice) are essentially similar, except for hyperleptinemia, which is present in fa/fa and db/db animals. It is noteworthy that NAFLD occurs in both leptin-deficient and in hyperleptinemic animals with impaired leptin signaling. In leptin deficient animals, leptin restoration leads to NAFLD reversal [36]. Another important consideration in NAFLD development is the contribution of central versus peripheral leptin activity. To address this question, animals with tissue-specific leptin receptor knockouts were created. In contrast to the db/db and the neuron-specific knockout (KO) mice, the livers of hepatocyte-specific leptin receptor knockout animals were normal [37]. Unfortunately, the experimental approach does not exclude the possibility that fatty liver development is influenced by autocrinous leptin signaling in adipose tissue itself, or by endocrinous action of the leptin on cytokine releasing cells. So, the question of peripheral versus central involvement of leptin signaling in NAFLD development remains unanswered.

TNF-α serum levels increase in all animal models of NAFLD. However, the source of TNF-α (i.e., adipose tissue versus monocytes and macrophages) is not entirely clear. In NAFLD, TNF-α level may be influenced by the lipotoxic effects of excess fat. In fact, hepatic steatosis seems to increase oxidative stress and activation of the NF-κB pathway, leading in turn to an increase in TNF-α production. Once initiated, this vicious cycle of NF-κB/TNF-α becomes self-perpetuating, contributing to a "pro-inflammatory" state in patients with NASH [25]. Evidence supporting this concept is provided by anti-TNF-α treatment in ob/ob mice, which can improve liver histology, reduce hepatic total fatty

acid content, and decrease serum alanine aminotransferase (ALT) levels. In addition, fatty acid beta-oxidation and uncoupling protein (UCP)-2 expression decreases with anti-TNF-α treatment in ob/ob mice, both of which suggest an improvement in oxidative stress and probably insulin resistance [38]. Similarly, metformin, which is known to inhibit hepatic expression of TNF-α and TNF-inducible factors, also seems to improve fatty liver disease [39]. This has led to the hypothesis that chronic exposure to TNF-α prompts accumulation of inflammatory cells in the liver parenchyma, thereby exposing hepatocytes to other damaging factors released by activated mononuclear cells.

In animal models, adiponectin decreases insulin resistance by decreasing triglyceride content in the muscle and liver tissue and by increasing the ability of sub-physiological levels of insulin to suppress glucose production by inhibiting hepatic gluconeogenic enzymes [40, 41]. This results from increased expression of molecules involved in both fatty-acid metabolism and energy dissipation in muscle tissue. It has been shown that mice chronically fed high-fat, ethanol containing food, have lower levels of adiponectin. Experimental replenishment of adiponectin dramatically alleviates hepatomegaly and steatosis in these animals, and attenuates inflammation by suppressing the hepatic production of TNF-α [42]. Similar effects are achieved in nonalcoholic ob/ob mice [42]. Experiments with adiponectin-knockout mice conducted by Kamada and coworkers demonstrated that adiponectin is capable of attenuating liver fibrosis that develops after carbon tetrachloride administration [43]. Adiponectin also alleviates lipopolysaccharide (LPS)-induced liver injury [44]. Suppressing local TNF-α production and signaling provides a protective effect in all cases of adiponectin-related decreases in the extent of acute liver injury.

Studies of the effects of resistin in animal models of NAFLD are substantially less relevant to human fatty liver disease due to functional differences between resistin encoding genes in animal models and humans. The expression of resistin mRNA in human adipose tissue and its serum content are substantially lower (1/250) than that in rodents. Moreover, the resistin-α encoding gene is absent in humans [45].

Adipokines in Patients with NAFLD

The following sections review the current clinical data on specific adipokines in NAFLD: adiponectin, resistin, leptin, and TNF-alpha, as well as several other soluble molecules.

Adiponectin

Adiponectin is the most frequently studied adipokine associated with NASH. Many authors have suggested that hypoadiponectinemia may contribute to the development of NASH in obese individuals. This hypothesis is supported by a study of 257 healthy individuals, which reports a negative correlation between adiponectin serum levels and two markers of liver injury, alanine aminotransferase (ALT) and gamma-glutamyltranspeptidase (GGT) before and after adjustment for sex, age, body mass index (BMI) and insulin resistance [46]. Pagano and coworkers showed that plasma adiponectin levels were significantly lower in NAFLD patients than in the matched controls (5.93 ± 0.45 versus 15.67 ± 1.60 ng/ml). However, there was no difference in adiponectin levels between patients with simple steatosis and those with NASH (6.16 ± 0.78 versus 5.69 ± 0.49 ng/ml) [47]. The authors reported an inverse correlation between adiponectin and homeostatic model assessment (HOMA) of insulin resistance ($P = 0.008$), but no correlation between adiponectin and serum transaminases or lipid values [47]. A second study confirmed the protective effect of adiponectin against the development of radiologically proven steatosis [48]. Finally, a study of 113 obese children confirmed the protective role of adiponectin against NAFLD in pediatric populations [49].

Despite increasing evidence supporting the association between hypoadiponectinemia and steatosis, the role of adiponectin in distinguishing NASH from simple steatosis remains controversial. A study by Bugianesi and colleagues related decreased levels of circulating adiponectin in NAFLD to hepatic insulin sensitivity and to the amount of hepatic fat content, but not to liver disease severity as measured by necroinflammation and fibrosis [50]. On the other hand, Hui and coworkers found that low serum adiponectin is associated with increased grades of hepatic necroinflammation independent of insulin resistance [51]. Our own observations suggest that higher serum adiponectin concentrations also protect against the progressive form of fatty liver disease, NASH ($P=0.024$) [31]. Finally, a report by Musso and colleagues is of special interest [52]. This study of 25 nonobese, nondiabetic patients with biopsy-proven NASH showed that adiponectin was protective against histologically proven NASH (NASH 5,476 ± 344 versus matched controls 11,548 ± 836 ng/ml; $P=0.00001$) [52]. Adiponectin was negatively correlated with the presence of necroinflammation (OR = 5.0; $P=0.009$), and fibrosis (OR = 8.0; $P=0.003$). On logistic regression controlling for all important confounders, hypoadiponectinemia remained an independent predictor of severe necroinflammation and of stage 3 fibrosis [51, 52]. In the same cohort of patients, the magnitude of postprandial lipemia was significantly higher in NASH than in controls and was related to fasting adiponectin ($\beta=-0.78$; $P=0.00003$) [53]. Controls showed a significant increase in serum adiponectin in response to the fat load, whereas patients with NASH showed a slight decrease. Postprandial free fatty acids response correlated inversely with adiponectin response in both groups and independently predicted the severity of liver steatosis in NASH ($\beta=0.51$; $P=0.031$). In other words, the dynamic adiponectin response to an oral fat load is strikingly different in healthy subjects and patients with NASH and is related to the postprandial FFA response. These findings suggest that hypoadiponectinemia precedes the overt manifestation of diabetes and is linked to impairment of the postprandial lipid metabolism.

Circulating levels of adiponectin has a strong genetic component demonstrated by an additive genetic heritability of 46 % [54]. The regulation of serum adiponectin levels has been linked to regions on chromosome 5p (logarithm of odds [LOD] = 4.06) and 14q (LOD = 3.2) in a predominantly northern European population [54] and to chromosome 9p (LOD = 3.0) in Pima Indians [55]. In addition, four haplotype-tagging single-nucleotide polymorphisms (SNPs) have been identified at the adiponectin-encoding APM1 locus itself. One of them, +276G>T, is associated with serum adiponectin levels collected from nondiabetic, Caucasian individuals ($P=0.032$). Individuals homozygous for the +276T allele have higher adiponectin levels than other subjects [56]. Individuals with an allelic combination of +45T and +276G ("TG" haplotype) have higher body weight ($P=0.03$), waist circumference ($P=0.004$), systolic ($P=0.01$) and diastolic blood pressure ($P=0.003$), total to HDL cholesterol ratio ($P=0.01$), and insulin resistance as measured by HOMA scores ($P=0.003$) as well as fasting serum glucose ($P=0.02$) and serum insulin ($P=0.005$) levels [57]. Subsequent studies of adiponectin polymorphisms in obese and diabetic subjects suggest similar trends. In NAFLD, the homozygous "GG" genotypes at positions −11377 and +45 were significantly more prevalent than in matched controls [58]. Moreover, the presence of the "G" allele at these positions was associated with a necroinflammatory grade [58].

It is important to note that adiponectin is secreted into the circulation as three oligomeric isoforms, including (low molecular weight, LMW), hexamer (middle molecular weight, MMW) and the high molecular weight (HMW) oligomeric complex. Obesity-related metabolic complications, including NAFLD, are especially tightly associated with lower concentrations of HMW adiponectin [59]. This observation is not incidental as HMW oligomer mediates the insulin-sensitizing effects of adiponectin on suppression of hepatic gluconeogenesis. In one of the studies, after adjustment for gender, age, and total body fat, the content of the fat in the liver and within the muscles is associated only with HMW adiponectin ($r=-0.35$, $P=0.012$), but not with total-, MMW-, or LMW adiponectin [60]. Levels of HMW adiponectin negatively correlate with the expression of nuclear receptor peroxisome proliferator-activated receptors-γ (PPAR-γ) expression in the liver, a prosteatotic factor in fatty liver disease [61].

In addition to the studies focusing on serum adiponectin levels, some recent publications looked into the role of the adipokine receptors. The results are contradictory. Kaser and colleagues showed that immunostaining of the adiponectin receptor adipoRII as well as its mRNA expression level were

significantly reduced in liver biopsies of patients with NASH compared to patients with simple steatosis, but found no differences in adipoRI mRNA expression between the two groups [62]. Similar finding were reported by Shimizu and colleagues [63]. On the other hand, Vuppalanci and colleagues reported an increase in the mRNA expression levels of adiponectin receptor AdipoRII in liver specimens of patients with NASH compared to normal liver tissue [64]. These investigators reported several other contradictory findings. The Kaser group reported adiponectin expression both in endothelial cells of portal vessels and in hepatic sinusoids [62], but Vuppalanci found no adiponectin mRNA expression in any of the liver samples studied [64]. The recent work of Carazo and co-authors corroborated findings of Vuppalanci in a larger group of morbidly obese patients ($N=60$) showing that NAFLD progression is associated with increase in the hepatic expression of both adiponectin receptors [65]. Two other studies had not registered any difference in expression of adipoRI and adipoRII in NASH and steatosis only groups [66, 67].

In connection to NAFLD, the allelic states of the human adiponectin receptor encoding gene ADIPOR1 and ADIPOR2 have been studied as well. A longitudinal study showed that a common haplotype of −8503A and −1927C ADIPOR1 alleles were associated with higher liver fat at follow-up as determined by proton magnetic resonance spectroscopy ($P=0.02$) compared with the haplotype consisting of −8503G and −1927T alleles. These observations were independent of basal measurements, sex, and baseline versus follow-up percentage of body fat [68]. In Northern European populations, the polymorphism of ADIPOR2 (rs767870) was significantly associated with liver fat content measured with (1)H-MRS after adjusting for age, gender, and BMI and related to serum gamma glutamyltransferase concentrations [69]. In subjects with diabetes Type II, the at-risk alleles for the common −64241T/G and +33447C/T SNPs in ADIPOR2 were associated with increased serum ALT and AST [70]. Both findings were confirmed by replication studies in larger cohorts.

Further studies of allelic states of adiponectin and its receptor in association with NASH and NAFLD are clearly warranted.

Resistin

Resistin has been implicated in the pathogenesis of obesity-mediated insulin resistance and Type II diabetes mellitus. In addition, resistin also appears to stimulate macrophage secretion of TNF-α and IL-12 to the same extent as lipopolysaccharides. Most likely, its proinflammatory action is an induction of the nuclear translocation of NF-κB transcription factor [71]. Both pro-inflammatory properties and association with insulin resistance suggest that resistin may play an important role in the pathogenesis of NASH. One study showed that plasma resistin concentrations in serum positively correlate with hepatic fat content ($r=0.66$, $P<0.001$) [72]. In pediatric NAFLD, hepatic progenitor cells express higher levels of resistin, and this increase is proportional to the degree of fibrosis ($r=0.432$, $P<0.05$) [73]. Another study of pediatric NAFLD indicated that serum resistin levels are lower in children with advanced liver steatosis (grade 3, $N=10$) compared to patients with mild steatosis (grade 1–2, $N=23$) [74].

However, a number of studies of adult NAFLD cohorts failed to see any NASH-related differences in plasma or serum resistin concentrations [31, 52]. The role of resistin in the pathogenesis of NAFLD requires further clarification.

Leptin

Leptin is release by adipocytes into circulation that transfers it to the central nervous system where it regulates food intake. However, leptin receptors encoded by the LEPR gene are expressed both centrally and peripherally, as they were found in many peripheral tissues. Human livers express high

mRNA levels for both short and long isoforms of the leptin receptor Moreover, there is a trend toward lower levels of mRNA encoding the long form of the leptin receptor in hepatic tissue from patients with NASH as compared to those with steatosis only [75]. In human blood, the bioavailability for leptin is modulated by the so-called soluble leptin receptor (SLR), a product of ADAM10-dependent shedding of the extracellular domain of the leptin receptor [76]. This cleavage is enhanced by treatment with lipotoxic agents and apoptosis. On the other hand, when leptin levels and/or ER stress are high, the levels of SLR in the serum are reduced, which might reflect a decrease in the membrane expression of leptin receptors.

As the SLR seem to directly block leptin action, the profiling of the serum leptin levels might be difficult to interpret. So far, only two NAFLD-related studies profiled both leptin and SLR. Huang and co-authors found that enhanced release of leptin is accompanied by a decrease in SLR concentration, which suggests higher resistance of peripheral tissues towards the action of leptin [77]. Medici and co-authors found that the extent of steatotic changes in the liver could be predicted by the Free Leptin Index (FLI) calculated as the ratio of leptin to SLR, while levels of SLR specifically, were correlated to the stage of fibrosis [78].

Leptin contributes to the development of NASH in many ways. First, abnormalities in serum leptin or its receptor promote insulin resistance. Second, leptin-dependent changes in insulin signaling increase fatty acid influx into hepatocytes, promoting lipotoxicity [79]. In the later stages of pathogenesis of NASH, leptin enhances the systemic low-grade inflammation, thus providing the "second hit" that advances simple steatosis to steatohepatitis. In fact, levels of leptin are independently associated with that of C-reactive protein (CRP) after adjustment for age, gender, BMI, waist-to-hip ratio, smoking and alcohol consumption ($F = 12.39$, $P = 0.0007$) [80]. Additionally, leptin acts as a profibrogenic cytokine in several liver diseases. As a profibrogenic agent, leptin influence both endothelial and Kupffer cells [81].

It is important to remember that NAFLD is commonly seen in conjunction with lipodystrophy, a condition characterized by the partial or complete absence of adipose tissue and hypoleptinemia. In patients with congenital lipodystrophy, leptin administration improves insulin resistance and corrects liver steatosis and hepatocellular ballooning injury [82, 83]. It also corrects elevation of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels [84]. Interestingly, the degree of liver fibrosis in NASH patients treated with recombinant leptin remains unchanged [83].

Genetics-wise, polymorphisms in both leptin and leptin receptor encoding genes were reported to have an effect on NAFLD-related phenotypes. For intronic SNP rs6700896 located within an intron of the LEPR gene, significantly different allelic frequencies were reported between NAFLD with or without T2DM and non-steatotic controls [85]. Frequencies of mutant LepR polymorphism were also significantly associated with IR increments [85]. Similar finding were also reported by Lu and co-authors [86]. The study of Aller et al. showed a substantial influence for the Asparagine-656 variant on leptin receptors (Lys656Asn polymorphism within LEPR gene) with obesity-related, rather than NAFLD-related parameters [87]. Larger, better controlled studies of the polymorphisms of leptin and leptin receptor-encoding genes in NAFLD are warranted.

TNF-alpha

TNF-α is a proinflammatory cytokine that orchestrates the synthesis, secretion, and activity of other proinflammatory molecules. Macrophages are a major source of TNF-α in humans. TNF-α is also produced by many other tissues in response to various pathological processes such as infection, ischemia, and trauma. Adipocytic production of TNF-α is very low [88]. Nevertheless, an overall increase in the adipose mass usually leads to substantial, cumulative production of this cytokine that is achieved by stromavascular cells and macrophages infiltrating expanded adipose. Increased amounts of TNF-α released by excessive adipose tissue may contribute to the development of obesity-related NAFLD.

Several studies have demonstrated that serum levels of TNF-α are significantly higher in patients with NASH than in healthy controls (see [30, 31] for review). A comprehensive study of TNF-α in steatohepatitis shows remarkable increases in the expression levels of mRNA encoding TNF-α in both hepatic and adipose tissues in NASH patients as compared to obese controls [89]. Similar mRNA increases were observed for the p55 receptor, but not for the p75 receptor of TNF-α. Furthermore, the degree of hepatic fibrosis correlated with TNF-α expression levels in adipose tissue and levels of mRNAs encoding p55 in the hepatic tissue [89]. Recent meta-analysis suggests that TNF-α gene promoter polymorphism at position −238 but not −308 might be a risk factor for NAFLD (GA/AA versus GG [odds ratio = 2.06, 95 % confidence interval = 1.58–2.69, $P < 0.00001$]) [90].

Additional indirect evidence of TNF-α involvement in NASH comes from a 12-month trial of pentoxifylline, a methylxanthine that can suppress both TNF-α at the level of mRNA accumulation and the bioactivity of its secreted form, possibly through the generation of intracellular cAMP. A study by Adams showed that both alanine and aspartate aminotransferase levels are significantly lower after 12 months of therapy compared to baseline, indicating a significant improvement in treated patients [91]. Another study examined 18 patients with histologically proven NASH who received pentoxifylline (400 mg three times per day) for 6 months. After 6 months of therapy, the mean AST and ALT improved significantly ($P < 0.0001$ and <0.0001, respectively). In fact, ALT levels normalized in 23 % of patients at month 1, 35 % at month 2, and 60 % at month 6 of treatment. The insulin resistance index also improved ($P = 0.046$) and the serum TNF-α was also reduced significantly after therapy ($P = 0.011$), while serum triglyceride, cholesterol, and body mass index (BMI) remained unchanged [92]. Both of these studies suggest a potential role for TNF-α in NASH as well as potential interventions targeting TNF-α. The most recent randomized intention-to-treat pentoxifylline trial ($N = 26$, pentoxifylline arm; $N = 29$, placebo arm) showed a decrease of ≥2 points in the NASH activity score (NAS) in 38.5 % of patients on PTX versus 13.8 % of those on placebo ($P = 0.036$); this effect was accompanied by a significant decrease in steatosis and lobular inflammation [93]. Despite the encouraging tone of these reports, recent systematic reviews show that pentoxifylline reduces AST and ALT levels and may improve liver histological scores in patients with NAFLD/NASH, but does not appear to affect TNF-α or other cytokine levels [94]. It is currently unclear whether the effects of pentoxifylline on NAFLD phenotypes are due to its suppression of TNF-α or to its non-TNF-α related anti-inflammatory properties.

Other Cytokines

Obesity is a chronic state of low-grade inflammation which predisposes obese individuals to both insulin resistance (IR) and NAFLD. A progressive infiltration of classically activated (M1) macrophages into obese adipose tissue leads to a release of proinflammatory cytokines. In lean individuals macrophages are in an alternatively activated (M2) state; these cells secrete IL-10, an anti-inflammatory cytokine, which may protect against inflammation. Differential activation of resident macrophages defines net inflammatory/anti-inflammatory balance of secreted peptides released by adipose. Additionally, overproduction of inflammatory cytokines in adipose could be explained by a decrease in the production of miRNAs that regulate their synthesis. Concerted decrease of mature miRNA levels has recently been reported in adipose of patients with advanced stages of NASH [95].

In obese subjects, both visceral and subcutaneous adipose tissues release potent pro-inflammatory cytokines interleukin-6 (IL-6) and interleukin-8 (IL-8) [34, 96]. In fact, explanted human adipose tissue releases even more IL-6 and IL-8 than adiponectin [97], probably due to an obesity-related increase in the TNF-α production involving p38 MAPK and NF-κB pathways [98]. Unfortunately, little is known about IL-6 and IL-8 in patients with NAFLD or NASH. Some studies showed that serum IL-6 and IL-8 levels in patients with NASH are significantly higher than the healthy controls [31, 99, 100].

On the other hand, IL-6 seems to have hepatoprotective effects [101]. In particular, it prevents sinusoidal endothelial cell damage and associated changes in hepatic microcirculation and decreases hepatocyte death [102]. Moreover, in vitro, IL-6 treatment improves the outcomes for patients with liver transplants for alcohol-related liver disease [102]. These contradictory data emphasize our incomplete understanding of the role of these cytokines in NAFLD.

Another significant focus of interest is on monocyte chemoattractant protein-1 (MCP-1)/CCL2. This potent chemoattractant is secreted mainly by macrophages known to infiltrate adipose tissue in obese humans. Levels of MCP-1 are elevated in both the adipose tissue and the plasma of obese mice [103]. Interestingly, MCP-1 deficiency in mice fed a high-fat diet decreases insulin resistance and hepatic steatosis whereas mice overexpressing MCP-1 in adipose tissue show increased insulin resistance and hepatic triglyceride levels [104]. In mouse models of acute and chronic hepatic injury, pharmacological inhibition of MCP-1 suppresses the infiltration of macrophages into the liver and intrahepatic production of proinflammatory cytokines [105]. In humans, data on the role of MCP-1 in NAFLD are sparse. MCP-1 serum levels are elevated in patients with ultrasound-diagnosed NAFLD and positively correlate with body-mass index and fasting glucose [106].

Recently, the presence and accumulation of the CD11c(+)CD1c(+) dendritic cells has been demonstrated in the adipose tissue of obese individuals [107]. These cells are capable of inducing the differentiation of IL17-producing type of T-helpers (Th17) that functionally oppose T(reg)-mediated responses. In turn, Th17 cells may infiltrate the liver and facilitate the transition from simple steatosis to steatohepatitis [108]. This line of thought is augmented by observations of Th17 cells acting synergistically with FFAs to induce IL-6 production in cultured hepatic cells and of an increase in the hepatic expression of Th17 cell-related genes encoding for retinoid-related orphan receptor gamma (ROR)γt, IL-17, IL-21, and IL-23 in NASH patients as compared to healthy controls [108]. Moreover, in mice, neutralization of IL17 with specific antibodies improved their resistance to LPS-induced liver injury as measured by lower serum alanine aminotransferase (ALT) levels and reduced inflammatory cell infiltrates in the liver [108]. These data suggest that Th17 cell expansion and hepatocyte-generated IL-6, particularly in the presence of FFAs, such as would occur in NAFLD, may contribute to a vicious cycle leading to increased levels of hepatic inflammation and steatosis. Targeting the balance between Th17 cells and T(regs) may lead to novel strategies in preventing NAFLD progression.

The Role of Adipokines in Promoting Hepatic Steatosis, Insulin Resistance Oxidative Stress, and Hepatic Fibrosis

As previously noted, hepatic steatosis, insulin resistance and oxidative stress all play critical roles in the pathogenesis of NAFLD, forming the basis of the "two-hit" or "multi-hit" hypotheses [23, 24]. The following paragraphs focuses on the potential role of adipokines in inducing hepatic steatosis, enhancing insulin resistance, and promoting oxidative stress or hepatic fibrosis.

Adipokines and Steatosis

Fat accumulation in hepatocytes may result from an increase in the delivery of FFAs to the liver, increased FA synthesis, decreased FA degradation, impaired triglyceride release from the liver, or a combination of these factors. The role of several key adipokines in each of these steps is described below.

Adiponectin as an Anti-steatotic Agent

One hypothesis linking low serum adiponectin to the development of NAFLD focuses on an increase in hepatic lipid retention, a consequence of adiponectin-dependent suppression of very-low-density lipoprotein (VLDL) synthesis, the chief route of hepatic lipid export. One of the rate-determining steps in hepatic VLDL production is the synthesis of apoB-100. The absolute synthesis rates of apoB-100 in patients with NASH are lower (31.5 ± 3.4 mg/kg/day) than in either obese (115.2 ± 7.2 mg/kg/day, $P < 0.001$) or lean non-NAFLD controls (82.4 ± 4.1 mg/kg/day, $P = 0.002$) [109]. In fact, plasma adiponectin concentrations are inversely associated with both VLDL-apoB-100 concentrations ($r = -0.337$) and VLDL-apoB-100 production rates ($r = -0.373$) [110]. Additionally, results reported by Ng and coworkers [111] indicate that lower than normal adiponectin levels may weaken its beneficial effects on the accumulation of triglycerides and on the concentration of fatty acids in skeletal muscle. Earlier work showed that adiponectin enhances fatty acid oxidation both in liver and muscle tissue through activation of acetyl CoA oxidase, carnitine palmitoyltransferase-1, and 5′-AMP activated protein kinase (AMPK) [112]. Adiponectin increases lipoprotein lipase (LPL) translocation to the cell surface where it could be released [113]; decreased serum adiponectin is associated with LPL deficiency and acts independently of systemic inflammation and/or insulin resistance [114, 115]. Seemingly, these data indicate that the decrease in serum adiponectin concentrations may stimulate the accumulation of fat in the liver by promoting LPL deficiency and subsequent increase in free fatty acid flux to the liver. However, there are other data suggesting that the logic of relationship between adiponectin and LPL levels may be inverted. In patients with loss-of-function LPL gene variants, plasma adiponectin concentrations are significantly lower than in matched controls [116]. In fact, in the LPL mutation group, lower levels of adiponectin explained a proportion of the variance in metabolic covariates and, after adjustments for anthropometrics, lipids, glucose and other factors, substantially contributed to risks of obesity-associated disorders [116].

Other researchers have suggested an alternative mechanism for the steatogenic effects of low levels of adiponectin. One mechanism involves an increase in FA synthesis and/or a decrease in FA degradation within the liver. Through PPARalpha, adiponectin stimulates the expression of carnitine palmitoyltransferase 1 (CPT1), a rate limiting enzyme involved in the transport of long-chain fatty acids into the mitochondrial matrix of liver cells [117]. At the same time, adiponectin decreases the activity of two key enzymes in the hepatic lipogenesis pathway, namely, acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS) [117]. In transgenic mice that overexpress adiponectin via the aP2-promoter (ADNTg mice), lipogenic gene expression is reduced as well [118]. These data suggest that an increase in adiponectin concentrations should stimulate beta-oxidation of fatty acids in the liver, decreasing the intrahepatic lipid load, while low adiponectin levels should promote hepatic steatosis, further supporting the anti-steatotic properties of adiponectin.

Leptin as an Anti-steatotic Agent

Leptin protects against steatosis and lipotoxicity in non-adipose tissues, including the liver, but the molecular mechanisms underlying these effects are not fully understood. Most likely, the mechanism for this protection is peripheral. In cultured pancreatic islets, leptin lowers triglyceride content by increasing FFA oxidation and preventing its esterification [119]. A similar mechanism may be at work in the liver, as it expresses leptin receptors. In fact, tissue-specific over expression of wild-type leptin receptors in steatotic livers of leptin-receptor-null fa/fa rats reduces TG accumulation in the liver but not anywhere else [120].

Early studies demonstrated that, in hepatocytes, the stimulation of the long isoform of the leptin receptors provides IL-6-like signals, as it synergizes with IL-1 and TNF-alpha to activate STAT proteins and synthesize acute-phase plasma proteins [121]. In addition, leptin dramatically suppresses the

expression of hepatic stearoyl-CoA desaturase-1 (SCD-1), the rate limiting enzyme in the biosynthesis of monounsaturated fats [122, 123]. SCD-1 suppression, in turn, supports resistance to both hepatic steatosis and obesity due to marked increase in energy expenditure. The proposed mechanisms for the metabolic effects mediated by leptin induced SCD-1 deficiency include the blocking of triglyceride synthesis and the export of VLDL [122, 124]. This, in turn, leads to a concomitant increase in the pool of saturated fatty acyl CoAs, which allosterically inhibits acetyl CoA carboxylase (ACC) and reduces the amount of malonyl CoA. As a result, inhibition of the mitochondrial carnityl palmitoyl shuttle system is relieved, stimulating the import and oxidation of fatty acids in mitochondria. Thus, leptin administration de-represses fatty acid oxidation, leading to increased fat burning [122]. Other proposed mechanisms of anti-steatotic effects of leptin involve increases in peroxisome proliferator-activated receptor-α (PPAR-α) signaling [125] and/or activity of AMP-activated protein kinase (AMPK) [126].

Beyond this, leptin seems to promote the elimination of plasma cholesterol by decreasing cholesterol biosynthesis. Cholesterol elimination is achieved by down regulating the hepatic activity of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, up-regulating the activities of both sterol 27-hydroxylase and cholesterol 7α-hydroxylase and diminishing the cholesterol fraction bound to VLDL by limiting triglyceride supply [127]. Lowered leptin signaling may even be responsible for the increased prevalence of cholesterol gallstones in obese patients as compared to the general population [128].

Because obesity is associated with leptin resistance, exogenous leptin administration does not diminish liver steatosis. The development of both central and peripheral leptin resistance depends on the liver. In animal models, chronic leptin treatment in leptin-naïve animals induces leptin receptors and subsequent increases in the serum concentration of the soluble leptin receptor protein (SLR). This increase occurs as a consequence of ectodomain shedding from the membrane-bound isoforms. SLR shedding sequesters leptin and prevents its productive interactions with the receptor. In this way, the hepatic circuit limits peripheral leptin activity all over the body. To reflect available leptin as opposed to any leptin, the free leptin index (FLI), calculated as the ratio of leptin to SLR, was developed. Studies correlating FLI and hepatic steatosis, however, contradict each other; some studies show that FLI scores are lower in steatosis as compared to non-NAFLD obese individuals [78], while others point at the opposite [77, 129].

Resistin as a Pro-steatotic Agent

In rodents, resistin is capable of influencing lipid metabolism. Adenovirus-mediated resistin over expression leads to an increase in plasma triglycerides in mice and rats [130, 131]. Loss of resistin ameliorates hyperlipidemia and hepatic steatosis in leptin-deficient mice [132]. In healthy men, serum resistin levels were found to negatively correlate with high-density lipoprotein cholesterol (HDL-C) levels [133]. When human hepatocytes are treated with resistin, at levels observed in human obesity, the secretion of apoB dramatically increases along with hepatocyte lipid content. These increases are due to the stimulation of de novo lipogenesis via the SREBP1 and SREBP2 pathways [134]. This suggests that higher than normal resistin levels, typically seen in obesity and type II diabetes, might contribute to the development of fatty liver through its dyslipidemic effects.

TNF-α as a Pro-steatotic Agent

The list of the pleiotropic effects of TNF-α includes changes in lipid metabolism. In the liver, TNF-alpha stimulates the expression of genes involved in de novo synthesis of FAs, while suppressing those responsible for FA oxidation. This activation is achieved through interactions with the insulin-Insig-Srebp signaling pathway. The direct consequences to steatogenesis include reduction of

HDL-cholesterol, increase of LDL-cholesterol, the accumulation of potentially harmful precholesterol metabolites and the suppression of cholesterol elimination through bile acids [135]. It also enhances the secretion and metabolic processing of VLDLs [136].

Over the years, mice expressing T cell-targeted human TNF-α transgenes have served as an animal model for persistent low-grade exposure to TNF-α typical of morbid obesity. In these mice, hepatic triglyceride and cholesterol levels are increased, despite concomitant serum cholesterol lowering [137]. In addition, mitochondrial β-oxidation is inhibited in the livers of hTNFα-transgenic mice [137], as well as the activities of carnitine palmitoyltransferase-II (CPT-II) and mitochondrial HMG-CoA synthase [137]. Peroxisomal β-oxidation is also lowered [137]. These changes are probably mediated by low mRNA levels of peroxisome proliferator-activated receptors α and δ (PPARα and PPARδ) [137]. On the other hand, there is no increase in de novo fatty acid synthesis. In fact, both fatty acid synthase (FAS) activity and the gene expression of acetyl-CoA carboxylase 2 (ACC2) are reduced [137]. Therefore, liver-specific steatogenic effects of TNF-α are predominantly caused by lipid retention due to the suppression of fatty acid decomposition. It is important to mention that both the control and the transgenic mice in this study were fed ad libitum. Thus, this model does not allow us to determine whether these effects are direct consequences of TNF-α, peripheral consequences of elevated TNF-α levels, or secondary effects related to other TNF-α-mediated mechanisms such as appetite.

In addition to pronounced inhibition of mitochondrial and peroxysomal β-oxidation, TNF-α stimulates VLDL production in the liver [136] and inhibits the activity and mRNA expression of lipoprotein lipase in adipocytes [138]; these processes favor lipolysis in visceral and subcutaneous fat depots that contribute to the development of TNF-α dependent hypertriglyceridemia and associated NAFLD.

Adipokines and Insulin Resistance

There is a striking association between NASH and insulin resistance. As insulin resistance is thought to lead to the accumulation of triglycerides in the liver [139]; thus, any factor promoting a vicious cycle of insulin signaling can be steatogenic and factors counteracting insulin resistance can be protective against the development of NAFLD.

Adiponectin as Insulin Sensitizer

Hyperinsulinemia caused by insulin resistance increases fatty acid synthesis and impairs both mitochondrial β-oxidation and the export of triglycerides in multiple ways. Early studies indicate that adiponectin decreases insulin resistance by increasing oxidation of FAs, thereby reducing the triglyceride content in peripheral tissues [112]. Adiponectin also suppresses glucose production in the liver and enhances the hepatic action of insulin. These glucose-lowering effects of adiponectin require liver-specific activation of AMP-activated protein kinase (AMPK) [140], a central component of the protein kinase cascade that plays a key role in the regulation of energy control. It is tempting to speculate that the AMPK-mediated antiglycemic effects of adiponectin in the liver may play a role in the prevention of NAFLD, but this seems unlikely. Recent work indicates that short-term over expression of a constitutively active form of AMPK in the liver can lead to the development of steatosis even in the presence of lowered hepatic glycogen synthesis and circulating lipid levels [141]. Most likely, the NAFLD-like disorder in these animals develops from the hepatic accumulation of lipids released from adipose tissue in response to the relative scarcity of glucose. Therefore, additional stimulation of AMPK provided by a sudden increase of adiponectin (e.g., due to thiazolidinedione (TZD) treatment) may aggravate early stages of the steatogenic processes in the liver. This may also explain the infrequent but potentially serious hepatotoxic side effects of chronic administration of TZDs [142] and the pronounced exacerbation of hepatic steatosis in mice with polygenic obesity treated by rosiglitazone [143].

Insulin-sensitizing effects of adiponectin are not limited to AMPK signaling events. For example, in primary mouse hepatocytes, the absence of AMPK, or other components of the same signaling cascade did not prevent adiponectin from inhibiting glucose output or reducing gluconeogenic gene expression [144]. It is also important to note that adiponectin inhibits autophagy, while AMPK stimulates it, pointing at other signaling pathways sensitive to the presence of adiponectin. One potential contributor to AMPK-independent adiponecting response is newly discovered suppressor of glucose by autophagy (SOGA) that both inhibits autophagy and contributes to the inhibition of glucose production in hepatocytes [145].

Interestingly, various treatments successful at improving insulin response (thiazolidinediones (TZDs), *n*-3 polyunsaturated fatty acid (PUFA) supplementation) also stimulate adiponectin production [146].

Leptin as an Insulin Sensitizer

It is widely accepted that leptin exerts a systemic insulin-sensitizing effect. The interaction between the insulin and leptin signaling cascades in peripheral organs have been studied both in vitro and in vivo [147]. However, the results are inconsistent in different cell lines and the complete mechanism remains unclear. Most likely, these cross-cascade interactions involve down-regulation of the PKR-like endoplasmic reticulum (ER) kinase/eukaryotic translation inhibition factor 2α (PERK-eIF2α) arm of ER stress in liver, skeletal muscle, and adipose tissue [148]. One way of inhibiting the peripheral effects of leptin is through the feedback inhibition by SOCS3 via phosphorylation of Tyrosine 985 on its receptor [149]. When this tyrosine is mutated, hence, abrogating this inhibitory signaling, the insulin sensitivity is enhanced throughout the body via increased insulin action on the suppression of hepatic glucose production [150]. The liver is probably central to this mechanism, as some studies suggest that leptin selectively improves insulin receptor (IR) activation only in this organ, but not in the skeletal muscle or fat [151]. Unfortunately, the insulin-related branch of the leptin-dependent signaling in obese livers is profoundly suppressed [152]. Therefore, it is unlikely that therapeutic administration of leptin would alleviate liver steatosis through improved insulin sensitivity.

Resistin as an Inductor of Insulin Tolerance

Hyperresistinemia certainly contributes to impaired insulin sensitivity in obese rodents. In mice, resistin elimination reduces hepatic glucose production due to decreased gluconeogenic enzyme expression in the liver and to the activation of AMPK [153]. In humans, the situation is much more difficult to trace, because serum resistin levels are related to sex, age, testosterone and estradiol levels [154]. These fluctuations in resistin levels and the relatively low homology between resistin and resistin-like molecules in humans and rodents complicate the study of resistin involvement in the development of insulin resistance in the liver and NAFLD.

TNF-α Impairs Insulin Signaling

TNF-α alters systemic energy homeostasis in a way that closely resembles the insulin resistance phenotype. Mice with a complete knock-out of TNF-α signaling show significantly improved insulin sensitivity in both diet-induced and leptin-deficient obesity [155]. Molecular studies show that long-term exposure to TNF-α completely abolishes insulin-induced glycogen synthesis in hepatocytes [156]. TNF-α inhibits *tyr* phosphorylation of IRS-1 that promotes the transmission of the insulin signal, and stimulates *ser* phosphorylation instead, thus blunting peripheral insulin response [157, 158].

It was also shown that TNF-α-mediated insulin resistance of glucose uptake occurs through a MEK/Erk-dependent activation of CDK5 [159]. This compelling evidence demonstrates that abnormal production of TNF-α may predispose obese individuals to the development of insulin resistance and NAFLD.

Other Adipokines Influencing Insulin Resistance

The secretion of the extracellular form of nicotinamide phosphoribosyltransferase (NAMPT), also known as *visfatin*, is upregulated in obesity and has been shown to help in the regulation of glucose homeostasis. Visfatin regulates insulin secretion, insulin receptor phosphorylation and intracellular signaling and the expression of a number of beta-cell function-associated genes in the pancreas [160]. In rat livers, visfatin is strongly expressed, while in visceral fat its expression is significantly lower, and in subcutaneous adipose, it is undetectable. When visfatin was downregulated in rat hepatocytes by RNAi, a significantly decrease in glucose uptake after stimulation with insulin was observed, thus pointing at substantial autocrine effects on the sensitivity of liver cells to insulin action, possibly through its effects on NAD biosynthesis [161].

Another interesting adipokine with potential effects on the pathogenesis of NASH is *vaspin*, visceral adipose tissue-derived member of serine protease inhibitor (serpin) family [162]. Vaspin administration in obese mice fed with high-fat high-sucrose chow normalizes their serum glucose levels by reversing altered gene expression related to insulin resistance, including all other adipokines discussed above [162]. In humans, the role for vaspin in metabolic regulation is unclear at present. Serum vaspin concentrations display a circadian rhythm, along with a preprandial rise and postprandial fall, similar to that of ghrelin, while unscheduled meals lead to a decrease in vaspin levels [163]. The study in normoglycemic ($N=259$) and diabetic Japanese patient ($N=275$) showed that serum vaspin levels closely correlate with HOMA scores for insulin resistance rather than with BMI and other anthropometric parameters. Moreover, the minor (A) allele of SNP rs77060950 in the promoter region of the vaspin-encoding gene SERPINA12 appears to be closely linked to increased levels of serum vaspin and higher HOMA scores [164]. Additionally, insulin sensitivity was shown to be the strongest determinant of vaspin mRNA expression in human subcutaneous adipose [165]. These data point at vaspin as an important insulin sensitizer of adipocytic origin that may play an instrumental role in NAFLD.

Apelin inhibits glucose-stimulated insulin secretion both in vivo and in vitro by acting on its receptor, which is expressed in beta-cells of pancreatic islands [166]. The expression of apelin in fat cells and apelin plasma levels are largely increased in all the hyperinsulinemia-associated obese states of mice, independent of diet composition [167]. In obese patients, plasma apelin levels are also significantly higher than in normal controls. When apelin levels were studied in patients with NAFLD and healthy controls of the same gender, apelin levels correlated with and HOMA indexes positively ($r=0.4$, $P=0.008$). Importantly, adjustments for BMI and HOMA indices eliminated the differences in apelin concentrations between compared groups [168].

Chemerin, encoded by the retinoic acid receptor responder 2 gene RARRES2, exacerbates glucose intolerance, lowers serum insulin levels, and decreases tissue glucose uptake in obese/diabetic but not normoglycemic mice when administered exogenously [169]. The disruption of the chemokine-like receptor-1 (CMKLR1) gene that encodes the receptor for chemerin leads to glucose intolerance as evidenced by decreased glucose stimulated insulin secretion as well as decreased skeletal muscle and white adipose tissue glucose uptake [170]. Studies using hemerin-deficient murine islands and a chemerin-ablated β-cell line showed that chemerin regulates β-cell function via maintaining expression of MafA, a pivotal transcriptional factor that stimulates insulin gene promoter activity [171]. In adipocytes, chemerin potentiated insulin-stimulated glucose uptake concomitant with enhanced insulin signaling [172]. Intravenous administration of the chemerin analog in sheep led to a dramatic increase in the insulin levels and a drop in glucose levels, along with an immediate increase in the

level of triglycerides [173]. In humans, receptors for chemerin, CMKLR1, could be detected in primary human hepatocytes (PHH), Kupffer cells, bile-duct cells, and hepatic stellate cells; its amounts were strongly induced by treatment with exogenous adiponectin [174].

It seems that the *NUCB2*-encoded *nesfatin* play important roles in both central and peripheral branches of the glucose homeostasis regulation. In the brain, it stimulates the PEPCK/InsR/IRS-1/AMPK/Akt/TORC2 pathway, thus contributing to increased peripheral and hepatic insulin sensitivity by decreasing gluconeogenesis and promoting hepatic glucose uptake in vivo [175]. In β-cells, nesfatin exerts a direct, glucose-dependent insulinotropic action [176, 177]. It acts by promoting Ca(2+) influx through L-type Ca(2+) channels independently of PKA and PLA(2) [177]. Levels of nesfatin are elevated in newly diagnosed type 2 diabetes patients and glucose intolerant subjects [178], but are decreased in patients with insulin resistance-associate polycystic ovary syndrome (PCOS) [179]. In line with the above, there is some evidence of the reduction of sensitivity to nesfatin in obese individuals, possibly due to the saturation of transporters [180].

Omentin is expressed in stromal vascular cells of the visceral adipose, but not in the fat cells per se [181]. In human adipocytes grown in vitro, omentin enhances insulin-stimulated glucose uptake, while not affecting basal influx of glucose [181]. In omental adipose tissue explants, both insulin and glucose significantly and dose-dependently decrease the secretion of omentin [182]. Fasting serum omentin levels negatively correlate with HOMA-IR scores and positively correlate with serum levels of adiponectin [183].

In serum samples of patients with NAFLD or with NAFLD-associated disorders, the concentration of these, newly emerging, adipokines are altered. The interplay between novel insulin-sensitizing and insulin suppressing soluble molecules may represent an important avenue for future studies of the pathogenesis of NAFLD and NASH.

Adipokines and Oxidative Stress

Several lines of evidence support the role of adipokines in the increased oxidative stress seen in patients with NASH. Most studies converge on CYP2E1, peroxisomal release of reactive oxygen species and mitochondrial dysfunction with high proton potential. Within the liver, the reactive oxygen species (ROS) and reactive nitrogen species (RNS) are generated by the parenchymal cells, by activated Kupffer cells and by both resident and attracted inflammatory cells, which further mobilize cellular defense mechanisms and contribute to liver injury and necrosis. Here we will review ROS/RNS-promoting and antioxidant properties of adipokines, leaving out their cytokine-mediated proinflammatory/anti-inflammatory effects as these are reviewed in detail in other chapters.

Antioxidant Role of Adiponectin

The potential role of adiponectin in the prevention of oxidative stress in the liver lacks direct evidence. Nakanishi and coworkers presented the most compelling indirect evidence of such an influence [184]. The authors used an oral glucose test to study 259 Japanese Americans with normal glucose tolerance, impaired glucose tolerance, or diabetes. Concentrations of 8-iso-prostaglandin F(2-α) (isoprostane), a marker of oxidative stress, were measured in the urine and adiponectin concentrations were measured in the serum. Adiponectin levels negatively correlated with urinary isoprostane levels (adjusted for age, gender, and smoking status). This association was attenuated but remained significant after adjusting for waist-to-hip ratio, body mass index, percent body fat, C-reactive protein levels, glucose tolerance status, or HOMA scores [184].

A different line of evidence suggests a reverse causative relationship between oxidative stress and levels of adiponectin, such that the production of adiponectin might be suppressed in pro-oxidative conditions. For example, in differentiated murine adipocytes exposed to increasing concentrations of glucose oxidase, adiponectin mRNA expression is suppressed [185]. Moreover, a significant inverse correlation between the formation of 4-Hydroxynonenal (4-HNE), an important by-product of lipid peroxidation, and adiponectin secretion was observed. Additionally, 4-HNE inhibits adiponectin production when administered alone [185]. Researchers have proposed a direct transcriptional effect of 4-HNE on the adiponectin gene and/or effects mediated by NF-κB activation through the phosphorylation of Iκ [185].

On the other hand, there is a certainty that adiponectin exerts antioxidant effects in non-hepatic cell types. For example, it inhibits the generation of ROS in human glomerular mesangial cells treated with high concentrations of glucose; it also stimulates eNOS activity, which has additional protective effects [186]. Similar effects were observed in primary human phagocytes; these effects were invoked by full-length adiponectin only, while the globular fragment of adiponectin enhanced the production of ROS [187]. The list of other cellular types protected by full-size adiponectin from an excess of oxidative stress include: β-cells of the islets, endothelial cells, cardiomyocytes, and adipocytes. It is likely that in the human livers the HMW or full-size adiponectin is exerting similar effects, thus protecting from the progression of simple steatosis to NASH.

The most recent, intriguing observation on possible liver-specific anti-inflammatory function of adiponectin is that, in the liver cells, it stimulates the expression of UCP-2, the mitochondrial inner membrane transporter uncoupling protein 2 with hepatoprotective effects. A recent study of adiponectin knockout (ADN-KO) mice treated with adiponectin found that it stimulates mitochondrial superoxide production that, in turn, facilitates the transportation, stabilization and translation of UCP2 mRNA in nonparenchymal cells of the liver [188]. Livers of untreated AND-KO mice readily accumulate fat and have dysfunctional mitochondrial, while the replenishment of adiponectin restores the oxidative capability of the mitochondrial respiratory chain (MRC) complexes, thereby preventing the accumulation of lipid peroxidation products without a direct effect on mitochondrial biogenesis [189]. LPS induces elevation of TNFα and ALT levels in the livers of ADN-KO mice, but not in the livers of UCP2-KO mice [189]. These evidences suggest a UCP-2 dependent beneficial effect of adipose-derived adiponectin on the levels of oxidative stress in the liver (Fig. 17.3).

Pro-oxidant Role of Leptin

Leptin inhibits antioxidant systems and enhances lipoperoxidation in the liver and other tissues. Administering leptin to experimental animals increases hepatic acetyl-coenzyme A carboxylase phosphorylation, fatty acid oxidation and ketogenesis [189] along with the hepatic levels of thiobarbituric acid reactive substances (TBARS), which are markers of lipoperoxidation [190]. On the other hand, this same treatment decreases antioxidant GSH levels and the activities of glutathione-*S*-transferases (GSTs), superoxide dismutase (SOD) and catalase [191]. These differences are more pronounced when hyperleptinemia is induced in alcohol-treated animals, suggesting that leptin may augment liver injury mediated by free radicals via other mechanisms [191].

Intravenous injections of leptin induce the release of nitric oxide (NO) by both inducible NO synthase (iNOS) and endothelial nitric oxide synthase (eNOS) [192]. When overexpressed eNOS remains uncoupled, it changes from a protective enzyme to a contributor to oxidative stress, thus adding up to a pro-oxidative environment [193]. Therefore, leptin induced stimulation of eNOS and iNOS may be a pro-oxidative event.

Leptin also upregulates CYP2E1 expression, a cytochrome P450 responsible for the oxidation of alcohol and production of activated oxygen species leading to oxidative stress. Proofs that leptin regulates CYP2E1 activity come from observations that livers of leptin-deficient mice express much lower levels of CYP2E1, while short-term leptin replacement completely reverses suppression of CYP2E1 [194].

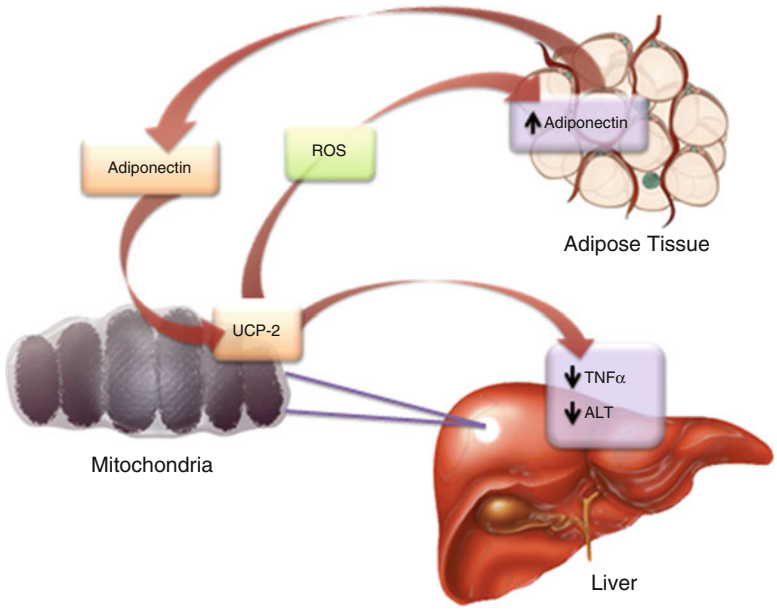

Fig. 17.3 Mutual regulation of adiponectin and UCP2 across the liver and adipose tissue

Paradoxically, CYP2E1-dependent production of ROS inhibits apoptosis but accelerates necrosis, stimulated by polyunsaturated fatty acids [195]. This latter observation is consistent with the neuroinflammatory features seen in patients with NASH. Finally, higher hepatic CYP2E1 expression and activity have been frequently observed in the context of NAFLD (see [196] for review).

Pro-oxidant Role of Resistin

Despite the scarcity of observations showing that resistin directly influences oxidative stress, there is a body of evidence suggesting a pro-oxidative role for this adipokine. In porcine coronary arteries, resistin evokes an increase in the production of superoxide radical [197], while in rats, the long-term overexpression of resistin in cardiomyocytes in vivo results in larger amounts of TNFα release, along with increased phosphorylation of IκBα and intracellular ROS content [198]. It is of interest that individuals with high expression levels of NAD(P)H:quinone reductase (NQO), a prototypical phase II antioxidant enzyme, tend to have higher levels of resistin mRNA in their subcutaneous adipose tissue [199]. The positive effects of NQO are exercised only in individuals with at least one −180G allele that confers significant increase to the basal activity of the resistin promoter [199]. It is possible that antioxidant enzymes and oxidative stress-promoting resistin are co-regulated. Importantly, levels for both gene and protein expression of resistin are substantially higher in the liver than in the adipose [200]. Accordingly, it is important to find out wither locally produced resistin plays its pro-oxidative role within the liver parenchyma.

Pro-oxidant Role of TNF-α

The important role of TNF-α in the enhancement of ROS production, observed in steatotic livers, is certain. Key components of TNF signaling include sphingolipids, particularly ceramide, generated from acidic sphingomyelinase activation [201]. Mitochondria isolated from TNF-α-treated liver cells

show a two- to threefold increase in the amount of ceramide when compared with mitochondria from untreated cells. Ceramide, in turn, influences the mitochondrial electron transport chain and evokes hydrogen peroxide overproduction [202], one of the most potent sources of oxidative damage. In addition, ceramide induces necrosis through the mitochondrial membrane permeability transition (MMPT) mechanism [203]. In mitochondria, treatment with TNF-α induces pronounced morphological changes, decreased mitochondrial membrane potential and reduced production of intracellular ATP [204]. These changes are accompanied by accumulation of significant amounts of reactive oxygen species (ROS) [204].

Some conditions sensitize liver cells to TNF-α-induced cell death; one of these is the depletion of reduced glutathione [205]. It is peculiar that chronic exposure to low levels of TNF-α, similar to that observed in NAFLD patients, profoundly modulates GSH metabolism. Reduced levels of catalase and glutathione peroxidase (reflecting impaired redox buffering capacity) were observed in the livers of mice with low, persistent expression of TNF-α in the T-cell compartment [206]. GSH depletion driven by TNF-α may further enhance oxidative stress, constituting a vicious circle augmenting parenchymal injury. Consistent with this notion, the oxidized/reduced glutathione ratio (GSSG/GSH) is increased in NASH patients, while superoxide dismutase, glutathione peroxidase and glutathione reductase activities remain unchanged [207].

Interestingly, TNF-α enhances UCP-2 expression in hepatocytes [208]. In these effects, the action of TNF-α is similar to that of adiponectin (see argument in respective subchapter). On the one hand, the up-regulation of UCP-2 may compromise cellular ATP levels and worsen liver damage by augmenting cell death; on the other hand, it may have a protective role by reducing ROS. Studies of adiponectin-dependent upregulation of UCP-2 [188, 189] point toward the second option. It is also possible that these two effects cancel each other out. The latter suggestion has been supported by the finding that serum alanine aminotransferase (ALT) levels and steatohepatitis scores in UCP-$2^{-/-}$ mice remains similar to those of wild-type controls, both in genetically and diet-induced obesity [209]. Little is known about the state of UCP-2 activity in NAFLD and NASH patients. There is only one study that covers hepatic expression of UCP-2 in humans; in this study, the staining intensity of TNF-α and UCP-2 were correlated with the severity of liver disease [210]. If the majority of NAFLD patients, indeed, overexpress UCP-2, as do NAFLD mice, and if this over expressed molecule remains functional, it seems that it is unable to protect against steatohepatitis, possibly due to the overwhelming effect of ROS that exceeds the compensatory potential of mitochondrial uncoupling. As mitochondrial uncoupling sensitizes cell to TNF-α induced death, it might be that this effect outweighs the simultaneous decrease in the ROS production.

Other Adipokines and Oxidative Stress

The effects of *NAMPT/visfatin* at the levels of oxidative stress are unclear at best. On the one hand, nicotinamide mononucleotide (NMN), a product of the NAMPT reaction and a key NAD(+) intermediate, restores sensitivity to glucose in diabetic mice through enhancement of hepatic insulin sensitivity and restoration of gene expression related to oxidative stress and inflammatory response [211]; On other hand, in both human primary pulmonary artery endothelial cells and A549 lung epithelial cells, the overexpression of visfatin affects intracellular ROS production in either the presence or absence of IL1-β. These effects are abrogated by visfatin-specific siRNA and by treatment with rotenone, a NADH oxidase complex I inhibitor [212]. In mouse models of ischemia and reperfusion in vivo, pharmacological inhibition of NAMPT with FK866 reduced both neutrophil infiltration and reactive oxygen species (ROS) generation within infarcted hearts. Sera from FK866-treated mice showed reduced circulating levels of the neutrophil chemoattractant, CXCL2, and impaired capacity to prime migration of these cells in vitro; these effects were reduced by either FK866, or sirtuin (SIRT) inhibitors, implying that visfatin stimulates the production of this chemokine [213].

In glomerular injury, often observed in diabetic patients, visfatin promotes the formation of lipid raft redox signaling platforms, which produces local oxidative stress resulting in the disruption of microtubular networks in glomerular endothelial cells and an increase in the glomerular permeability [214]. Similar effects are observed in coronary circulation, when visfatin-dependent lysosome-associated molecular trafficking and consequent ceramide accumulation in cell membrane mediates the assembly of NOX subunits and their activation, producing endothelial dysfunction [215]. The observations mentioned above might be explained by the pleiotropic action of visfatin. Being beneficial as an NMN-producing enzyme, visfatin may serve as a soluble signal detrimental to the oxidative state within the cells comprising a particular tissue. It is unclear whether hepatocytes or others cells of the liver are sensitive to the ROS-augmenting effects of visfatin.

Through its receptor APJ, *apelin* mediates oxidative stress in blood vessels, thus contributing to atherogenesis [216]; however, in cardiomyocytes, the same protein inhibits the hypertrophic response to oxidative stress in a dose-dependent manner through stimulation of the activity of catalase [217]. As pertains to NAFLD, treatment with apelin increases fatty acid oxidation and mitochondrial biogenesis in muscles of apelin-treated mice [218]. Apelin is highly expressed by stellate cells and its receptor is expressed in the parenchyma of the liver. Whether these insulin-sensitizing effects of apelin take place in the liver, or whether they increase oxidative stress in the liver, is currently unknown.

Studies of *chemerin* levels measured in portal venous (PVS), hepatic venous (HVS) and systemic venous (SVS) blood of patients with liver cirrhosis showed that its levels seem to be associated with inflammation rather than BMI, and that this adipokine is also released by the liver [219]. Serum chemerin concentrations are significantly higher in NAFLD patients as compared to healthy volunteers, and in NASH patients as compared to simple steatosis [220]. Almost nothing is known about the involvement of chemerin in the pathogenesis of NAFLD.

Treatment with *omentin* prevents TNF-α-induced COX-2 expression and subsequent vascular inflammation in human endothelial cells through stimulating eNOS [221]. Similar to chemerin, the influence of omentin levels on inflammatory features of NAFLD and NASH were never studied.

It is clear that thorough studies of inflammation-related effects of newly discovered adipokines are needed.

The Role of Adipokines in Hepatic Fibrosis

Liver fibrosis is a wound healing response that involves several cell types. It is characterized by inflammation, activation of matrix-producing cells, extracellular matrix (ECM) deposition and remodeling, and epithelial cell regeneration or an attempt thereof. The major matrix producing cells in the liver are hepatic stellate cells (HSC) that may undergo a phenotypic transition to myofibroblast-like cells that synthesize various ECM components. This fibrogenic response might result in cirrhosis in NASH livers and may instigate the progress to hepatocellular cancer and liver-related death. Despite a strong association between necroinflammatory activity and fibrosis on cross sectional studies, fibrosis progression in many NASH patients may still occur in the presence of an improvement in inflammation, ballooning, and steatosis [222].

Leptin as Fibrogenic Agent

The first evidence of the fibrogenic effects of leptin comes from studies of leptin deficient animals that are resistant to fibrosis resulting from long-term thioacetamide administration [223]. Ablation of leptin also alleviates profibrogenic effects of exposure to carbon tetrachloride [224, 225], Schistosoma mansoni infection [225], or as a consequence of steatohepatitis. Subsequent studies revealed a

profound positive influence of leptin on TGF-β [223] and collagen I and III mRNA and protein production in HSCs [226]. Leptin effects on collagen production are at levels seen in obese individuals [226]. Leptin also augments the effect of TGF-β1 on collagen production [213].

The mechanism of leptin-dependent stimulation of collagen production includes the Janus kinase-phosphatidylinositol 3-kinase-Akt (JAKs-PI3K-Akt) pathway [227] and peroxide-dependent components coupled to the ERK1/2 and p38 pathways [228], thus opening the door to possible antioxidant therapy of liver fibrosis. Moreover, in HSCs, the same leptin-induced signaling leads to enhanced production of the tissue inhibitor of metalloproteinase TIMP-1 that suppresses collagen degradation [229]. To induce expression of the smooth muscle actin αSMA, leptin synergizes with IL-6 [230].

Taken together, these data indicate that leptin is a potent hepatic fibrosis promoter. In obese individuals, the increase in the leptin levels observed along with increasing fatty mass leads to persistent hyperleptinemia that may be a detriment to their livers through leptin-dependent profibrogenic effects. Observations in lipodystrophic patients treated with recombinant leptin support this conclusion. Despite significant improvements in hepatic steatosis, ballooning injury and NASH activity scores, there were no changes in hepatic fibrosis in patients treated with recombinant leptin [83, 231].

Adiponectin as Antifibrotic Agent

Kamada and co-authors revealed that adiponectin attenuates liver fibrosis in a carbon tetrachloride administration model [43]. In cultured hepatic stellate cells, adiponectin suppresses PDGF-induced proliferation and migration and attenuates the effects of TGF-β1 [43]. Another recent study demonstrates that adiponectin can inhibit proliferation and induce apoptosis in activated HSCs, but not in quiescent HSCs [232]. Local adiponectin production has been demonstrated in quiescent HSCs. Both AdipoR1 and AdipoR2 receptors are present in both quiescent and activated HSCs; however, AdipoR1 mRNA expression is reduced by 50 % in activated HSCs as compared to quiescent HSCs [232]. This data indicates that adiponectin is essential to either maintaining the quiescent phenotype of HSCs or is capable of reversing hepatic fibrosis by hampering the proliferation of activated HSCs and by inducing HSC apoptosis.

Importantly, in activated HSCs, adiponectin dampens the profibrogenic signaling through leptin receptor and prevents excess extracellular matrix production. Adiponectin-dependent suppression of the proliferation and the migration of HSCs is achieved through activation of AMPK [233, 234] and subsequent inhibition of leptin-mediated Stat3 phosphorylation and SOCS-3 binding to the leptin receptor [234, 235]. In addition, adiponectin-stimulated AMPK inhibits transforming growth factor (TGF)-β-induced fibrogenic properties of HSCs by regulating transcriptional coactivator p300 [236]. Adiponectin also stimulates PTP1B expression and activity, thus inhibiting JAK2/STAT3 signaling at multiple points [235].

Resistin as a Potential Fibrogenic Agent

Resistin has no known connection with hepatic fibrosis, except indirect evidence. In particular, resistin levels are associated with fibrosis severity in patients with chronic hepatitis B and C [237]. In liver cirrhosis, the plasma resistin levels are elevated as well [238]. On the other hand, resistin has been implicated in pulmonary fibrosis induced by bleomycin. In fact, microarray profiling has revealed a 17–25-fold induction of the RELM-alpha encoding gene FIZZ1 in the alveolar and airway epithelium of bleomycin treated rats. Co-cultures of FIZZ1-expressing epithelial cells and fibroblasts stimulate alpha-smooth muscle actin and type I collagen expression independently of transforming growth factor-beta. Similar effects were achieved in experiments transfecting a FIZZ1-expressing plasmid into fibroblasts [239]. Similar resistin-dependent responses might be produced in HSCs, if resistin indeed contributes to the development of NASH.

TNF-α and Fibrogenic Responses

There is direct evidence of the involvement of TNF-α in fibrogenic responses. When double knockout mice lacking both TNF receptors (TNFRDKO mice) are fed methionine- and choline-deficient (MCD) diets, they develop less pronounced liver steatosis than their wild-type counterparts [240]. The livers of these mice show significantly decreased numbers of recruited Kupffer cells, together with the extent of centrizonal fibrosis; stellate cell activation is also diminished [240]. Similar findings in TNFRp55 knock-out mice indicate that even partial suppression of TNF-α signaling can alleviate liver fibrosis [241]. Diet-driven induction of α1(I) collagen and TIMP-1 in TNFRDKO livers was also significantly lower, indicating TNF-α involvement in the stimulation of collagen deposition. Moreover, in primary cultures, TNF-α administration enhances TIMP-1 mRNA expression in activated hepatic stellate cells and suppresses apoptosis [241]. It seems that TNF-α increases the recruitment of Kupffer cells that, in turn, produce extra TNF-α and hasten fibrosis in either an autocrine or paracrine manner, thus contributing to NASH progression to cirrhosis. It seems that TNF regulates the profibrogenic effects of HSC through its binding to TNFR1, which is required for both HSC proliferation and MMP-9 expression. Both in vivo liver damage and fibrogenesis after bile-duct ligation were reduced in TNFR-DKO and TNFR1 knockout mice, compared to wild-type or TNFR2 knockout mice [242].

Other Adipokines and Fibrogenic Responses

Recent studies on rats have shown that *apelin* is overexpressed in activated HSCs and its receptor APJ—in the hepatic parenchyma of animals with cirrhosis [143]. The treatment of these animals with antagonist of APJ lead to the decrease in both grade of hepatic fibrosis and vessel density [243]. Similar effects of apelin signaling suppression were observed in rats treated with carbon tetrachloride [244]. Interestingly, the treatment with profibrogenic agent TNF-α stimulates the expression of apelin in cardiomyocytes and adipocytes [245, 246]. It is important to note that, in cardiovascular tissues, apelin prevents fibrosis by inhibition of the TGF-β-mediated expression of the myofibroblast marker α-SMA and the production of collagen. The prevention of collagen accumulation by apelin is mediated by a reduction in the activity of sphingosine kinase 1 (SphK1) [247]. Similarly, the decrease in the extent of the cardiovascular fibrosis and the levels of mRNA encoding plasminogen activator inhibitor type-1 (PAI-1) was observed in mice treated with both apelin and profibrotic peptide angiotensin II [248]. It is possible that the opposing effects of apelin on the liver and non-hepatic tissues are due to its differential regulation.

It seems that *visfatin* has at least some fibrogenic effects. In cardiac fibroblasts, visfatin stimulates collagen I and III production, procollagen I and III mRNA expression and protein production via p38MAPK, PI3K, and ERK 1/2 pathways in a dose- and time-dependent manner [249]. Importantly, in the livers of NAFLD patients, the expression levels of visfatin were significantly higher in patients with fibrosis ($P=0.036$) and were positively correlated with the fibrosis stage ($r=0.52$, $P=0.03$), while no difference in intrahepatic visfatin levels were registered when patients with NASH were compared to those with simple steatosis [250]. These observations point at the necessity of further investigations of possible fibrotic effect of visfatin in the liver.

Conclusions

The past decade has produced a great deal of knowledge about the epidemiology and pathogenesis of NAFLD and NASH. It is increasingly clear that the development of NASH is a complex process involving multiple mechanisms including insulin-resistance, oxidative stress, abnormal free fatty acid metabolism, and inflammatory cytokines and adipokines. Increasing evidence indicates that the pathogenesis of NAFLD and NASH is hastened by a disturbance of adipocytic production of adipokines.

Table 17.1 Positive and negative effects of adipokines to particular cellular processes contributing on NASH

	Cellular processes contributing to NASH				
Adipokine	Lipid accumulation in liver (steatosis)	Insulin resistance	Oxidative damage	Fibrotic responses	Role in NASH progression
Adiponectin	Suppresses	Suppresses	Suppresses	Suppresses	Prevents NASH
Leptin	Suppression effects are low due to leptin resistance	Suppression effects are low due to leptin resistance	Pro-oxidant	Fibrogenic action	"Wolf in sheep's clothes"
Resistin	Possibly steatogenic	Possibly involved in insulin resistance; difficult to study in humans	Possibly pro-oxidant	Possibly fibrogenic	Unclear
TNF-α	Steatogenic	Impairs insulin signaling	Pro-oxidant	Fibrogenic action	Augments NASH
Visfatin	Unknown	Regulates insulin secretion, insulin receptor phosphorylation	Contradictory data	At least some fibrogenic effects	Unclear
Vaspin	Unknown	Insulin sensitizer	Unknown	Unknown	Unclear
Apelin	Unknown	Inhibits glucose-stimulated insulin secretion	Unclear	Profibrotic in the liver; anti-fibrogenic in cardiac tissue	Unclear
Chemerin	Unknown	Exacerbates glucose intolerance, lowers serum insulin levels, and decreases tissue glucose uptake	Unknown	Unknown	Unknown
Nesfatin	Unknown	Exerts both central and peripheral insulin sensitizing effects	Unknown	Unknown	Unknown
Omentin	Unknown	Enhances insulin-stimulated glucose uptake	Unknown	Unknown	Unknown

Decreased production of adiponectin and increased production of TNF-α, characteristic of obesity, seem to contribute to all major NASH-related cellular processes (Table 17.1). Leptin, on the other hand, behaves as a "wolf in sheep's clothing." Its NASH suppressive effects are diminished by the widespread effects of leptin resistance, and it becomes potentially pro-oxidant and fibrogenic. Resistin's involvement in NASH is documented in rodent models, which may not be applicable to the human disease of NAFLD. Therefore, the molecular effects of resistin in patients with NASH remain to be investigated. In addition, other adipokines that seem to be involved in insulin signaling, such as vaspin, visfatin, apelin, nesfatin, omentin and chemerin require further study in patients with NAFLD and NASH.

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Chapter 18
Obesity and Cardiovascular Disease

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Keywords Pro-inflammatory adipokines • Obesity • Cardiovascular disease • Cardiac remodeling • Adipose tissue

Key Points

- Cardiovascular disease is the number one killer in the world and obesity is a major modifiable risk factor that directly contributes to cardiovascular disease.
- More than one-third of US adults (35.7 %) are obese and 68 % are overweight or obese (1) which has major health implications, as well as economic costs from loss of life and productivity (2).
- Excess fat has effects on the cardiovascular system either as a major contributor to the metabolic syndrome or as emerging evidence suggests, directly by itself [3, 4].
- Contrary to popular notion, adipose tissue is not inert but rather a metabolically active organ producing various factors (such as adipokines) with targeted effect on other organs of the body.
- Investigation into adipose biology and mechanisms of obesity has gained momentum as new ways are being sought to combat the obesity epidemic.
- In January 2000, the Department of Health and Human Services launched *Healthy People 2010*, a comprehensive, nationwide health promotion and disease prevention agenda. However, no single state was able to achieve a 15 % reduction in obesity set forth by this initiative. In fact there was an increase in the number of states with obesity [5].
- In 2000, no state had an obesity prevalence of ≥30 %. However by 2009, nine states had obesity rates of ≥30 % with a further increase to 12 states in 2010. These numbers highlight the extraordinarily, rapid increase in the prevalence of obesity and the expected impact on cardiovascular disease.
- This has resulted in an unprecedented need to understanding the effects of obesity on the cardiovascular system. This chapter will discuss the direct and indirect effects of obesity on the cardiovascular system, and its impact on morbidity and mortality.

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Introduction

In June 2012, the Food and Drug Administration (FDA) approved a new anti-obesity medicine lorcaserin [6]. This was 2 years after the *Healthy People 2010* initiative was deemed a failure [5]. The need to aggressively tackle obesity, all point to the economic, social and health care impact caused by this epidemic. Though the cause of obesity is multifactorial with genetic and environmental interplay, the persistence of this problem can be directly related to abundance of food especially in the industrialized nations. Genetic causes of obesity are rare and most obese individuals likely acquire increased adiposity through excess caloric intake [7]. Psychosocial and socioeconomic factors are important elements that need to be address when dealing with the obesity epidemic. Cardiovascular disease is of specific interest as it remains the major cause of death with obesity being a major contributor.

Obesity: Public Health Crisis

During the past 20 years, there has been a dramatic increase in obesity in the USA and rates remain inordinately high. More than two-thirds of adult Americans are overweight or obese and more than one-third of US adults (35.7 %) are obese [8]. The health care implications of obesity cannot be overstated. According to the Centers for Disease Control and Prevention (CDC), obesity costs our health care system $147 billion in 2006 alone, with obese individuals requiring $1,429 in health care cost compared to normal weight persons in that year. This figure is estimated to increase by more than twice to $344 billion by 2018. As a leading component of cardiovascular disease, obesity also contributes to significant loss of lives and productivity. Recognizing the enormity of the problem, *Healthy People 2010* was established in 2000 with the intent of reducing obesity by 15 %. However this initiative failed [5].

Several organizations have their own definition of obesity but the most widely accepted is the criteria from the WHO based on Body Mass Index (BMI). Underweight is <20 kg/m^2, normal 20–25 kg/m^2, overweight 25–30 kg/m^2, class I obesity 30–35 kg/m^2, class II obesity 35–40 kg/m^2, and class III obesity >40 kg/m^2 [4, 9, 10]. BMI estimates total body fat with BMI >30 kg/m^{2v} approximately equal 30 % of body fat.

Obesity and Cardiovascular Disease

Obesity is associated with premature atherosclerosis, increased myocardial infarction and heart failure risk and cardiovascular deaths. Factors that contribute to cardiovascular disease in obesity include insulin resistance, hypertension, lipid abnormalities, premature coronary artery disease and are associated with adverse cardiac remodeling and impaired ventricular systolic and diastolic function; vascular endothelial dysfunction; increased sympathetic tone; pulmonary hypertension with right-sided heart strain; and arrhythmias [4]. Obesity is linked to a pro-inflammatory state where pro-inflammatory cytokines, from hepatic and adipose tissues, likely play a pathological role in cardiovascular disease progression. BMI is useful in predicting overall risk, but adiposity distribution, degree of visceral or ectopic fat burden, percent body fat, genetic factors, sex, and possibly qualitative features of adipose tissue, may be equally as important. We will review the cardiovascular disease associations and consequences of obesity.

Hypertension

A causal link exists between obesity and hypertension. Studies in adults have shown a link between obesity and hypertension in both men and women and the burden of hypertension attributable to obesity is very high [11]. Obese individuals have a twofold to threefold risk for developing hypertension [12]. Similarly observations in overweight and obese children with elevated blood pressure have shown evidence of end-organ changes such as structural arterial abnormalities, and increased left ventricular (LV) mass, suggesting a causal relationship between obesity and hypertension [13, 14]. By itself obesity-related hypertension is increasingly recognized as a distinct phenotype that requires a vigilant approach to diagnosis, treatment, and prevention.

Leptin, an adipokine, functions as a feedback regulator to suppress appetite centrally in the hypothalamus. Circulating leptin levels are elevated in obesity and levels are also correlated with adiposity [4]. Leptin resistance occurs in obesity and appetites are not suppressed despite higher leptin levels [15]. Similarly, leptin is linked to hypertension [16]. Several mechanisms have been proposed for hypertension in obesity such as increased renal Na^+ K^+-ATPase activity, activation of the renin–angiotensin–aldosterone and sympathetic nervous systems, insulin resistance, and activation of pro-inflammatory cytokines. The pro-inflammatory adipokines that are upregulated in adiposity include interleukin-6, tumor necrosis factor-α (TNF-α), plasminogen activator inhibitor-1, and C-reactive protein [4]. Conversely adiponectin, another adipokine confers cardioprotection against oxidative stress and insulin resistance. Adiponectin levels are decreased in obesity partly because its production is suppressed by pro-inflammatory adipokines. Hypoadiponectinemia is present in obesity, type 2 diabetes, and hypertension [17, 18] and in experimental aldosterone-induced hypertension exacerbates adverse cardiac remodeling and diastolic heart failure [19]. Administration of adiponectin in experimental models ameliorates hypertension [20]. Thus, depletion of cardioprotective adipokines and activation of pro-inflammatory cytokines creates a scenario of impaired vascular endothelial dysfunction and exacerbates the propensity to changes in the vascular wall, resulting in hypertension and further augmenting the risk for cardiovascular disease.

Adipose tissue is a source of angiotensinogen, angiotensin-converting enzyme, renin, and possibly even aldosterone and thus contributes to circulating levels of the components of the renin–angiotensin–aldosterone system. Thus, these neurohormones need to be an important consideration in the management of hypertension in the presence of obesity [21]. Adipocyte hypertrophy, low body weight, and low blood pressure are evident in angiotensin-deficient mice supporting a direct role of adipose tissue in the pathogenesis of hypertension [21].

Finally recent data suggest a paradox between obesity and hypertension. The effect of obesity on cardiovascular outcomes in treated hypertensive patients with known coronary heart disease was investigated. Survival was better in overweight and obese patients, despite less effective blood pressure control in these patients compared with the normal weight group [22]. Thus, obesity may be a powerful risk factor for hypertension and LV hypertrophy (LVH), but obese hypertensive patients may paradoxically have a better prognosis compared with lean hypertensive patients for reasons that are unclear at this time.

Effect on Cardiac Structure and Function

Hypertension induces LV wall thickening generally without chamber dilation when LV mass is not increased; or concentric LVH when LV mass is increased. Conversely the heart in obesity is characterized by LV chamber dilation without marked increases in wall thickness, a process that leads to eccentric LVH. Thus, some studies have concluded that obesity is independently associated with LVH [23–25].

Similarly, as with abnormalities in the vasculature, LVH and alterations in myocardial systolic function are observed in obese children and adolescents [26]. Alterations in cardiac structure and function in obese subjects include increased myocardial fatty acid uptake and utilization particularly those with insulin resistance [27]. Using endomyocardial biopsy, others have found only mild myocyte hypertrophy without evidence of abnormal collagen accumulation in the heart of obese subjects [28]. Conversely others have found elevated serum cardiac collagen turnover markers unrelated to LV mass in normotensive, nondiabetic obese subjects [29]. Therefore, there does not appear to be a specific pathological phenotype in the human heart which is clearly associated with obesity, other than mild myocyte hypertrophy and perhaps intra- and extracellular fat accumulation.

Systolic and Diastolic Dysfunction and Heart Failure

In the evaluation of LV systolic function in obesity, findings have been varied with some reporting depressed LV ejection fraction (EF) [30] and others normal EF [23, 24]. Despite a normal LVEF, myocardial function is often reduced in obesity as measured either by noninvasive measures such as midwall LV fractional shortening, systolic velocity measured with tissue Doppler, or systolic strain rate [4, 23, 25], or via invasive studies which demonstrate subclinical contractile abnormalities [31].

With regard to diastolic function which is a measure of relaxation and filling, tissue Doppler demonstrates reduced early diastolic tissue velocities and diastolic strain rate in obese subjects [24, 25] and likely reflects a slowing in the rate of LV relaxation. Interestingly although obese subjects have normal resting pulmonary capillary wedge pressures (PCWP) compared with normal-weight control subjects, obese subjects have an exaggerated rise in PCWP during exercise [32]. Thus, although obesity is associated with diastolic dysfunction at the myocardial level, LV filling pressures remain normal at rest (but not during exercise), the majority of obese patients do not have evidence of clinical heart failure.

In the Framingham Heart Study increased BMI was associated with an increased risk of HF in both men and women and that this risk was increased with increasing BMI. In a subset of patients, echocardiography performed within 30 days of the HF diagnosis, demonstrated reduced LVEF [33]. Brain natriuretic peptide (BNP) levels are elevated in HF; however, obese subjects have lower circulating BNP levels than normal-weight controls with similar PCWP [34]. It is unknown why they have lower levels since BNP is secreted from the ventricular during wall stress [35]. Wang et al., have suggested that these low BNP levels reflect impaired natriuretic peptide response in obese subjects and predisposes them to hypertension and hypertension-related disorders [36]. However, some obese subjects do have elevated BNP levels in HF and when this occurs BNP retains its prognostic capacity in this cohort predicting worse symptoms, impaired hemodynamics, and higher mortality at all levels of BMI [37].

Endothelial Dysfunction

The endothelium lines the interior of arterial and venous blood vessels and this single layer of cells is responsible for the maintenance of fluidity of blood and control of inflammation [38]. It serves as a physical barrier and produces several vasoactive factors to maintain homeostasis and vascular tone [39]. Endothelial dysfunction is evident in early atherosclerosis and is associated with several cardiovascular risk factors including obesity, diabetes, hypertension, and dyslipidemia. Obesity is associated with reduced endothelial cell numbers in the bone marrow [40]. There is both a reduction in early endothelial cell numbers and in mature cells in the blood [40]. Obesity induces endothelial dysfunction by dysregulation of adipocyte-derived hormones [41]. For instance, obesity has known

associations with reduced adiponectin levels, and hypoadiponectinemia has been shown to cause endothelial dysfunction through a variety of mechanisms such as inhibition of NFκB and apoptotic signaling [42, 43]. These are corrected in experimental models by supplementing with adiponectin [44]. Endothelium-dependent vasodilation was significantly reduced in adiponectin-deficient mice compared with wild-type mice in response to acetylcholine [17]. Several studies have emphasized the importance of nitric oxide (NO) for endothelial function. NO activity is reduced in obese individuals [39]. Deng et al. concluded that adiponectin improves endothelial function by increasing NO production through eNOS phosphorylation, and prevents NO inactivation by blocking superoxide production. Similarly, caloric restriction can promote revascularization in response to tissue ischemia via an AMPK-eNOS-dependent mechanism that is mediated by adiponectin [45].

Prothrombotic States

Obesity contributes to endothelial damage and a consequence of endothelial dysfunction is accelerated thrombosis. Obesity itself is also associated with the increased generation of thrombin, platelet hyperactivity and decreased fibrinolysis which are crucial in atherothrombosis [46]. Mean platelet volume (MPV) mimics platelet activation in vivo and is a measure of platelet function. MPV is increased in obese individuals, independent of other cardiovascular risk factors demonstrating a risk factor for atherothrombosis in obesity [47–49]. Several pro-inflammatory markers are involved in platelet activation, a common pathway in thrombosis. These include TNF-α, thromboxane, CD40 and CD40L. In obese patients, these markers are elevated [50, 51]. Several mechanisms are involved such as the binding of TNF-α to its ligand expressed on the platelet surface prompting activation, thus further enhancing thromboxane biosynthesis and thrombus formation [52]. In obese individuals, thrombosis is enhanced by an increase in pro-thrombotic factors and a decrease in some antithrombotic molecules such as adiponectin, which act as an endogenous antithrombotic molecule. Since adiponectin levels are decreased in obesity [53], supplementation of adiponectin in experimental models attenuates thrombus formation and inhibits platelet aggregation [54].

Dyslipidemia

The metabolic syndrome imparts a cardiovascular risk [55]. The metabolic syndrome constitutes a group of risk factors that occur together in an overweight or obese individual. These metabolic factors include central obesity, insulin resistance, hypertension, elevated triglycerides and reduced high density lipoproteins (HDL) cholesterol. Although obesity and dyslipidemia are major components of the metabolic syndrome, they carry their own independent cardiovascular risks. In obese individuals there is lipid dysregulation, which is characterized by increased production of low density lipoproteins (LDL), low HDL and increased triglycerides [56]. The contribution of triglycerides to cardiovascular disease has been controversial leading the American Heart Association (AHA) to release a statement in 2011 to address the issue [57]. The conclusion was that triglycerides do not directly cause atherosclerosis but is an important risk for cardiovascular disease due to association with apolipoprotein (Apo) CIII, an atherogenic remnant [57]. Several studies have validated HDL as important factor in coronary artery disease (CAD), with low HDL seen as an independent risk factor while high HDL is protective [58, 59]. A recent study has, however, challenged the notion that high HDL is protective and that raising plasma HDL cholesterol will uniformly translate into reductions in risk of myocardial infarction [60]. LDL cholesterol has consistently being shown as a cardiovascular disease risk factor with weight loss lowering LDL levels and improving cardiovascular risk [60, 61].

Coronary Artery Disease (CAD)

Obesity is an independent risk factor for most cardiovascular diseases, of which CAD is a major component. By itself, obesity is also an independent risk factor for CAD [62]. In addition, in the presence of established CAD, obesity is independently associated with risks for major adverse cardiovascular events, particularly in men [63]. Fat distribution is important, especially considering the effect of obesity on insulin resistance, diabetes and hyperlipidemia [64]. For example, central (abdominal) obesity has consistently been shown to increase CAD risk at any BMI [65]. But BMI remains an important risk factor for developing CAD irrespective of other traditional CAD risk factors. A meta-analysis of 21 cohorts studies involving >30, 000 individuals and 18,000 CAD events, found that for every 5 unit of BMI increase, there was a 29 % increase in CAD [66]. Even after controlling for traditional risks like hypertension and dyslipidemia there was still a 16 % increase risk in CAD [66]. This contribution to atherosclerosis is likely through the release of pro-inflammatory cytokines, depletion in protective adipokines, endothelial dysfunction and dyslipidemia as already described.

Atrial Fibrillation

Atrial fibrillation (AF) and obesity are epidemics. The prevalence of AF is increasing, and is expected to increase [67]. Similarly, obesity is an important risk factor for development of AF independent of other clinical risk factors such as aging [68]. In a meta-analysis of >75,000 subjects, obese patients had a nearly 50 % increased risk of developing AF that escalated with increasing BMI [69]. Obesity, with its attendant hemodynamic effects and impact on LV and left atrial structure and function, may also contribute to the higher prevalence of AF. This may be related to left atrial size since it increases as BMI increases and with weight loss there is regression of left atrial size [68] raising the possibility that weight loss may decrease the risk of AF.

Obstructive Sleep Apnea

Obstructive sleep apnea (OSA) is prevalent in 55–100 % of severely obese subjects [70]. However, only moderate weight gain is associated with an increased risk of sleep apnea [71]. Fat distribution in the neck alone [72], waist circumference [73] or BMI [70] have all been shown to be predictors of OSA. Weight loss improves OSA but does not cure it and this may relate to cervical fat deposition or abnormal distribution of neck fat in massively obese patients. Finally, OSA may contribute to the pathogenesis of hypertension and increase inflammation [74] and AF. A recent study showed that obesity and the magnitude of nocturnal oxygen desaturation, an important pathophysiological consequence of OSA, were independent risk factors for AF [75].

Obesity Paradox

Weight loss in obese persons is associated with a reduction in all cause mortality [76]. However, it is less clear if cardiovascular events and cardiovascular mortality improve [77–79]. On the contrary, there is mounting evidence over the last decade to support that in overweight and obese individuals with established cardiovascular disease, weight loss may actually worsen mortality, the so called "obesity paradox" [80, 81]. A possible explanation for the lack of cardiovascular disease mortality

benefit seen with weight loss is the difficulty to maintain weight loss especially for more than 5 years without surgery. In addition, randomized controlled trials are difficult to conduct with patients receiving bariatric surgery [82]. With the obesity paradox, there is convincing data, however, that the patient population studied should be taken into consideration. Most of the studies were in older and frailer patients. Younger obese patients may have better cardiopulmonary fitness, associated with improved survival. Furthermore, being younger may offer lead-time bias towards survival. Moreover, obese patients may become more symptomatic with the disease earlier, leading to early diagnosis and treatment [83, 84]. Some have also argued that fat distribution and not only BMI, should be considered when studying the obesity paradox. It is clear that this phenomenon will need further investigation with randomized controlled trials.

Conclusion

The contribution of obesity to cardiovascular disease is underscored by its contribution to the metabolic syndrome and its inherent risk factors. Obesity continues to be a major health problem, with associated risks for disease, disability, and reduced quality of life. The American Heart Association (AHA) designated obesity as a category II risk factor, a risk factor for which intervention will likely reduce CAD events. Similarly weight loss in obesity is associated with a reduction in incidence of diabetes [85], dyslipidemia [82], hypertension [86], endothelial dysfunction and inflammation [87, 88], and other cardiovascular disease risk factors.

Weight loss remains a primary end point for drug development. In addition to a reduction in obesity, it remains to be seen if it includes a decreased risk of cardiovascular and cancer events, sleep apnea, osteoarthritis, and depression and improved quality of life. The FDA approved lorcaserin [6], after initially rejecting it based on concerns over both safety and efficacy, after the BLOOM-DM trial [89]. This trial showed lorcaserin used for up to 1 year in obese and overweight patients with type 2 diabetes was associated with weight loss and improvements in glycemic control (37.5 % had weight loss ≥5 % with lorcaserin compared with 16.1 % with weight loss ≥5 % with placebo) [89]. Thus, the FDA officially approved lorcaserin for use in the treatment of obesity for adults with a BMI ≥30 or adults with a BMI ≥27 who "have at least one weight-related health condition," such as high blood pressure, type 2 diabetes, or high cholesterol [6]. It remains to be seen if the findings seen with type 2 diabetes translate to a reduction in cardiovascular diseases, cardiovascular events and cardiovascular mortality.

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Chapter 19
Respiratory Diseases in Obesity

David G. Chapman, Benjamin T. Suratt, and Anne E. Dixon

Keywords Lung function • Asthma • Chronic obstructive pulmonary disease • Pulmonary hypertension • Acute lung injury • Influenza • Pneumonia • Mechanical ventilation • Sleep apnea • Obesity hypoventilation syndrome

Key Points

- Obese individuals breathe at low lung volumes which increases airway resistance, and predisposes to airway closure and expiratory flow limitation.
- Obesity is a major risk factor for the development of asthma, which is characterized by poor control and poor response to standard therapies.
- Obesity is associated with increased health care utilization in chronic obstructive pulmonary disease.
- Obesity is associated with decreased response to influenza vaccine, and was a significant risk factor for mortality from H1N1 influenza.
- Obesity is associated with pulmonary hypertension; there are multiple factors that may contribute to the development of pulmonary hypertension in obesity.
- Obesity appears to increase risk of mortality in acute lung injury, but increase length of mechanical ventilation and hospital length of stay.
- Sleep apnea is strongly associated with central obesity, and may contribute to the development of insulin resistance.

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Abbreviations

ALI	Acute lung injury
COPD	Chronic obstructive pulmonary disease
FEV1	Forced expiratory volume in 1 s
FVC	Forced vital capacity
FRC	Functional residual capacity
RV	Residual volume

Introduction

While it has long been recognized that obesity is a risk factor for diseases such as type II diabetes and steatohepatitis, it has only recently been appreciated that obesity is a major risk factor for lung diseases such as asthma, sleep apnea and likely also pulmonary hypertension. Obesity also dramatically alters the clinical course of other common lung diseases such as chronic obstructive pulmonary disease and acute lung injury. The purpose of this chapter will be first to review the effects of obesity on lung function, and then to discuss the effect of obesity on the aforementioned pulmonary diseases.

Physiology

The dynamic nature of tidal breathing, whereby cycles of pressure changes across the lungs cause the chest wall, lung tissue and airways to repeatedly expand and contract, ventilates the lung tissue and allows respiration to occur. The volume at which tidal breathing occurs is the balance point between the deflationary and inflationary pressures in the lung, called functional residual capacity (FRC) (Fig. 19.1). When one considers that tidal breathing is dependent upon both continual movement of the thoracic region and the balance between pressures in the lung, it is not surprising that obesity has the potential to greatly affect respiratory mechanics. Indeed, the most pronounced and consistently reported effect of obesity on the respiratory system is a reduction in FRC, so that tidal breathing in the obese occurs at low lung volumes. This occurs because the increased adipose tissue around the rib cage and abdomen increases the deflationary pressures in the lung, resulting in a lower volume at which the balance between inflationary and deflationary pressures is reached [1, 2]. Substantial reductions in FRC are seen even in overweight subjects (BMI 25–30 kg/m^2) resulting in a negative exponential relationship between BMI and FRC [3]. In contrast, total lung capacity (TLC), the volume after full inspiration, is only mildly reduced in obesity, while residual volume (RV), the volume of gas trapped in the lungs after full expiration, is mostly unaltered in obesity [3]. However, it must be noted that although TLC is reduced in obesity, TLC often remains within the limits of normality even in severe obesity [3, 4]. Therefore, depending upon the effect of obesity on TLC, the RV to TLC ratio may be either normal or slightly increased, although the latter must be interpreted in light of any reduction in TLC.

The reduction in FRC in obesity has two important consequences. Firstly, airway caliber during tidal breathing is reduced in obesity as a consequence of reduced lung volumes. Since airway resistance is dependent upon volume, this reduction in airway caliber results in increased airway resistance, which is a potential determinant of symptoms. There is some contention as to whether the

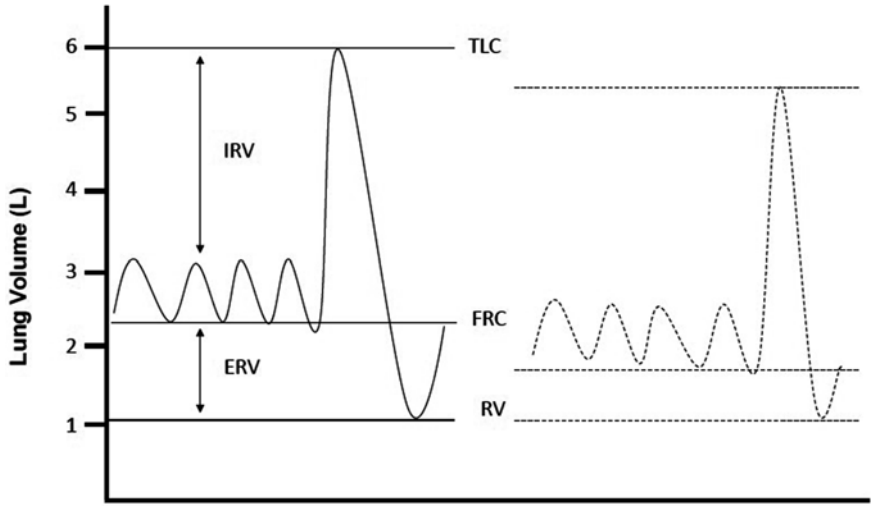

Fig. 19.1 Representative lung volume traces of a normal weight, healthy patient (*solid*) and a patient with obesity without airways disease (*dashed*)

increased airway resistance is purely due the reduction in lung volume as both normal [5] and increased [6, 7] values have been reports in the obese after adjustment for lung volume. Secondly, the reduction in FRC with obesity substantially reduces expiratory reserve volume (ERV) so that tidal breathing occurs near or below closing capacity (the volume at which airways begin to close) [8, 9]. The combination of reduced airway caliber and the close proximity to closing capacity may predispose obese subjects to increased airway closure during bronchoconstriction [10]. This physiological observation may be important since increased airway closure appears to be a determinant of symptoms [11].

Spirometric variables in the obese, such as the forced expiratory volume in one second (FEV_1) and forced vital capacity (FVC) are slightly reduced, and are reduced to the same extent so that the FEV_1/FVC ratio is preserved [12, 13]. However, as in the case of TLC, FEV_1, and FVC often remain within normal limits [12, 13]. Although maximal flows are mostly unaltered in obesity, the reduction in FRC places tidal breathing at a volume approaching maximal expiratory flows (Fig. 19.2a). Tidal breathing which reaches maximal expiratory flows, called expiratory flow limitation, is not a common finding in seated obese subjects [14–16]; however, the occurrence of expiratory flow limitation increases dramatically when supine and therefore has implications for patient populations such as the obese in the intensive care unit or during anesthesia. Furthermore, expiratory flow limitation is likely to be important in patients with airflow obstruction (Fig. 19.2b), such as in Chronic Obstructive Pulmonary Disease (COPD) patients or in asthmatics during episodes of bronchoconstriction. Expiratory flow limitation is commonly associated with COPD [17]; however, it is unknown whether obesity exaggerates the occurrence and/or magnitude of expiratory flow limitation in COPD. On the other hand, recent research suggests that while expiratory flow limitation is uncommon in obese asthmatics at baseline, its presence may explain reduced symptom control in obese asthmatics after the resolution of ICS-responsive airway inflammation [16]. Furthermore, obese patients, both those with and without asthma, are at increased risk of expiratory flow limitation during bronchoconstriction [16]. Taken together, this suggests that expiratory flow limitation may alter the perception of symptoms in obese asthmatics, thereby contributing to symptoms of wheezing and dyspnea which may not necessarily be reduced by standard ICS therapy.

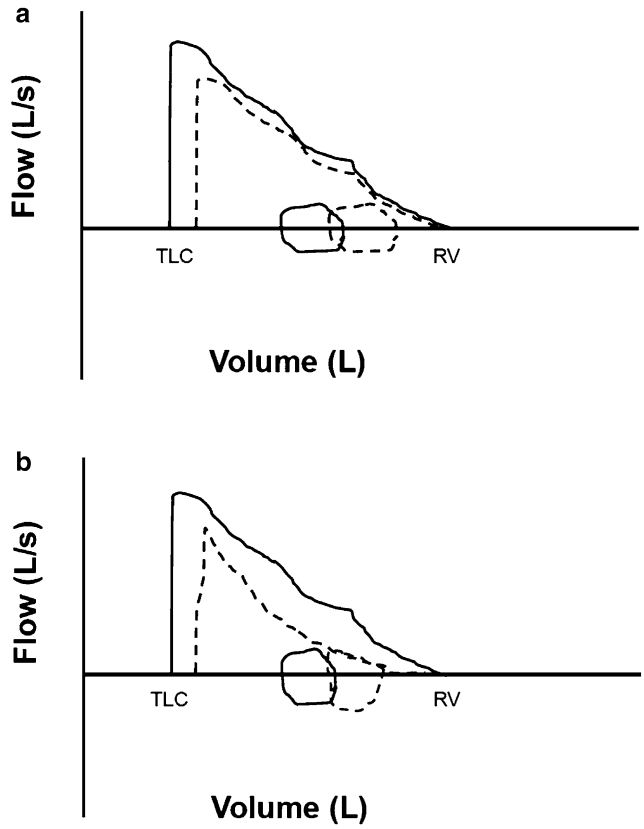

Fig. 19.2 Representative flow-volume traces showing maximal expiratory flow volume loops as well as tidal breathing loops. As is the convention, expiration is represented as positive on the *y*-axis. Compared to the normal weight, healthy patient (**a** and **b**, *solid*) obese patients without airways disease (**a**, *dashed*) are likely to have a small reduction in total lung capacity (TLC), similar maximal expiratory flow rates throughout expiration and a similar residual volume (RV). The reduction in functional residual capacity in the obese patient places tidal breathing close to RV, although tidal breathing will not usually reach the maximal expiratory loop under baseline conditions. In contrast, in patients with airway obstruction in which maximal expiratory flow rates are decreased and RV increased, obesity may lead to tidal breathing encroaching on the maximal expiratory loop (**b**, *dashed*). In this case expiratory flow limitation would occur during tidal breathing

Summary: Lung Function and Obesity

The presence of increase adipose tissue surrounding the lungs leads to dramatic effects on the mechanical properties of the lung, so that tidal breathing occurs at substantially reduced volumes. Although spirometry is largely unaffected, the reduction in tidal FRC leads to an increase in airways resistance during tidal breathing and increases the risk of expiratory flow limitation, especially during bronchoconstriction or during supine posture. Therefore the interaction between these mechanical effects of obesity with disease is likely to have important clinical implications, such as increased respiratory symptoms in asthmatic patients which are unlikely to be altered by standard therapy.

Interactions Between Obesity and Asthma

Obesity is a major risk factor for asthma. This has been described in reports from all over the world, in all ethnic groups, in adults as well as children [18–22]. There is also a dose dependent relationship between obesity and asthma, such that the greater the BMI, the greater the risk of asthma [22], indeed,

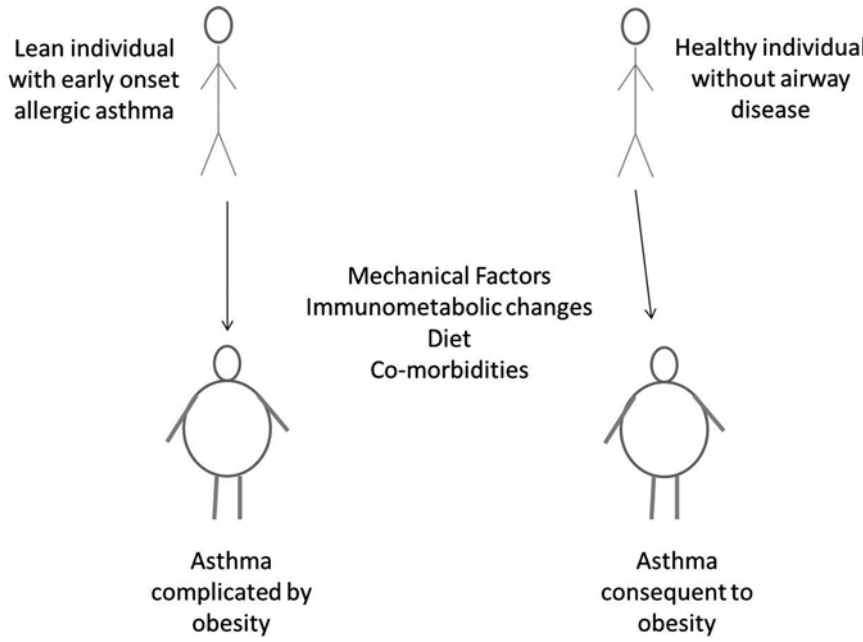

Fig. 19.3 Phenotypes of Asthma in Obesity. Obesity is a disease modifier of asthma in those with early-onset allergic asthma, and likely causes de novo airway disease in patients who have late-onset asthma in the setting of obesity

in patients with BMI's over 60 kg/m^2, the prevalence of obesity has been reported as over 30 % [23]. To put this into perspective, it is estimated that obesity causes 250,000 new cases of asthma per year in the USA [24].

When the first reports surfaced reporting an association between obesity and asthma, there were concerns that this was simply related to misdiagnosis: obesity was causing symptoms and increased ventilatory requirements that were being confused with asthma. However, there appears to be no difference in the rate of misdiagnosis of asthma between obese and lean individuals [25]. Another suggestion was that perhaps reduced exercise tolerance, or use of steroid medications in asthma could lead to the future development of obesity. However, obesity is a risk factor for the development of future asthma; obesity precedes the development of asthma and not the other way around [24]. Obesity is a risk factor for the development of incident asthma, and particularly a risk factor for the development of non-atopic asthma [26, 27].

Recent studies suggest that obese asthmatics are not a single, uniform group, but rather include at least two distinct phenotypes of asthma: those with early-onset asthma, and a relatively high prevalence of allergy and higher circulating immunoglobulin E (IgE), and those with late-onset disease, lower prevalence of allergy and lower IgE [28, 29]. It is likely that the former group represent patients who have asthma that is complicated by obesity, and the latter group likely develop asthma secondary to obesity (Fig. 19.3). This is likely why obesity is known to be particularly a risk factor for non-atopic asthma, as this is the group that develops asthma particularly in the setting of obesity. At the same time, there are clearly individuals with early-onset atopic asthma that develop obesity, given the high prevalence of obesity in the developed world, and obesity will complicate the presentation of their asthma.

Clinical Characteristics of Asthma in Obesity

Asthma in obese individuals has distinct characteristics compared with that in lean individuals. One particular characteristic that presents a challenge to clinicians is that asthma in obesity tends to be characterized by poor asthma control and increased risk of asthma exacerbations [30–35], indeed one

publication reported that asthmatics had a greater than fourfold risk of hospitalization compared with nonobese asthmatics [36]. This increased severity has major implications for public health given the number of obese asthmatic patients in countries like the USA.

While most studies have not distinguished between early-onset allergic asthmatics, and late-onset nonallergic asthmatics, two studies have examined clinical characteristics in these two phenotypes. Holguin et al. found that patients with early-onset asthma appeared to have an increased risk of asthma exacerbations [28], and Sutherland et al. that they had worse asthma control than the late-onset asthma patients [29]. We have shown that these groups also have a differing response to weight loss surgery [37]. Early-onset asthmatics with high IgE experience improved asthma symptoms with bariatric surgery, but no improvement in airway reactivity, whereas those with later-onset asthma and low IgE experience both improved symptoms and highly significant improvements in airway reactivity with bariatric surgery [34]. Future studies will need to more clearly separate out obese asthmatics into these different phenotypes of disease if we are to develop a better understanding of asthma in obesity, with the ultimate goal of developing more effective treatments for this population.

Pathogenesis of Asthma in Obesity

Asthma in obese individuals likely arises through a combination of mechanical effects, immunometabolic alterations and life-style factors that occur in the setting of obesity. As noted earlier, the obese breathe at low lung volumes [38], and breathing at low lung volumes may cause airway reactivity and expiratory flow limitation [39, 40]. Mediators produced by adipose tissue may also affect airway reactivity. For example, Shore et al. have shown that leptin increases, and adiponectin decreases, airway reactivity [41, 42] tumor necrosis factor α and interleukin-6 may also contribute to the development of airway reactivity in obesity [43, 44]. Diet may also be important in the pathogenesis of asthma in obesity. Recent data suggest that a high fat diet may increase airway neutrophilia [45]. The combination of these factors likely lead to a distinct form of asthma in those with late-onset nonallergic asthma, and alters disease on those with early-onset allergic disease.

Comorbidities which occur in obesity likely also contribute to the development of airway disease. For example, sleep apnea is common in obesity, and is associated with poor asthma control [46]. Depression is also increased in the setting of obesity [47], and associated with neuroendocrine abnormalities which may affect symptom perception and compliance with treatment [48, 49]. Gastroesophageal reflux disease (GERD) is increased in obesity [50] and may contribute to asthma symptoms either by direct reflux of acid into the airway or indirectly through reflux into the esophagus causing vagally mediated bronchospasm [51, 52]. GERD, sleep apnea, and depression are likely to be important in the pathogenesis of asthma in obesity.

Medications for the Treatment of Asthma in Obesity

Obese asthmatics appear to have altered response to some of the standard therapies used in the treatment of asthma. They have attenuated response to inhaled corticosteroid therapy. This was first described by Peters-Golden et al. [53], and has since been noted by a number of other investigators [54, 55]. Response to theophylline also appears to be altered in obesity, such that obese asthmatic patients treated with obesity appear to have increased asthma exacerbations compared with lean patients treated with theophylline [56]. Response to leukotrienes does not appear to be attenuated in obesity, though obese patients still have greater responsiveness to steroids than to leukotrienes in terms of lung function response [57]. When treated with high dose inhaled corticosteroids, airway inflammation and asthma symptoms do improve in obese asthmatics, but after treatment asthma symptoms are still independently related to BMI, suggesting that asthma control in obesity is related

to BMI independent of steroid responsive airway inflammation [58]. As obese asthmatics tend to have attenuated response to controller therapy they are a particularly challenging to treat this patient population.

Lifestyle and Weight Loss Interventions

Life style factors likely contribute to the pathogenesis of asthma in obesity. Given the data suggesting that high fat diet may aggravate airway inflammation [45, 59], dietary intervention is likely to be helpful. Weight loss is likely to be a useful intervention, and although there are no large studies of diet induced weight loss, small studies suggest that this is likely to be a useful lifestyle intervention [60]. Studies suggest that bariatric surgery will lead to highly significant improvements in asthma control, though clearly this is an expensive intervention associated with significant morbidity in its own right, so it is likely to be of use in only a small subset of patients [37, 61]. Lifestyle interventions such as dietary modification and weight loss are likely to be of critical importance in the treatment of obese asthmatics.

Summary: Asthma and Obesity

Obesity may cause asthma in an otherwise healthy patient, and modify the pathogenesis of disease in a patient with preexisting disease. Factors such as altered mechanics, metabolic mediators, altered immune function, diet and comorbidities such as sleep apnea, GERD and depression may all contribute to airway disease in obesity. Asthma in obese individuals tends to be severe, and these patients suffer with poor control. They respond poorly to standard controller therapy, likely because the pathogenesis of their disease is distinct from asthma in lean individuals.

Interactions Between COPD and Obesity

Epidemiology

Millions of people suffer with chronic obstructive pulmonary disease (COPD), in fact it is estimated that 11–25 % of the adult population of the world may be afflicted with COPD [62]. It is a debilitating respiratory disease characterized by airflow limitation. Airflow limitation is a result of both narrowing of diseased airways and loss of elastic recoil from an emphysematous lung parenchyma. The disease is caused by exposure to noxious particles and gases. In the USA this exposure is typically tobacco smoke, though worldwide, exposure to biomass fuel is a leading cause of COPD. This airflow limitation leads to symptoms of wheezing, dyspnea and cough, and eventually may cause hypoxemia, right heart failure and death. Patients with COPD are also at increased risk of lung cancer, respiratory infection and cardiovascular disease. In fact COPD is predicted to become the 3rd leading cause of mortality world-wide by 2020 [62].

By way of background, it is worth understanding the epidemiological relationship between these two diseases. It is noteworthy that the prevalence of obesity in COPD is not uniform, but varies with the severity of COPD such that the prevalence of obesity is increased in patients with mild disease, but is decreased in those with severe COPD [63]. The reasons for this are not known. One may speculate that obese patients with airflow limitation may come to medical attention more quickly than their lean counterparts because of increased ventilatory requirements with exercise. In the case of the decreased rate of obesity in patients with severe COPD, it has long been recognized that patients with severe

COPD may suffer with cachexia which has been attributed to high ventilatory requirements and an increase in circulating inflammatory mediators such as tumor necrosis factor alpha, which may cause appetite suppression [64]. In fact low body weight predicts mortality in patients with severe COPD [65], so there may be some protective effects of a slightly higher body weight than normal in this particular population, though the optimal BMI in patients with COPD is not known.

Health Care Utilization in Obese Patients with COPD

COPD and obesity are two of the most common diseases in the world, so it is critically important to understand the interaction between these two diseases if we are to effectively manage the millions of patients who suffer with both. Recent publications suggest that the rates of obesity are higher in COPD than in the general population and that the presence of obesity in COPD is associated with severe activity limitation and increased health care utilization [66–68]. Obesity is a major factor complicating the management of patients with COPD.

Effects of Obesity on Exercise Tolerance in COPD

Obesity has a significant impact on exercise tolerance in COPD, which differs by type of exercise. Although obese individuals tend to have reduced exercise tolerance when matched for level of lung function [68], obesity may actually improve lung mechanics and exercise tolerance during non-weight-bearing exercise. Obese patients have higher exercise capacity than nonobese patients during bicycle ergometry [69]. This is a result of the effect of obesity on lung mechanics. In COPD, lung volumes tend to increase; this hyperinflation can lead to a sensation of dyspnea. Obesity decreases lung volumes, so it may have beneficial effects on hyperinflation, reducing dyspnea. However, this needs to be balanced against the fact that obesity increases baseline metabolic and ventilatory requirements; when obese patients perform weight-bearing exercise (when their increased body mass leads to higher energy and metabolic demands compared with lean patients), they do not perform as well as lean patients [70]. Obesity increases exercise capacity for non-weight-bearing exercise, but decreases exercise capacity for weight-bearing exercise. Understanding the effects of obesity on different types of exercise in COPD is likely to be an important consideration when designing exercise programs for this population—a population in which pulmonary rehabilitation and exercise is an important intervention know to improve outcomes.

Metabolic Syndrome in COPD

The relationship between obesity and COPD is likely quite complex. Some recent studies have suggested that the prevalence of metabolic syndrome may be higher in patients with COPD than in age, and gender matched controls [71–73]. One could speculate that studies showing that hypoxemia causes insulin resistance may be very relevant to the pathogenesis of metabolic syndrome in COPD [74], though as yet no human studies have addressed this issue.

Summary: COPD and Obesity

COPD and obesity are two of the most common diseases in the world. Understanding the interaction between these diseases will be important in the coming years given the numbers of patients that suffer with both diseases. Recent work has highlighted the fact that rates of obesity differ by severity of

COPD, and that although obesity may have some beneficial effects in non-weight-bearing exercise and be associated with lower mortality in severe COPD, overall obesity is associated with significantly higher health care utilization in COPD. Recent work suggesting that the prevalence of the metabolic syndrome is increased in COPD suggests a bi-directional relationship that is likely to be of high clinical significance given the morbidity and mortality attributable to these two diseases.

Interactions Between Obesity and Pulmonary Hypertension

Pulmonary hypertension is a devastating disorder causing impaired exercise tolerance, syncope and death. Obesity is associated with pulmonary hypertension [75], and very recent work has reported on mouse models of pulmonary hypertension developing in the setting of obesity [76], suggesting a mechanistic relationship between these diseases.

Pulmonary hypertension is a disease in which remodeling of the pulmonary vasculature occurs raising resistance and pressures in the pulmonary circulation. Patients present with shortness of breath and decreased exercise tolerance. With advanced cases of pulmonary hypertension, syncope may occur with exercise due to impaired cardiac output, and eventually right heart failure ensues. Lung function testing show decreased diffusing capacity and, in advanced cases hypoxemia. There appear to be a number of factors that could contribute to the development of pulmonary hypertension in obese patients.

Left Heart Failure

The most common cause of pulmonary hypertension is left heart failure [77]. Certainly obese patients have increased rates of cardiovascular disease, so this may contribute to the development of pulmonary hypertension [78]. Obesity is also associated with the development of heart failure with preserved systolic function. Both forms of left heart failure (with and without preserved ejection fraction) are likely to contribute to the development of pulmonary hypertension in obesity [77, 79]. When an obese patients presents with pulmonary hypertension, it is essential to assess left heart function, as the most a likely contributor to the development of pulmonary hypertension.

Sleep Disordered Breathing

Another common disease that is increased in obesity, and has long been associated with pulmonary hypertension, is sleep apnea [80]. In this case, pulmonary hypertension likely develops as a result of intermittent hypoxemia, particularly if this persists during the day [80]. Hypoxemia causes pulmonary vasoconstriction. Some obese patients with obstructive sleep apnea also have daytime hypercapnia, this is termed obesity hypoventilation syndrome [81]. Hypercapnia may cause pulmonary hypertension through carbon dioxide mediated vasoconstriction [82]. Both sleep apnea and obesity hypoventilation syndrome are important risk factors for the development of pulmonary hypertension in obesity.

Thromboembolic Disease

Obesity is associated with a pro-coagulant state and endothelial dysfunction, and this has been reported even in obese children [83], so obesity is a significant risk factor for venous thromboembolism [84]. For example obese individuals have a threefold increased risk of pulmonary embolism after

foot and ankle trauma [85]. Chronic thromboembolic disease can lead to progressive occlusion and remodeling of the pulmonary vasculature, so thromboembolic disease is an important cause of pulmonary hypertension that should be evaluated in the setting of obesity.

Metabolic Factors

Metabolic changes in obesity may also contribute to the development of pulmonary hypertension. This is a new and active area of investigation. For example, adiponectin deficient mice develop pulmonary hypertension, and adiponectin overexpression can reverse pulmonary hypertension [76]. Adiponectin inhibits the expression of platelet derived growth factor, a potent vascular smooth muscle mitogen, which may be one mechanism linking the development of pulmonary hypertension in adiponectin deficient states [76]. Other mouse models have implicated insulin resistance [86, 87], and also deficiencies in peroxisome proliferator-activated receptor γ and apolipoprotein E pathways in the development of pulmonary hypertension [88, 89]. The role of metabolic factors in the development of pulmonary hypertension will be an important area of research in the coming decade.

Summary: Pulmonary Hypertension and Obesity

Pulmonary hypertension is a severe, life-threatening disease that is increased in the setting of obesity. There are likely to be multiple factors that could contribute to the pathogenesis of pulmonary hypertension in obesity, and appropriate evaluation of these factors is critical for the appropriate management of these patients.

Interactions Between Obesity and Pneumonia

Obesity is an important factor in modifying the host response to infection. As discussed elsewhere in this book, obesity has widespread effects on cells of the innate and adaptive immune system, so it is perhaps not surprising that obesity appears to be associated with altered response to respiratory infections.

Viral Pneumonia

Obesity alters the response to influenza infection. A classic example of this is the increased susceptibility and mortality that was reported in obese patients infected with the pandemic H1N1 influenza virus [90, 91]. The reasons for this are not known, though obesity has effects on cells involved in host defense against viral infections, and is also associated with impaired response to influenza vaccination [92]. Mouse models of diet induced obesity have also shown increased mortality following influenza infection with impaired cell-mediated responses, and cytokines responses [93–95]. There are fewer studies of the effects of obesity on response to other respiratory viruses, but given the impaired response to influenza vaccination, and the ever present threat of a global pandemic from changes in this virus, the public health implications of impaired immune response to influenza in the setting of obesity are potentially devastating.

Bacterial Pneumonia

It is not clear whether obesity is a risk factor for the development of bacterial pneumonia. When analyzing human studies reporting pneumonia in relation to BMI, it is important to realize that many chronic diseases which cause weight loss may increase the risk of developing bacterial pneumonia (such as malnutrition, emphysema, other chronic diseases), so it is likely important to exclude underweight patients in any analysis of the risk of bacterial pneumonia in relationship to BMI. Some studies have reported that increased BMI was associated with reduced mortality from bacterial pneumonia [96, 97], though one report from the nurse's health study reported that a 40 pound weight gain was associated with a twofold increase in the risk of community acquired pneumonia [98]. The data from human studies is somewhat contradictory, but there is valuable data from mouse models of bacterial pneumonia which have important implications for humans.

Studies in mouse models of leptin deficient mice (ob/ob mice) have shown that leptin deficient mice have increased mortality when challenged with *Klebsiella* and *Streptococcus*, two common causes of pneumonia in humans. This increased mortality is related at least in part to defective alveolar macrophage and neutrophil function [99, 100]. If this is also true in humans, this could have significant effects on the pathogenesis of bacterial pneumonia in obese patients.

Summary: Pneumonia and Obesity

Although there are as of yet few studies of respiratory infection in obesity, studies published so far indicate that obesity is likely to be a major factor which alters response to pneumonia in humans. This was illustrated dramatically by the H1N1 epidemic. Future studies will be needed to determine if immunization recommendations should be altered in obese patients. Future studies will also be needed to determine how response to bacterial pneumonia is altered in obese patients, and whether this has implications for the treatment of these patients.

Interactions Between Obesity and Acute Lung Injury

Acute lung injury (ALI) is a syndrome of bilateral pulmonary infiltrates causing hypoxemic respiratory failure in the absence of left heart failure. It is a devastating disease, afflicting 200,000 people in the USA every year, with a mortality rate between 30 and 40 %. Acute lung injury is characterized by a massive inflammatory response in the lungs in response to an inciting event such as sepsis, trauma, or aspiration. Obesity and metabolic factors may be important as moderating factors in the development of acute lung injury.

Diabetes and Risk of Acute Lung Injury

It has long been recognized that diabetes is associated with a reduced risk of developing acute lung injury, suggesting that metabolic factors may be important in the pathogenesis of acute lung injury [101–103]. This protective effect has also been shown in animal models of diabetes [104–107], though the underlying mechanisms remain unclear. Diabetes is associated with impaired innate immune response [108, 109], which although believed to drive the increased risk of infection in diabetics [110], might conversely attenuate inappropriate inflammatory states such as ALI.

Effect of Obesity on Outcomes from Acute Lung Injury

Obese patients appear to have either similar or perhaps improved survival from acute lung injury compared with lean individuals [111, 112]. In fact the highest mortality is typically reported in underweight patients [111], and this is thought to be related to the fact that underweight patients are suffering from significant other diseases that cause weight loss and increase their risk of dying from acute lung injury. Although survival tends to be better than might be anticipated, obese patients are often reported to have longer duration of mechanical ventilation and greater length of hospital stay [113].

Prolonged Mechanical Ventilation in Obese Patients with ALI

Results of clinical studies have prompted interest in investigating mechanisms by which obesity may influence ICU outcomes. Reasons for increased duration of mechanical ventilation and ICU length of stay in obese patients with ALI observed in some studies may be due to physiologic factors that lead to longer duration of care but do not increase mortality. For example, lung derecruitment due to the weight of the abdomen and chest wall and provider reluctance to extubate an extremely obese patient may contribute to longer duration of ventilation [114].

Improved Survival in Obese Patients with ALI

Why survival in obese patients with ALI is at least as good as, if not better than, that in normal weight patients is not clear. In the few published reports examining obesity's effects on acute lung injury models, obese mice and rats demonstrate reduced inflammation, lung injury, and mortality from LPS-, hyperoxia-, and ozone-induced ALI [104, 115–117], although in the case of ozone exposure, findings are mixed and appear to vary with the acuity of exposure [117–119]. Obesity may be somehow protective in ALI, but the mechanisms by which that might occur have not been elucidated. A recent study found that obese patients with ALI have lower levels of several proinflammatory cytokines (IL-6, IL-8. and surfactant protein D) that are known to be increased in ALI and to be associated with increased mortality [120], thus suggesting that innate immunity and the inflammatory response may be altered in obesity attenuating the development of acute lung injury.

Summary: Acute Lung Injury and Obesity

Diabetes reduces the risk of developing acute lung injury, and obesity appears to attenuate cytokine levels and mortality in ALI, yet these patients tend to have longer intensive care unit length of stays. This differential effect on outcome is likely related to the former being related to altered innate immunity, and the latter due to mechanical factors complicating treatment. Nevertheless, the data on mortality in obese patients with acute lung injury suggest that clinicians should be aware that these patients may have good outcomes in the long term, despite their length of stay in the intensive care unit.

Interactions Between Obesity and Sleep Disordered Breathing

Obesity is a major risk factor for obstructive sleep apnea (OSA) [121, 122]. Indeed, sleep apnea is rare in normal weight individuals. Men appear to have a greater risk of suffering with OSA than women [122], though the risk in women increases dramatically after menopause, some of the reasons for this will be discussed below.

Sleep apnea is a disease characterized by snoring and apneic episodes during sleep; this results from repetitive episodes of upper airway obstruction during sleep. Patients present with daytime somnolence, this can have catastrophic consequences for those with a profession that requires a high level of alertness, and it is thought that sleep apnea contributes to a significant proportion of motor vehicle accidents [123]. Sleep apnea is associated with increased cardiovascular morbidity and mortality [124], daytime somnolence and impaired quality of life [125].

Pathogenesis of Obstructive Sleep Apnea

Apneic episodes are caused by repetitive obstruction of the upper airway during sleep. These apneic episodes are associated with desaturation in oxyhemoglobin, decreases in the duration of stage 4 and REM sleep, and increased sympathetic nervous system activity (which persists into the day). These episodes of upper airway obstruction result from increased collapsibility of the upper airway, impaired neuroventilatory control, and impaired neuromuscular control of the upper airway.

The increased collapsibility of the upper airway is related in part to neck circumference. It is thought that increased soft tissue mass causes external loading on the airway and contributes to this tendency to collapse [126]. Central obesity aggravates the mechanical compromise of the upper airway, as central obesity acts to mechanically reduce the volume of the lung volume; this in turn tends to reduce external tethering of the upper airway, so central obesity is particularly a risk factor for the development of sleep apnea [126]. This may help explain why men have a higher risk of sleep apnea than women, but that the risk in women increases after menopause, a time in which they tend to develop increased central obesity.

Another factor that is critical in maintaining upper airway patency is neurological control of ventilation. Cytokines that are increased in the setting of obesity appear to have depressant effects on neurological control of ventilation (such as TNFα), and this is likely another important factor in the development of sleep apnea in obesity [127]. Leptin, which is obviously increased in the setting of obesity, is actually a central respiratory stimulant. This is somewhat counterintuitive given that leptin levels are increased in the setting of sleep apnea, even when controlled for the level of obesity [128], and decrease with treatment of sleep apnea. Whether the increased leptin in the setting of sleep apnea is a compensatory mechanism, a futile attempt to increase ventilation [129], or another manifestation of leptin resistance is not known [130].

A 3rd factor that is involved in the pathogenesis of upper airway collapse is muscle tone in the upper airway, upper airway muscles tone is a critical factor maintaining upper airway patency, and this is decreased during sleep [131, 132]. This loss of tone contributes to the tendency of the airway to collapse during sleep [133]. Fat deposition in the upper airway and possibly the muscles themselves may contribute to reduced function of these pharyngeal muscles in obesity [134], and this may be another mechanism linking the development of sleep apnea in the setting of obesity.

Relationship Between Sleep Apnea and Insulin Resistance

A number of studies published in recent years suggest that the insulin resistance is increased in patients with sleep apnea [74, 135]. Mouse models, and human studies of periodic hypoxemia show that periodic hypoxemia may contribute to insulin resistance and steatohepatitis [74, 136, 137]. There are also data suggesting that treatment of sleep apnea can improve insulin resistance [138]. Hence sleep apnea may contribute to the development of insulin resistance independently of obesity.

Treatment of Sleep Apnea

Therapy for sleep apnea can include dental devices, surgery, or continuous positive airway pressure (CPAP), all designed to maintain patency of the upper airway [139]. Another highly effective treatment for sleep apnea is weight loss [140].

Successful treatment of sleep apnea can improve hypertension, insulin resistance and significantly improve quality of life.

Obesity Hypoventilation Syndrome

Approximately 20 % of patients with OSA have obesity hypoventilation, a disease characterized by sleep apnea and daytime hypercapnia causing hypersomnolence [141]. This was classically described in Charles Dickens's description of "Joe the fat boy" in the Pickwick papers, hence the eponymous label of Pickwickian Syndrome [142]. Today it is more commonly, if less prosaically, known as obesity hypoventilation syndrome. This is a very serious condition, associated with increased mortality and significantly increased health care costs over that for patients with obstructive sleep apnea [143]. Patients can develop hypercapnic respiratory failure requiring hospitalization and ICU admission, they may develop right and left heart failure, so it is important to treat this condition aggressively [144]. This condition will improve with nocturnal positive airway pressure, though on occasion, tracheotomy may be required. The daytime hypercapnia will also improve with appropriate treatment of OSA.

Summary: Sleep Apnea and Obesity

Obesity, particularly central obesity, is a major risk factor for sleep apnea. Sleep apnea is a serious condition association with significant morbidity and mortality. Sleep apnea itself may contribute to the development of insulin resistance. Obesity hypoventilation syndrome is a severe form of OSA associated with hypercapnia and daytime hypersomnolence. All these conditions can be treated with positive airway pressure, and also respond well to effective weight loss interventions.

Conclusions: Respiratory Disease in Obesity

Obesity is a major threat to lung health. It fundamentally alters lung mechanics to produce respiratory symptoms even in otherwise "healthy obese" individuals. It leads directly to new-onset disease such as asthma, pulmonary hypertension, and sleep apnea, and significantly alters outcomes in those with COPD and asthma leading to significant increases in health care utilization. The observation that obesity may also alter host response to influenza infection and pneumonia is of major concern given the potential large numbers of patients that could be affected. At the same time, there is evidence that obesity and diabetes may actually have some beneficial effects in patients with acute lung injury, though in terms of health-care utilization this is offset by longer lengths of ICU stay. Obesity is fundamentally altering respiratory disease in the twenty-first Century.

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Chapter 20
Adiposity and Kidney Disease

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Keywords Adipose tissue • Obesity • Kidney disease • Insulin resistance • Cardiovascular outcomes

Key Points

- The prevalence of chronic kidney disease (CKD) is increasing worldwide and is a global public health challenge.
- Epidemiological data suggest a potential causal relationship with adiposity.
- Clinical and laboratory studies suggest that adiposity is involved in the development and progression of kidney disease.
- Mechanisms of kidney damage in obesity include adaptation to increased body mass, activation of sympathetic nervous and renin–angiotensin systems, insulin resistance, hyperlipidemia, and release of adipokines.
- Kidney disease may also effect the association of adiposity with cardiovascular outcomes.
- In this chapter, the interactions of adiposity and kidney disease and their effects on clinical outcomes are examined.

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Introduction

Chronic kidney disease (CKD) is increasingly common. The prevalence of CKD in the US population is 15.1 %, affecting nearly 20 million. At the end of 2009, the number of people with stage 5 CKD (glomerular filtration rate (GFR) <15 ml/min) requiring dialysis was 571,414 with a prevalence rate of about 1,700 per million population, representing over an 80 % increase in the past decade [1].

There is evidence that adiposity is involved in the development and progression of kidney disease. Although adiposity is a well known cardiovascular risk factor in the general population, epidemiological studies have raised uncertainties regarding the impact of adiposity on clinical outcomes in CKD and dialysis patients. In this chapter, we address these two issues: the effects of adiposity on kidney disease and the effects of kidney disease on the associations of adiposity on cardiovascular risk factors and cardiovascular disease.

Effect of Adipose Tissue on Progression of Kidney Disease

Epidemiological Data

In many populations, the rising trend of kidney disease has mirrored that of obesity [2, 3]. Obesity, which is mainly caused by increase in adipose tissue, has a direct relationship to the development and progression of diabetes mellitus, hypertension, and dyslipidemia. Diabetes and hypertension are well known to be the two most common causes of renal impairment. The United States Renal Data System (USRDS) lists diabetes mellitus as the etiology for end stage renal disease (ESRD) in 47 % of the prevalent dialysis population and hypertension accounts for nearly 28 %. Analyses of the Modification of Diet in Renal Disease (MDRD) [4] and Atherosclerosis Risk in Communities (ARIC) studies [5] showed that high triglycerides and low high density lipoprotein (HDL) are related to the development of CKD.

The above data raises the question whether the association of adiposity with CKD is a mere reflection of the other obesity related comorbidities such as diabetes mellitus, hypertension, and dyslipidemia or whether adiposity is an independent risk factor for kidney disease. In an analysis of the ARIC data, the odds ratio (OR) of developing CKD during a 9-years follow-up period in participants with metabolic syndrome was 1.43 and remained at 1.23 after adjusting for subsequent development of diabetes mellitus and hypertension. Compared with participants with no traits of metabolic syndrome, those with one, two, three, four, or five traits of the metabolic syndrome showed a graded increased in the OR for CKD from 1.13 to 2.45. Thus, metabolic syndrome is independently associated with an increased risk for incident CKD in non diabetic adults [6]. Johnson et al. also confirmed the earlier observation that the prevalence of metabolic syndrome increased with decreasing creatinine clearance suggesting that metabolic syndrome is an independent predictor of CKD [7].

Kidney damage is clinically manifest as loss of albumin in urine (albuminuria) or decline in GFR. Analysis of Third National Health and Nutritional Examination Survey (NHANES) data further showed that abdominal obesity was associated with both a decrease in GFR and microalbuminuria (24 h urinary excretion of albumin excretion in the range of 150–300 mg/d) [8]. This association was also seen with each of the other elements of metabolic syndrome (insulin resistance, hypertension, hypertriglyceridemia, and low HDL). Furthermore, there was a graded relationship between the components present and the corresponding prevalence of CKD and microalbuminuria. Microalbuminuria is a well known predictor of adverse cardiovascular outcomes.

Thus, metabolic syndrome is independently associated with the development and progression of CKD and microalbuminuria.

Table 20.1 Mechanisms of kidney damage

Cardiovascular	Hypertension
Renal	Altered vascular structure and function
	Enhanced renin–angiotensin–aldosterone system
	Enhanced sympathetic nervous system
Metabolic	Hyperinsulinemia/insulin resistance
	Dyslipidemia
	Hypercortisolemia
Inflammatory	Hyperleptinemia
Hematological	Hypercoagulability
	Altered kallikrein–kinin system

Renal Pathology in Adiposity

Histologically, renal biopsies of obese patients with renal failure have shown glomerulomegaly and focal and segmental glomerulosclerosis [9, 10]. In patients with morbid obesity and a mean body mass index of 52 kg/m^2 glomerulomegaly, podocyte hypertrophy with expansion of mesangial matrix and mesangial proliferation were observed [11]. Metabolic syndrome is also known to be associated with greater tubular atrophy, interstitial fibrosis and vascular damage on renal histology [12].

Mechanisms of Kidney Damage in Adiposity

There are several biological mechanisms through which adiposity could lead to kidney damage (Table 20.1). The pathophysiology of renal dysfunction in obesity is a combination of hemodynamic and metabolic abnormalities that include glomerular hyperfiltration, increased renal venous pressure, glomerular hypertrophy, and increased synthesis of vasoactive and fibrogenic substances (including angiotensin II, insulin, leptin, and transforming growth factor [TGF]-β). The following discussion elaborates on these mechanisms.

Adaptation to Increased Body Mass

An increase in body mass leads to an increased excretory load of nitrogen and metabolic waste. As the nephron number is fixed, this leads to an increased work load with hyperperfusion and hyperfiltration of each nephron. Obese patients have an increase in renal plasma flow and glomerular filtration rate by 31 and 51 %, respectively, leading to an increase in filtration fraction and glomerular hypertension [13]. It has been shown that obesity related glomerular hyperfiltration ameliorates after weight loss [14].

Adverse Effects of Obesity-Induced Sodium Retention

Obesity leads to activation of the sympathetic nervous system, in part by hyperleptinemia that stimulates the hypothalamic pro-opiomelanocortin pathway [15]. The renin–angiotensin–aldosterone system (RAAS) is also upregulated in obesity. The increased sympathetic nervous and renin–angiotensin systems lead to volume expansion and increased blood pressure. Further, the excess visceral adipose tissue may lead to physical compression of the kidneys causing increased intra renal pressures and increased tubular reabsorption of sodium [15].

The increased tubular reabsorption of sodium leads to afferent arteriolar vasodilatation and glomerular hyperfiltration [16]. The afferent arteriolar vasodilation and increased systemic arterial pressure cause an increase in hydrostatic pressure and contribute to glomerular capillary wall stress. These changes along with hyperlipidemia and hyperinsulinemia may cause glomerular injury with increased matrix accumulation and eventually glomerulosclerosis and loss of nephron function in obese subjects.

Direct or Indirect Effects of Hyperinsulinemia/Insulin Resistance

Insulin resistance and hyperinsulinemia are important pathophysiological factors in the development of metabolic syndrome. Hyperinsulinemia contributes to renal vascular injury by stimulating smooth muscle cell proliferation [17]. Hyperinsulinemia also has direct and indirect effects on the progression of glomerular dysfunction. The direct effects include irreversible glycosylation of glomerular protein, inhibition of phosphatidylinositol-3 kinase pathway (PI-3K), and activation of mitogenic activated protein (MAP) kinase pathway leading to increased atherogenesis and endothelial dysfunction [18]. Hyperinsulinemia is also associated with decreased endothelial production of nitric oxide and increased oxidative stress leading to vascular endothelial injury. The indirect effects include activation of the renin–angiotensin–aldosterone system leading to increased angiotensin II and aldosterone levels [19]. In addition to sodium retention, elevated aldosterone promotes fibrosis and target organ dysfunction by stimulating plasminogen activator inhibitor, reactive oxygen species and TGF-β1 [19]. In vitro studies have shown that hyperinsulinemia can also induce glomerular hypertrophy both directly and indirectly via insulin like growth factor (IGF)-1 [20].

Renal Lipotoxicity

Increased cellular lipid content leads to intracellular shunting of excess fatty acids towards synthesis of products that induce cell damage [21–23]. This impairs function of the individual cells and causes inflammation, apoptosis and cell necrosis. Lipotoxicity is associated with progression of metabolic syndrome and can involve multiple organs including kidney, liver, skeletal, pancreas and cardiac cells [22, 23]. In the kidneys, dyslipidemia increases the amount of lipoprotein being filtered in the Bowman's capsule, damaging glomerular and tubular cells, promoting fibrosis and enhancing endothelial dysfunction and atherosclerosis [24, 25].

Adipose Tissue as an Endocrine Organ

Adipose tissue secretes a wide range of protein and non proteinfactors, termed adipokines. A number of adipokines including leptin, adiponectin, adipsin, resistin, visfatin, tumor necrosis factor (TNF)-α, transforming growth factor β (TGF-β), interleukin(IL)-1β, IL-6, monocyte chemoattractant protein-1, macrophage migration inhibitory factor, nerve growth factor, vascular endothelial growth factor, plasminogen activator inhibitor 1, insulin like growth factor-1, retinol binding protein are secreted by adipose tissue. An imbalance of these adipokines is observed in patients with kidney disease leading to chronic inflammation which implicated in the development and progression of hypertension and endothelial dysfunction.

Leptin is a proinflammatory adipokine that is anorexigenic and is primarily cleared by the kidney [26, 27]. Leptin levels are elevated in patients with obesity who are predisposed to glomerulosclerosis. In glomerular endothelial cells, leptin stimulates cellular proliferation, TGF-β1 synthesis, and type IV collagen production [28]. In the mesangial cells, leptin upregulates synthesis of TGF- β2 receptor and

type 1 collagen production [29, 30]. These result in focal glomerulosclerosis and mesangial proliferation. Leptin also activates the sympathetic nervous system and enhances sodium reabsorption leading to hypertension, proteinuria and progression of kidney disease [31]. There is also evidence to suggest that leptin and TGF-β1 promote mesangial sclerosis by different mechanisms and act synergistically to potentiate mesangial matrix production.

Adiponectin is a peptide secreted exclusively by adipocytes that has antiatherogenic, anti-inflammatory, and insulin sensitizing effects. Plasma adiponectin level is negatively associated with fat mass [32]. Three adiponectin receptors AdipoR1, AdipoR2 and T-cadherin have been identified. AdipoR1 is most abundantly expressed in muscle, AdipoR2 in the liver and T cadherin on vascular endothelial and smooth muscle cells [33, 34]. These receptors are linked to activation of AMP-activated kinase (AMPK) pathways. Activation of AMPK by adiponectin results in stimulation of fatty acid oxidation in the skeletal muscles, inhibition of hepatic gluconeogenesis and stimulation of nitric oxide production in the endothelial cells [35, 36]. Adiponectin displays anti-inflammatory properties by inhibiting NF-κβ activation and TNF-β synthesis and by inducing anti inflammatory cytokines such as IL-10, IL-1 receptor antagonist [37, 38]. The insulin sensitizing effect of adiponectin is explained by stimulation of glucose uptake and oxidation of fatty acids in skeletal muscles and liver cells, induction of insulin signaling in skeletal muscle cells and suppression of liver gluconeogenesis.

Metabolic syndrome correlated positively with leptin and inversely with adiponectin levels. Serum adiponectin is inversely associated with increased cardiovascular risk [39, 40]. The adiponectin/receptor system is upregulated in ESRD likely as a counter regulatory response to the uremic milieu. In animal studies, administration of adiponectin was shown to decrease albuminuria and mesangial sclerosis.

Resistin is secreted by both adipocyte and immunocompetent cells [41]. Plasma resistin level increases with progressive renal insufficiency [42] and early studies suggest an association with obesity and insulin resistance [43] though the exact pathophysiological role is unknown.

Visfatin is a proinflammatory adipocytokine [44]. In uremic patients, visfatin level was independently associated with sVCAM-1, a marker of endothelial damage [45]. The relationship between visfatin and insulin resistance is unknown.

Management

There is substantial evidence that adipose tissue and obesity are related to the progression of renal disease. If managed effectively in the early stages, most of the physiological and structural changes may be reversible.

Weight loss and physical activity are recommended as first line therapy. The reduced calorie DASH (dietary approaches to stop hypertension) diet and a Mediterranean diet have both been demonstrated to reduce risk of metabolic syndrome [46, 47]. Fiber and other phytonutrients in fruits and vegetables have been shown to reduce cholesterol and markers of inflammation. Increased dietary intake of fiber was associated with decreased C reactive protein and mortality in patients with CKD [48]. An inverse association between the intake of dairy and metabolic syndrome has also been reported. As patients with metabolic syndrome are salt sensitive, dietary restriction of sodium may be beneficial by lowering blood pressure [49]. In patients with CKD, nonsurgical weight loss interventions reduce proteinuria and blood pressure and prevent decline in renal function [50]. In morbidly obese individuals with glomerular hyperfiltration, surgical interventions normalize GFR and reduce blood pressure and microalbuminuria [50].

As obesity is associated with increased activation of the renin–angiotensin system, treatment with angiotensin receptor blocking agents should be considered especially in patients with hypertension and proteinuria.

Adiponectin is an anti-inflammatory and antiatherogenic adipokine and interventions to improve adiponectin levels may be considered to improve long term outcomes. Improvement in adiponectin levels and insulin resistance was observed with RAAS blockade with either angiotensin converting enzyme blocker or angiotensin receptor [51]. Peroxisome proliferator activated receptor (PPAR) gamma ligands such as thiazolidinediones have also been shown to increase adiponectin levels and improve insulin resistance [52]. Further prospective studies are required to address the potential therapeutic role of adipokines is progression of renal disease.

Dietary management and physical activity remain cornerstones of therapy and early interventions targeted towards hypertension, adiposity and insulin resistance might minimize renal damage associated with obesity.

Effects of Kidney Disease on Associations of Adiposity with Cardiovascular Disease

In contrast to the data in the general population, dialysis patients with higher body mass index have lower mortality compared to those dialysis patients with normal body mass index [53]. Strikingly, these data have been consistent in several studies patients [54–63]. Thus, it has been suggested that obesity is protective in dialysis patients [56]. In other words, as the associations of body size with mortality appear to vary depending upon the presence or absence of advanced kidney failure, it can be said that kidney disease is an effect modifier of this association.

However, there are three problems with the suggestion that adiposity is protective in dialysis patients. First, the real paradox of the "BMI paradox" in dialysis patients is the possible association of high BMI with inflammation yet decreased mortality. Adipocytes are rich sources of pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α), which in turn stimulate the production of C-reactive protein (CRP) in the liver [64]. It was shown in a cross-sectional study that abdominal adiposity is strongly associated with elevated CRP levels in dialysis patients [65]. Further, the cross-sectional associations of high BMI, abdominal adiposity and other components of metabolic syndrome [66–68] with inflammation in stage III CKD have been demonstrated. Therefore, the current evidence suggests that in stages III and V of CKD, obesity is associated with inflammation as in the general population.

Second, high BMI might result from high muscle mass, fat mass or both. It is possible that high BMI due to high muscle mass might be more protective than high BMI due to high fat mass. In 70,028 patients initiated on hemodialysis in the USA from 1/96 to 12/98 with reported measured creatinine clearances at initiation of dialysis, BMI in conjunction with 24-h urinary creatinine excretion (an indicator of muscle mass) was used to estimate body composition and the effects of estimated body composition on all-cause and cardiovascular mortality were examined [54]. High body size was associated with better survival. However, compared to normal BMI, normal or high muscle patients, those with high BMI and low muscle mass had increased mortality, whereas those with high BMI and normal or high muscle mass had decreased mortality. These data suggest that high BMI is not uniformly associated with better survival and the body composition is important in high BMI dialysis patients. In another study of incident peritoneal dialysis patients, similar results were shown [68].

Third, previous studies have shown that in dialysis patients, adiposity and high BMI is associated with diabetes [69], inflammation [70], coronary calcification [71, 72] and carotid atherosclerosis [73]. These data raise the question that if adiposity is associated with diabetes, inflammation, coronary calcification and atherosclerosis in dialysis patients, how is adiposity associated with better survival in dialysis patients?

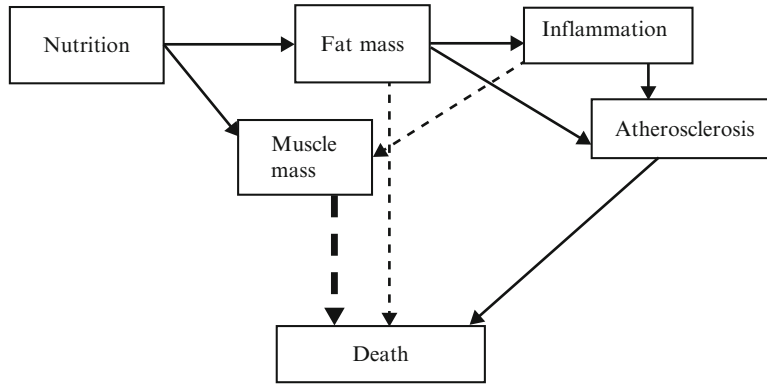

Fig. 20.1 Directed acyclic graph of the hypothesized associations of nutritional status with inflammation, atherosclerosis, and death. Dotted lines represent a negative effect, whereas the *unbroken lines* represent a positive effect

We propose the following framework (Fig. 20.1) to integrate these seemingly contradicting data. In Fig. 20.1, dotted lines represent a negative effect, whereas the unbroken lines represent a positive effect.

When the association of high BMI with survival is examined in dialysis patients, there might actually be two issues that are examined- what is the effect of nutrition on survival and what is the effect of adiposity on atherosclerotic events and cardiovascular events? We hypothesize that the effects of nutrition on survival are much stronger than the effects of atherosclerotic events on survival in dialysis patients. Further, we also propose that the effects of nutrition on survival might differ based on body composition (muscle versus fat). Better nutrition as evidenced by higher muscle mass decreases the hazard of death from concomitant cardiovascular and non-cardiovascular events resulting in the lowest cardiovascular and non-cardiovascular deaths. On the other hand, fat mass has dual effects; a negative effect on death as a result of nutrition and a positive effect on death mediated through its association with inflammation and atherosclerosis. Thus, compared to undernutrition, adiposity decreases the hazard of death from concomitant disease processes but is associated with inflammation, oxidative stress and atherosclerotic events in dialysis patients as in the general population. In other words, adiposity confers a survival advantage over undernutrition but not compared to higher muscle mass in dialysis patients.

Further, as shown in Fig. 20.1, the above paradigm could also incorporate the current theories on the association of inflammation with malnutrition, in particular, the observed associations of inflammation with decreased muscle mass in dialysis patients [74, 75]. In other words, the association of inflammation with loss of muscle mass does not contradict adipose tissue as a source of inflammation in CKD.

Conclusion

In summary, obesity is a risk factor for renal dysfunction, as evidenced by albuminuria and loss of GFR. Potential mechanisms include hemodynamic changes, lipotoxicity, and inflammation. The association of adiposity with cardiovascular outcomes in ESRD remains controversial and further studies will shed light on this complex issue.

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Chapter 21
Obesity and Joint Disease

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Keywords Obesity • Joint disease • Osteoarthritis • Weight loss

Key Points

- The prevalence of osteoarthritis, compounded by the current alarming obesity epidemic, is rapidly rising.
- This is associated with significant socioeconomic burden given the close obesity–osteoarthritis relationship.
- Obesity plays a major role in the pathogenesis of osteoarthritis in both weight-bearing and non-weight-bearing joints through joint loading and obesity-related meta-inflammatory process.
- This chapter examines the evidence for a role of obesity in joint pathology and the role of weight loss, using osteoarthritis as a disease paradigm.

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Prevalence of Osteoarthritis (OA)

Obesity and musculoskeletal conditions represent emerging global socioeconomic and public health problems. Both are chronic conditions with direct and indirect effects on quality and quantity of life. Up to 50 % of Americans 65 years or older had arthritis in 2007–2009 [1]. It was estimated about 27 million Americans in 2007 had OA [2], and the figure will rise to an estimate of 67 million Americans aged 18 years or older by the year 2030 [3]. In Australia, the National Health Survey 2007–2008 reported approximately 7.8 % of Australians have OA [4]. The prevalence increases with age and is more common in females (33.5 %) than in males (21.9 %) [5]. Compounded with the fact that there is currently no treatment for OA, the prevalence of OA will only continue to rise, fuelled by increased life expectancy in our aging society, as well as the obesity epidemic.

Prevalence of Osteoarthritis in Obese Population

The incidence of OA, a nonfatal but disabling condition, is on the rise with the increasingly aged population and the obesity epidemic [6, 7]. Up to 66 % of adults with arthritis are overweight or obese [8]. The proportion of OA attributable to obesity ranges from 8 % in China to 50 % in the USA, depending on the prevalence of obesity in the population [9]. In Australia, between the years 2000 and 2025, the number of normal weight adults is estimated to decrease from 40.6 to 28.1 % and number of obese adults to increase from 20.5 to 33.9 %, such that if current trends continue, normal weight adults will constitute less than a third of the population by 2025 [10]: this would have a significant impact on the prevalence and incidence of OA.

Socioeconomic Burden of Osteoarthritis

OA is the most common musculoskeletal disease, representing a significant burden to society. In the USA, OA has been the main cause of disability for the past 15 years [11]. It is also the fourth leading source of nonfatal health burden, accounting for 3 % of total years lived with a disability [12], with knee OA being the most prevalent and disabling condition [13]. In fact, obesity and knee OA are among the most frequent comorbid conditions in older Americans aged between 50 and 84 years [13, 14]. It is estimated that up to 3.5 quality-adjusted life-years lost per-person in those affected by both obesity and OA, resulting in a total of 86 million quality-adjusted life-years lost due to either or both the conditions in older Americans aged 50–84 years [13]. In addition, the total cost attributed to musculoskeletal conditions in 2003 was 128 billion dollars, or approximately 1.2 % of the US gross domestic product, up from 86.2 billion dollars in 1997 [15].

As a result of the close obesity–OA relationship, it is not surprising that the cost of musculoskeletal diseases were the highest among people with a Body Mass Index (BMI) of 27.5 kg/m^2 or greater in the workplace [16]. Despite the significant economic impact, the association between obesity and joint disease is not mechanistically well understood. Both metabolic and biomechanical factors have been shown to play important roles in the pathogenesis of OA, although the relative importance of different aspects of obesity may differ in the various affected joints.

This chapter aims to examine the evidence for a role of obesity in joint pathology using OA as a disease paradigm.

Definition and Assessment of Osteoarthritis

OA is a disease of the whole joint, previously characterized by cartilage loss, but also known to affect the underlying bone and surrounding soft tissues. The most widely used definition of OA is that of the American College of Rheumatology (ACR) which states that OA is "a heterogeneous group of conditions that lead to joint symptoms and signs, which are associated with defective integrity of articular cartilage in addition to related changes in the underlying bone margins" [17].

Radiology

Conventional X-rays have been used to assess structural changes of osteoarthritis, particularly joint space width (JSW), as an indirect measure of articular cartilage. A number of studies have shown an association between knee JSW narrowing and obesity [18, 19]. However, the use of X-rays to assess and quantify structural changes has limitations as it does not permit direct visualization of the cartilage [19], and other structure have been shown to account for some of the change in JSW observed. For instance, meniscal extrusion has been shown to account for some of the change in joint space narrowing [20]. Additionally, radiographs employ a one-dimensional measure to assess change in a three-dimensional structure such as the joint space. Therefore, the validity of the radiographic JSW as a measure of articular cartilage is limited.

Magnetic Resonance Imaging

MRI has revolutionized the field of clinical research in OA as it allows direct visualization of all diarthrodial tissues, including cartilage, bone, menisci, ligaments, and synovium. Thus, MRI provides a substantial advantage over X-rays, as it permits quantitative direct measures of joint structure, including cartilage morphology, enabling a sensitive evaluation of the progression of OA. Knee cartilage volume measured by MRI has been shown to be valid and reproducible [21–23], sensitive to change in both normal subjects [24] and those with OA [25], correlate with radiographic grade of OA [26, 27] and predict the clinically important outcome of pain [25, 28] and joint replacement [29]. MRI has therefore provided the first opportunity to directly examine articular knee cartilage noninvasively.

Assessment of Obesity

To date, the majority of studies have measured the BMI (kg m^{-2}) as an indicator of obesity. A major downside of using BMI to measure obesity is that BMI does not provide information on the independent contributions of fat and lean tissue mass to the total body mass. Recent studies have highlighted the importance of body composition, suggesting that muscle and fat mass have a differential effect on joint health [30, 31]. This has resulted in a shift from use of traditional measures of obesity such as weight and BMI to parameters of body composition, such as fat distribution and lean body mass when examining the relationship between obesity and joint disease. In addition, over recent years, Magnetic Resonance Imaging (MRI) has been increasingly used as a sensitive, noninvasive method for assessing the state of the joint [21, 22]. Combining these more sensitive methods for assessing body composition and joint structure have enabled significant insights into the relationship between obesity and joint health.

Association Between Obesity and Knee Osteoarthritis

The association between obesity and OA is arguably strongest and most consistent at the knee joint. In 1958, Kellgren and Lawrence found that knee OA was more common in obese people, particularly women [32]. Since then, cross-sectional studies have consistently shown an association between obesity and knee OA, which has been stronger for women than men [33–35]. Among obese middle-aged females with knee OA, it has been reported that the proportion of the disease attributable to obesity is approximately 63 % [36].

Longitudinal studies have consistently demonstrated an association between obesity and knee OA, when either symptoms or structural change is considered. A 35-year follow-up study demonstrated a strong association between being overweight and the development of OA, particularly in women [37]. Likewise, an increased BMI at a young age was a risk factor for knee OA in males [38]. Twin studies have also demonstrated that a twin with tibiofemoral and patellofemoral OA is likely to be 3–5 kg heavier than their co-twin. Moreover, twin studies have also demonstrated a 14 % increased risk of developing tibiofemoral osteophytes and a 32 % increased risk of developing patellofemoral osteophytes for every kilogram gain in body-weight [39]. In addition to the development and structural changes of OA, gaining 5 % of initial body weight or more was also associated with worsening knee pain, stiffness and function [40]. Furthermore, BMI and knee pain was shown to have a linear relationship while weight loss was associated with a beneficial effect on knee symptoms [41–43].

Given that the knee is composed of distinct compartments, the association between obesity and knee OA may differ between the different knee compartments. Of the few studies have examined the association between obesity and compartment OA, one cross-sectional study of middle-aged women demonstrated obesity was an important risk factor for both medial tibiofemoral and patellofemoral joint disease [39]. Another study demonstrated an association between obesity and tibiofemoral OA, but failed to show a relationship at the patellofemoral joint [44]. These contrasting results highlight the need to clarify the association between obesity and OA at the different compartments in the knee complex. Nevertheless, a detrimental association between obesity and knee OA is unequivocal.

Since the advancement of the use of MRI in evaluating OA, data to date have demonstrated inconsistent associations between articular cartilage volume or thickness and obesity measured by BMI [23, 45, 46]. However, this has largely been determined by the populations being investigated. In pre-OA populations, there is evidence showing significant association of increase risk and progression of cartilage defect and volume reduction with increased body weight [23, 34, 47]. However, less consistent findings have been observed when populations with OA have been examined [23, 34, 46]. In OA populations, some studies showed higher rates of cartilage loss, particularly at medial tibial-femoral condyle region in subjects with a high BMI [19, 35, 48]. A recent study has demonstrated improvements in the quality and quantity of medial articular cartilage with weight loss [49]. These findings suggest that the effect of obesity may differ in early versus late disease, where other factors, such as biomechanical factors, may have a stronger role.

Association Between Obesity and Hand Osteoarthritis

The results of studies examining the association between obesity with hand OA are conflicting. Data from the National Health Examination Survey demonstrated an association between the BMI and the presence of hand OA in men after adjustment for age, race and skin fold thickening [50]. However, this relationship was not significant after adjustment for waist girth and seat breadth. A case–control study found that obesity and hand OA were associated [51]. Longitudinal data also confirmed an association between radiographic hand OA and BMI in men [52], although the New Haven Survey demonstrated that finger OA and obesity were more strongly associated in woman than in men [53]. In the Chingford study, obesity was only moderately associated with distal interphalangeal and

carpometacarpal OA, but not with proximal interphalangeal OA in women [36]. Another study found that there was no significant difference in weight within twin pairs discordant for osteophytes at the distal and the proximal interphalangeal joints, although there was a 9 % increased risk for developing carpometacarpal osteophytes for ever kilogram increase in body weight [39]. Other studies, such as the National Health Examination Survey did not find a significant association between the BMI and hand OA [50]. A lack of association was also identified between indices of obesity and hand OA in men in the Baltimore Longitudinal Study of Aging [54].

Association Between Obesity and Hip Osteoarthritis

Similar to the hand, data regarding the association between obesity and hip OA is equivocal. A case control study that examined the BMI at 10-year intervals in men who had received a hip prosthesis because of OA demonstrated that a BMI greater than one standard deviation above the mean was associated with the development of severe OA [55]. Relative weight was only weakly associated with OA of the hips when examining data from 4,225 persons in the National Health and Nutrition Examination Survey (HANES) [56]. Data from the First National Health and Nutrition Examination Survey (NHANES-I) failed to demonstrate an association between obesity and hip OA [57]. Nevertheless, those studies (HANES and NHANES-I) used only radiological evidence in the assessment of hip OA without accounting for clinical symptoms. In a systematic review by Lievense et al., moderate evidence was found for a positive association between obesity and the occurrence of hip OA [58]. In fact, a few studies have demonstrated a clear dose–response relationship [58]. In contrast, Lievense et al. found no association or only a very weak positive association with hip OA in those studies using only an X-ray as evidence of hip OA, suggesting people with obesity may suffer more from the same radiological degree of hip OA than the nonobese people do [58].

Obesity as a Risk Factor for Progression of Osteoarthritis

Despite the significant limitations associated with the radiological assessment of OA, X-Rays have been routinely used as the gold standard to assess the progression of OA, with a reduction in joint space width (JSW) or joint space narrowing (JSN) are used to indicate disease progression. Whereas cross-sectional studies examining the association between the BMI and radiological JSW have reported conflicting results [59, 60], obesity has been consistently associated with a longitudinal reduction in the JSW. A 12 year follow-up study found that among people with knee OA, larger body mass indices were a risk for a reduction in the JSW, and therefore the radiological progression of knee OA (OR 11.1; 95 % CI 3.3–37.3) [61]. Also, there was another systematic review that examined BMI in relation to OA progression up to the year 2010 found that BMI was a strong predictor for long term OA progression (>3 years) clinically or radiologically [62].

While obesity is a risk factor for longitudinal narrowing of radiological JSW, the assessment of the JSW as an outcome measure for the progression of OA is often insensitive. Raynauld et al. found that over a 2-year period, radiological assessment was unable to distinguish significant changes in the JSW in people with knee OA, despite a significant loss of articular cartilage volume [63]. In contrast, MRI studies have revealed that as little as 2 % change in cartilage volume may be reliably detected when a maximum of 6 individuals (patella), 10 (femur) 28 (medial tibia) and 33 (lateral tibia) are followed longitudinally [64]. While obesity is a risk factor for the progression of radiological JSN, it would appear that the assessment of cartilage volume measured from MRI is a more sensitive indicator of disease progression in OA. Nevertheless, only one study has directly assessed the relationship between obesity and longitudinal loss of articular cartilage volume from MRI assessment. This showed that reduced cartilage

thickness loss was seen in those who lost >7 % of their body weight over 12 months [49]. However, no longitudinal study has examined the specific parameters of body-composition, such as fat distribution, and the risk of the progression of knee OA and cartilage loss. Further work is required in these areas.

Assessing the Role of Body Composition in Osteoarthritis

In contrast to the use of BMI as a measure of obesity which does not discriminate adipose from non-adipose body mass, body composition is used to examine the contribution of fat and muscle mass to the pathogenesis of osteoarthritis.

Relationship of Muscle Mass and Osteoarthritis

Muscle mass has generally been found to have a positive effect on knee structure. Lower limb muscle mass, muscle mass in all limbs, and total body muscle mass have been shown to be associated with the magnitude of medial tibial cartilage volume. Loss of muscle mass is also associated with longitudinal loss of medial and lateral tibial cartilage volume [65]. Similarly, some studies have demonstrated beneficial effects of increased muscle mass, where it not only protects against OA development [66], but also loss of tibial cartilage [34, 67].

Relationship of Fat Mass and Fat Distribution with Osteoarthritis

In the past, the association between OA and body fat distribution has remained unclear, with some studies showing no association [36, 68]. However, over recent years, there has been emerging evidence to support the link between obesity, body composition and fat distribution and osteoarthritis. An analysis of the National Health and Nutrition Examination Survey III data in the USA by Puenpatom et al., 2009 showed that 63 % of the OA population had abdominal obesity versus 38 % of the non-OA population [69].

There are also increasing evidence to show deleterious effect of fat mass on knee cartilage properties in healthy individuals [34]. Similarly, Berry et al. [47] demonstrated that fat mass was a risk factor for cartilage defects and BMLs, which are features of early knee pathology. This study showed that for every 1 kg increase in total body fat, there was an increased risk of cartilage defects [47]. Recent data has shown that there was a three- to fourfold increased risk of primary joint replacement associated with body weight, BMI, fat mass and percentage fat, waist circumference and waist-to-hip ratio, all relates to both adipose mass and central adiposity [70]. While the BMI is associated with OA, the evidence has become clear that body composition, in particular fat mass and its distribution mediate this relationship.

Mechanisms for Obesity in the Pathogenesis of Osteoarthritis

Although obesity is a well-established risk factor for OA [71], the mechanisms by which obesity leads to OA are still unclear. Recent studies have provided new insights into a number of potential mechanisms, which are now increasingly recognized as very important in the pathogenesis of OA. These include (1) obesity leading to chronic loading, coupled with increased or abnormal stress across the

joint and resultant deterioration of the joint structures with altered joint biomechanics; (2) body composition, in particular increased fat mass and reduced muscle mass in obesity leading to reduced muscle strength and resultant loss of protective joint support; (3) meta-inflammation caused by the inflammatory and metabolically active effect of adipose tissue in obesity. The emerging evidence suggests that the effect obesity on osteoarthritis is most likely due to a combination of both biomechanical and metabolic factors, as discussed below.

Obesity, Joint Loading and Biomechanics

Under a normal physiologic condition, joints of the body are subjected to loading resulting in forces up to ten times body weight passing through the joints [72]. For example, the medial compartment of the knee joint is subjected to approximately four times body weight. Thus, obesity leads to increased loading and resulting in detrimental effect on the weight-bearing joints, which includes alteration in the composition, structure, metabolism, and mechanical properties of the articular cartilage and other joint tissue [72].

The effect of loading on knee, be it a healthy knee or an osteoarthritic knee, can also be magnified by malalignment, particularly the varus knee [73, 74], due to unequal load distribution between the medial and lateral compartments, with up to 70 % of load passes through the medial compartment [74]. Hence, it is not surprising to find that varus increases the risk of initial development of knee OA. Furthermore, BMI and OA severity is correlated with varus malalignment, but not valgus knees. BMI is also correlated with the severity of varus malalignment [59, 74]. However, the correlation between BMI and OA severity was greatly reduced after accounting for the severity of varus malalignment, suggesting that part of the BMI effect could be explained by the severity of varus malalignment [59]. Varus malalignment may also intensify the effect of excess body weight on the medial tibiofemoral compartment, further contributing to the OA development, progression, and increasing bone marrow lesions over time at this compartment [59, 74, 75].

Apart from loading stress, obesity also leads to increased shear stress, tensile stress and hydrostatic pressure. Studies have shown that chondrocytes, which produces the extracellular matrix, are highly sensitive to mechanical loading, which may activate cytokines, growth factors, and metalloproteinases. Theses excessive chronic stresses have been shown to disrupt the homeostasis of anabolism and catabolism within the cartilage [76–78]. Higher stress on the joint from chronic loading has been associated with younger age at total hip replacement surgery [79]. Nevertheless, moderate exercise is still shown to be beneficial for the cartilage constitution [76].

Altered Biomechanics in Obese Individuals

Apart from the abnormal loading, the accompanied unfavorable joint biomechanics, including abnormal gait, malalignment and hyperextension also play a significant role in the obesity–osteoarthritis relationship. A recent systematic review concluded that obese individuals adjust their movement strategy of everyday movements, walking on average 0.3 ms^{-1} slower, with a smaller stride length by 0.2 m, a greater step width by 0.1 m and a 7° greater toe-out angle when compared with normal weight subjects [80]. These altered gait patterns due to obesity could be associated with cartilage degeneration secondary to change in the load-bearing regions, leading to osteoarthritis [80, 81].

At the knee, medial compartment OA is more common than lateral compartment OA (75–25 %), which may be due to the medial compartment bearing the majority of the load during weight bearing (60–70 %) [59, 82]. In addition, dynamic knee loading during walking also plays an important role in the structural progression of knee osteoarthritis. Studies have shown that higher medial knee load not

only predicts greater loss of medial tibial cartilage volume over time, but also increased the odds of having BML at the medial tibia, evident by the measurement of knee adduction moment impulse [83–85]. The "knee adduction moment impulse" is a measurement of loading that takes into account of both the magnitude and duration of the stance phase [86].

A Metabolic Component in Osteoarthritis

The increased risk of osteoarthritis in non-weight-bearing joints such as hand and fingers in obese population suggests that loading and biomechanical factors alone are insufficient to explain the obesity–osteoarthritis relationship [72, 87]. This new insight suggests that the systemic inflammatory mediators may contribute to the pathogenesis of obesity and osteoarthritis.

Adipose tissue in obesity acts as a rich source of pro- and inflammatory cytokines, in which their inflammatory and metabolically active effects could exert various detrimental systemic effects on the body. This is termed "meta-inflammation." Adipokines, which are cytokines produced by adipose tissue, include leptin, adiponectin, resistin, and visfatin. In addition, adipose tissue is also capable of producing many other pro-inflammatory cytokines, for example interleukin-1 (IL-1), IL-6, IL-8, IL-18, and TNF∝, which may play a role in structural changes of the osteoarthritis and perception of pain [72]. For example, IL-1, IL-6, IL-8, IL-18, and TNF-∝ are all associated with synovitis, IL-1 and TNF∝ are associated with cartilage breakdown and IL-6 is associated with ostophyte formation. On the contrary, IL-10, which is *decreased* in obesity, normally has chondroprotective effects by counteracting the effects of other cytokines [88]. With emerging researches focusing on the role of adipokines in the pathogenesis of osteoarthritis, it is believed that elevated adipokines in obesity mediates synovial tissue inflammation and up-regulate cartilage matrix synthesis and degradation [78, 89, 90].

Adipokines

Leptin

Leptin, primarily secreted by adipocytes, is a 16-kDa polypeptide hormone encoded by the obese (ob) gene [24] and is directly correlated with white adipose tissue mass. It functions as an afferent signal in a hypothalamic negative-feedback loop to regulate adipose tissue mass and body weight, acting as one of the hunger-inhibiting hormones, as well as regulating physiological processes like infection, inflammation, and autoimmune diseases [78, 91, 92]. Leptin binds to the leptin receptor (Ob-R), which has been found to be present in cultured human articular chondrocytes and native human cartilage, as well as osteoblasts, stromal cells, and disk cells in the musculoskeletal system [91].

Obesity has been linked to have higher circulating levels of leptin in serum, as well as in osteoarthritic synovial cartilage, which correlates with the severity of joint cartilage destruction [91]. The higher synovial fluid to serum concentration of leptin also suggests an important role for infrapatellar fat in intra-articular leptin production [93]. Apart from acting as a pro-inflammatory adipokine, leptin is also found to have a catabolic role on cartilage metabolism via the upregulation of proteolytic enzymes and acts synergistically with co-stimulation of other pro-inflammatory stimuli (IL-1, TNF∝, IFN) [78, 92].

Nevertheless, in the obesity–osteoarthritis pathogenesis, it is unclear of how much role leptin in mediating the onset versus the progression of OA [78]. In animal models using obese mice, it has been shown that leptin-impaired mice, when become morbidly obese, do not exhibit knee OA, suggesting the critical role of leptin in obesity–osteoarthritis pathogenesis [94]. On the other hand, what we

do know from human study is reduction of leptin level through weight loss and exercise is directly correlated with symptomatic relief in knee OA [88].

These suggest that the interaction among joint loading and local or systemic inflammation may be responsible for the obesity–osteoarthritis process [72].

Adiponectin

Adiponectin, is one of many other adipokines secreted by adipose tissue. It is also known as acrp30 or adipocyte complement related protein of 30 kDa, which exist in various isoforms, which have different, and sometimes counteracting functions.

People with obesity and diabetes are found to have lower circulating levels of adiponectin [91, 95]. There are growing evidence showing various effects of adiponectin in cardiovascular disease, type 2 diabetes, and metabolic syndrome. Its role in inflammation could be anti-inflammatory rather than pro-inflammatory [91]. There is evidence showing it has negative correlation with plasma C-reactive protein (CRP) level, which suggests its anti-inflammatory role [96]. Moreover, adiponectin has been shown to down-regulate MMP-13 and up-regulate tissue inhibitor of Metalloproteinases-2 (TIMP-2); where both actions protect cartilage from degradation [91]. In an OA population, synovial fluid adiponectin levels were found to be 100-fold less than plasma levels. The synovium and infrapatellar fat pad are believed to be the main sources of adiponectin in the OA-affected joint, suggesting that adiponectin may have a protective role against OA [88, 91].

Nevertheless, adiponectin may play a dual role in arthritis, as demonstrated in some other studies. It was found that RA patients have higher adiponectin level compared to healthy controls, which suggests a mechanistic link between obesity and RA [91, 95]. Adiponectin may also contribute to pro-inflammatory and matrix-degrading, suggested by its effect in stimulating IL-6 and pro-MMP-1 production (key mediators of destructive arthritis) in vitro [97]. Similarly, in the OA population, higher serum levels of adiponectin is associated with erosive OA, further suggesting its role in matrix degradation, facilitating inflammation and joint destruction [91, 95].

Therefore, the role of adiponectin in obesity–osteoarthritis is still controversial. More research is required to show whether adiponectin has a protective or pro-inflammatory role in osteoarthritis.

Resistin

Resistin, another adipokine, is also known as macrophage/monocyte-derived pro-inflammatory mediator, and is mainly secreted by peripheral-blood mononuclear cells. It is also produced by adipocytes, lung, heart, and synovial tissue [91, 95, 98]. Resistin levels are increased, not only in obese rodents in animal model, but also in patients with osteoarthritis, and even more so in patients with rheumatoid arthritis [88, 91]. Resistin plays a role in inflammation, whereby in vitro, it stimulates the synthesis and secretion of TNF-alpha and IL-12 and the activation of NK-kB transcription factor in macrophages. In humans, resistin has been detected joints affected by both OA and RA [91, 93, 95]. Following knee trauma, resistin levels quickly rise in the synovial fluid and serum, leading to matrix degradation and inflammatory cytokine release from articular cartilage [88, 91]. Hence, resistin may well be a pro-inflammatory mediator in joint inflammation in the obesity–osteoarthritis relationship.

Visfatin

Visfatin, also expressed by adipocytes, has previously been known as pre-B cell colony-enhancing factor (PBEF). This synergizes with IL-7 to promote the differential of B-cell precursors [91, 95].

Skeletal muscle, liver and bone marrow also express visfatin [91]. In vitro, visfatin was closely correlated with the regulation of insulin secretion and induced production of IL-1β, IL-6, IL-10, and TNF∝ in human monocyte, further suggesting its action as inflammatory mediator [91, 95]. Serum and synovial fluid levels of visfatin have been strongly associated with the severity of rheumatoid arthritis [91, 95]. Visfatin may also have catabolic effects on cartilage as it stimulates matrix metalloproteinase production and is expressed in osteoarthritic chondrocytes [88, 95].

The Role of Weight Loss in Osteoarthritis

Weight loss has an important role in the conservative management of OA.

It has been shown that weight loss provides range of beneficial effects on OA: these include reducing the risk of OA, alleviating symptoms and function and reducing long term OA progression. The Framingham Study found that for every 5 kg/m^2 increase in BMI at baseline, the risk for incident knee OA is increased by 60 % over 7–10 years [99], while a decrease in BMI of 2 kg/m^2 or more over 10 years decreased the odds for the development of knee OA by over 50 % in women [37]. BMI was found to be a strong predictor for long term OA progression (>3 years) in a systematic review that examined BMI in relation to OA progression [62].

Despite the evidence that weight loss alleviates symptoms and improves function, relatively few studies that have examined the impact of weight loss on structural progression, particularly cartilage loss. A few studies that examined the association between weight loss and cartilage biomarkers had showed some correlation between weight loss and cartilage turnover [43, 100]. It has been shown that weight loss is beneficial in the improvements in cartilage quality and thickness in the medial femoral compartment and the percentage of weight loss was positively correlated with medial dGEMRIC index, which is a measure of cartilage integrity [49].

The most effective forms of weight loss to date have been surgical approaches, shown in a Cochrane Review [101]. Whilst the most commonly performed operations are laparoscopic gastric banding and gastric bypass, biliopancreatic diversion/duodenal switch [102], sleeve gastrectomy, and vertical banded gastroplasty are also used [101].

It was shown in a study that people who underwent bariatric surgery had less cartilage thickness loss and improved delayed gadolinium enhanced MRI of cartilage (dGEMRIC) measures than the non-surgical group, with these differences presumed secondary to the higher weight loss in the surgical cohort [49]. Nevertheless, whether the greater weight loss achieved surgically translate to better OA symptomatic relief compared to less intense weight loss achieved by lifestyle interventions remains unknown. There are studies that showed when a reduction in the total percentage of body fat occurs via physical activity, symptomatic and functional improvement of knee OA can be achieved and the benefit is proportionate to the degree of weight loss [41, 103].

Evidence for Weight Maintenance in Knee Osteoarthritis

The long term results of weight loss are less promising with many regaining weight despite the availability of numerous weight loss strategies having been shown to be effective over the short term period [104]. It has been estimated that only one in six formerly obese dieters are able to sustain long term weight loss of at least 10 % [105], although recipients of bariatric surgery show a higher proportion maintaining weight loss over the longer term (>3 years) [106]. As a result, interventions at critical time periods during adulthood, in particularly in those age 25–45, which represents the most common time for weight gain, are needed as modest weight loss early in the disease process is more effective

in the reduction in OA incidence and progression. For example, we have recently shown that weight gain over mid life was significantly associated with structural damage at both the tibiofemoral joint [23] and patellofemoral joint (Under review) in healthy middle aged women.

Ideally, the role of weight management should precede the onset of symptomatic and radiographic OA. Interventions to prevent weight gain require small adjustment to daily dietary intake and activity which are more likely to be acceptable and feasible for most people [107, 108]. Therefore, when facing the reality of combating obesity in OA population, strategies to reduce weight, preventing excessive weight gain or the very least of maintaining a stable weight whilst increasing lean body mass and lower limb muscles strengthening training should be emphasized and instituted early. This is supported by our recent findings that weight gain was associated with increased knee symptoms over 2 years in a community-based population [40].

Conclusions

Obesity is a major risk factor for OA at both weight-bearing and non-weight-bearing joints. The mechanism for this relationship is likely to be through both loading on the joints and an obesity related, meta-inflammatory process. Weight loss is important in order to improve both joint symptoms and structural effects of obesity on the joint. Loss of fat mass is important, but maintaining muscle mass must be considered. Weight gain should be prevented since this is potentially more achievable for patients and is associated with benefits on joint.

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Chapter 22
Obesity and Colon and Postmenopausal Breast Cancer

Patricia M. Sheean, Sandra L. Gomez-Perez, Puja Agarwal, and Carol Braunschweig

Keywords Colon cancer • Breast cancer • Obesity • Body composition

Key Points

- To describe the epidemiologic links between colon and postmenopausal breast cancer with obesity
- To detail the obesity-induced mechanisms for colon and postmenopausal breast cancer
- To describe the potential variation in risks induced by the ectopic fat depot locations for colon and postmenopausal breast cancer

Introduction

Obesity (Body Mass Index, BMI ≥ 30) is currently the most serious public health problem in the US and occurs in 36 % of the adult population [1]. Accompanying the rise in obesity has been an increase in some types of cancer [2]. It has been estimated that overweight (BMI ≥ 25) and obesity (BMI ≥ 30) cause approximately 20 % of all cancers (Fig. 22.1). A systematic review of the evidence by the World Cancer Research Fund and American Institute for Cancer Research concluded obesity was an established risk factor for colon, esophageal, pancreatic, endometrial, kidney, and postmenopausal breast cancer (http:www//dietandcancerreport.org/cup/index.php). Further, a landmark prospective cohort study of 900,000 US adults showed a BMI ≥ 40 was associated 52 % greater cancer-related mortality rates in men and 62 % higher in women compared to rates in normal weight men and women [3].

The most common obesity related cancers in the US are colon for men and postmenopausal breast cancer in women. This chapter will describe the epidemiologic studies that have examined the influence of obesity and its proposed biological mechanisms in these two cancers. Additionally, because the location of adipose tissues influences dysmetabolism, and therefore cancer risk, studies that have explored the association between waist circumference (WC), waist-to-hip ratio, and specific adipose depots (visceral vs. subcutaneous) and risks for these diseases are also described.

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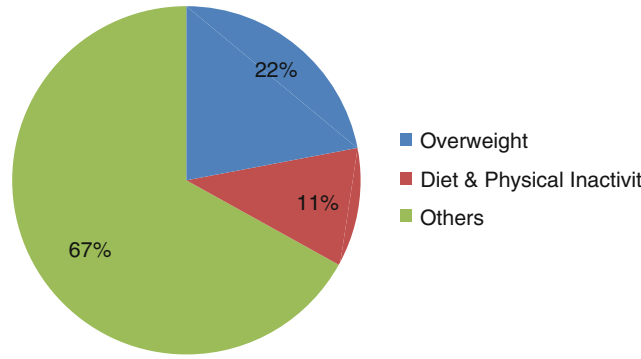

Fig. 22.1 Nongenetic causes for cancer in the US population including overweight, diet, and physical inactivity and others (tobacco, alcohol, environment, infection, medication, sun and radiation, reproductive factors, and family history) (adapted from Wolin et al. [4])

Obesity and Postmenopausal Breast Cancer

Breast cancer is the most frequent malignancy and cause of cancer-related death in women in the US (http://www.cancer.org/research/cancerfactsfigures/cancerfactsfigures/cancer-facts-figures-2013). Postmenopausal breast cancer is much more common than premenopausal breast cancer (441 vs. 75/10,000 women). Almost 70 % of postmenopausal women are overweight or obese in the US [1] and this excess adiposity is estimated to increase their risk of breast cancer by 50–250 % compared to their lean counterparts [5]. A pooled analysis of seven prospective cohort studies conducted in four nations (Sweden, Canada, the Netherlands and four in the US) including 337,819 women and 3,208 incident postmenopausal invasive breast cancer cases found a positive association between BMI and postmenopausal breast cancer (p value for trend = 0.0001) [6]. Women with a BMI above 28 had a relative risk (RR) of 1.26 (95 % CI = 0.34–0.85) compared to those with BMI's ≤ 21. Three years of follow-up data in the Women Health Initiative observational study ($n = 1{,}030$ cases, 84,887 controls) found women that did not take hormone replacement therapy (HRT) with a BMI above 31.1 had 2.5 times (95 % CI = 1.6–3.9) greater risk of developing postmenopausal breast cancer than women with BMI below 22.6 [7]. Similar results between BMI among non-HRT users (RR = 1.6, 95 % CI = 1.1–2.3; p trend ≤ 0.0001) was reported by Nurse's Health Study which had 16 years of follow-up [8]. The Malmo diet and cancer study followed postmenopausal Swedish women for a mean of 5.7 years ($n = 246$ cases; 11,913 controls) also found positive association between BMI and breast cancer (p trend = 0.01) [9].

Waist Circumference, Waist-to-Hip Ratio, and Skinfold Thickness and Risk for Postmenopausal Breast Cancer

The location of adipose tissue is considered a more important predictor of metabolic alterations than total adiposity. Waist circumference (WC) reflects abdominal fat mass and hip circumference largely reflects subcutaneous adipose tissue (SAT). The waist–hip ratio (WHR) is often done in large studies as proxy measures for these body fat distributions. Some, but not all, studies have found participants with larger WC and WHR had greater risks for postmenopausal breast cancer. The Nurses' Health Study reported both WHR (RR = 1.28, 95 % CI = 1.02–1.61) and WC (RR = 1.34, 95 % CI = 1.05–1.72) were

significantly associated with postmenopausal breast cancer after adjusting for other risk factors [8]. This association was much stronger among women not using hormone replacement therapy (WHR RR = 1.85, 95 % CI = 1.25–2.74 and WC RR = 1.88, 95 % CI = 1.25–2.85). The Women's Health Initiative Observational Study reported similar results for WC but not for WHR [7]. A pooled analysis of four cohorts found a 39 % reduction in breast cancer risk among postmenopausal women with lowest WC compared to those with the highest measures, after controlling for important confounders [10]. Pooled relative risk of five cohorts measuring WHR was 0.76 (0.67–0.86) when comparing smallest WHR to the largest [10]. In both these analyses the significant relationship between WC and WHR with breast cancer risk was abolished when adjusted for BMI. While there has been considerable focus on abdominal adiposity, this suggests that total adiposity remains an important cancer risk predictor.

Two case–control studies have used skinfold thickness to assess adiposity risks for postmenopausal breast cancer [11, 12]. Both studies found "android obesity" (i.e., central obesity) had greater risks for breast cancer than "gynoid obesity" (i.e., lower body fat). Schapira et al. [11] assessed biceps, triceps, midaxillary, suprailiac, subscapular, abdomen, and thigh skinfolds using Lange skinfold calipers in 648 women (78 % of participants were postmenopausal). Results from logistic regression models indicated measures of upper body fat deposition (larger WC, triceps, and suprailiac skinfolds and relatively smaller hip circumference and thigh skinfold) were significant predictors of breast cancer. Ballard-Barbash et al. [12] calculated a central adiposity ratio (the sum of chest, subscapular, and abdominal skinfolds divided by the sum of triceps and thigh) in female participants of the Framingham study. These authors also that the distribution of central rather than peripheral body fat predicted breast cancer occurrence.

Dual-Energy-X-ray Absorptiometry and Computed Tomography Scans for Body Fat Measures and Risks for Postmenopausal Breast Cancer

A recent report from the Women's Health Initiative compared anthropometric measures of adiposity (BMI, WC and WHR) to dual-energy-X-ray-absorptiometry (DXA) measures of body fat and their association with breast cancer risk in 10,960 postmenopausal women followed for a median of 12.9 years [13]. All baseline measures of body fat were strongly associated with breast cancer risk. The multivariable-adjusted hazard ratio for the highest versus the lowest quintile levels for DXA measures of trunk fat were 1.82 (95 % CI = 1.34–2.47) and 1.53 (95 % CI = 1.13–2.07) for whole body fat mass. The anthropometric measures were 1.97 (95 % CI = 1.45–2.68) for BMI, 1.97 (95 % CI = 1.46–2.65) for WC and 1.91 for WHR (95 % CI = 1.41–2.58). Similar results were found in both ever and never users of HRT. The positive predictive value for the DXA results was limited to women with estrogen receptor (+) tumors, which is similar to findings reported in a 2009 meta-analysis [14].

The visceral adipose tissue (VAT) is present in the abdominal cavity, mainly in the omentum and the mesentery. It is more innervated, vascular and has greater numbers of inflammatory and immune cells than SAT. VAT is also more strongly correlated with systemic inflammation, insulin resistance, and dyslipidemia [15]. Measurement of VAT and SAT can only be done precisely with computed tomography (CT) or magnetic resonance imaging (MRI) [16]. Both of these techniques are expensive and not practical for large studies. One small case control study ($n = 40$ cases; 40 controls) including both pre and postmenopausal women matched for age, bodyweight, and WC measured areas of VAT, SAT, and total fat at L4 vertebral region using CT scans [17]. They found 45 % ($p = 0.01$) higher VAT in cases than healthy controls and the SAT:VAT area ratio was lower among the cases ($p < 0.0001$). More studies with larger sample sizes are needed to confirm the associations, specifically for postmenopausal women.

Mechanisms Linking Obesity and Postmenopausal Breast Cancer

The established links between obesity and postmenopausal breast cancer are illustrated in Fig. 22.2. These include (1) inflammation, (2) enhanced estrogen bioavailability due to increased estrogen production and decreased SHBG, (3) insulin resistance and IGF-1, and (4) adipocytokines—leptin and adiponectin. Various arms of this diagram depict these links between obesity and breast cancer and also include detailed pathways based on different types of studies (in vitro, in vivo, animal, and human studies). Explanations for each of these pathways are provided.

Inflammation

Obesity is characterized by adipocyte hyperplasia and hypertrophy which leads to high levels of circulating proinflammatory cytokines [18] that promote infiltration of macrophages and T cells and further increase local and systemic cytokines [19]. In obesity adipose tissue is both an endocrine and immune organ that creates a chronic low grade inflammation. The cross-talk between immune cells, macrophages, adipocytes and breast epithelial cell in obesity increase breast cancer risks through promotion of vascularization, endothelial cell proliferation and apoptotic process [20–22].

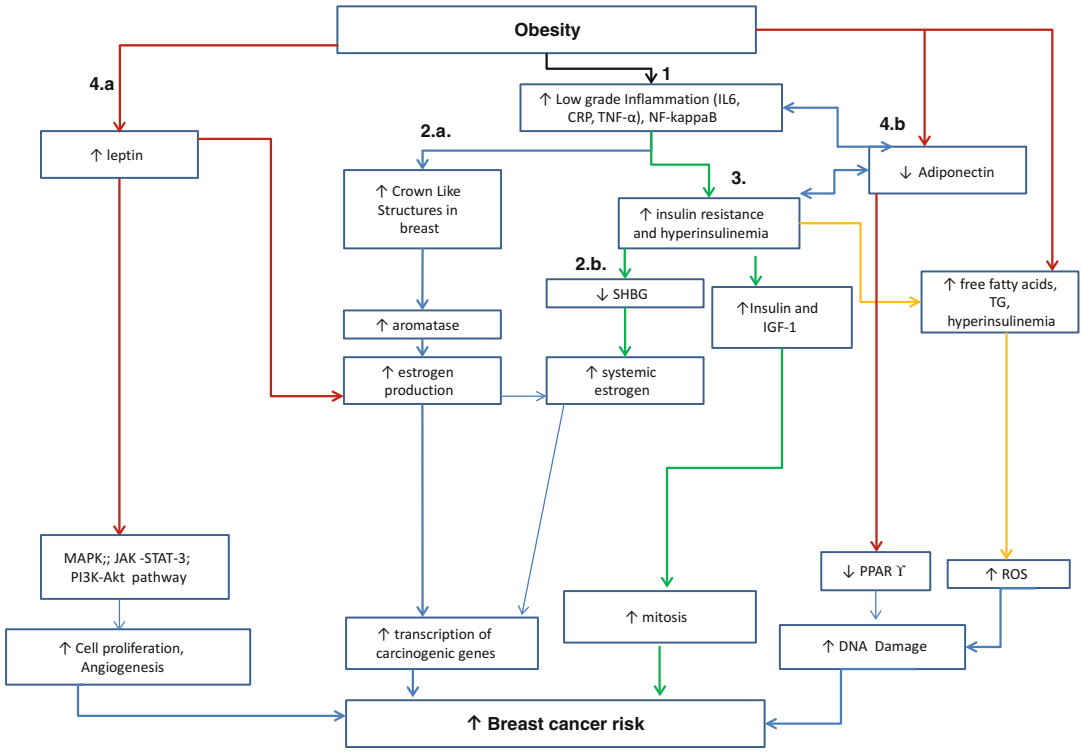

Fig. 22.2 Established mechanistic links between obesity and breast cancer. Pathways: (*1*) Enhanced estrogen synthesis via (*1a*) increased estrogen production and (*1b*) decreased SHBG due to hyperinsulinemia, (*2*) Insulin Resistance and IGF-1 signaling, (*3*) Adipocytokine—Leptin and Adiponectin and (*4*) Inflammatory signaling. *CLS* crown-like structures, *SHBG* sex hormone binding globulin, *ROS* reactive oxygen species alterations

IL-6 and Breast Cancer

One of the pleiotropic cytokines produced by adipose tissue is IL-6 [23] and serum levels are significantly positively correlated with elevated BMI and adiposity [24, 25]. The relationship between IL-6 and breast cancer has been studied in both population and in vitro studies. Women with breast cancer have elevated systemic IL-6 compared to healthy women that is associated with the extent of tumor invasion and TNM disease staging [26, 27]. Gonollu et al. [28] reported elevated serum IL-6 in obese and overweight postmenopausal women with early stage breast cancer compared to healthy BMI-matched controls. Further, IL-6 levels at diagnosis in women with metastatic breast cancer independently predicted recurrence and worse prognosis [29, 30], as well as worse therapeutic success [31, 32].

Associations between tumor tissue IL-6 levels and outcomes are inconsistent. Some studies have found elevated tissue IL-6 was a negative prognosticator for the disease (i.e., more expression in the advance tumors than the in-situ) [33, 34] while others found the opposite relationship [35, 36]. IL-6 works in paracrine and/or autocrine manner to alter the functions of its target cells. Overexpression of IL-6 in MCF-7 cells (breast cancer cell line) induced epithelial-mesenchymal transition as well as increased invasiveness [37]. Another study showed that adding IL-6 and estrogen sulfate simultaneously to breast cancer cell line enhanced cell proliferation [38]. Shen et al. [39] reported IL-6 inhibited molecules used by growth factor receptors to promote cell division signifying a protective effect of IL-6 at tissue level. These findings suggest within the tissue level IL-6 may act as a double edged sword for breast carcinogenesis and other factors may determine its action.

IL-6 may also promote breast tumor growth in obesity through its influence on *insulin resistance* as well as *estrogen synthesis*. When IL-6 binds to its receptor, it activates the STAT3-SOCS(3) pathway that is responsible for inducing insulin resistance [40]. Local IL-6 production in normal and malignant breast tissue has also been linked with endogenous estrogen production via its activation of aromatase, the key enzyme responsible for estrogen synthesis [41].

Local Breast Tissue Inflammation in Obesity

Healthy breast tissue of obese women has greater local inflammation and macrophage infiltration compared to normal breast tissue [42]. Breast tissue lesions in mammary adipose tissue of obese and overweight women had necrotic adipocytes rimmed by macrophages known as crown like structures (CLS) [43]. Such CLS represent inflammatory foci and activate nuclear factor κβ (NF-κβ). Breast tissue from obese women have increased local inflammation which is associated with elevated estrogen synthesis compared to nonobese women [44].

Systemic Estrogen and Breast Cancer Risk

Estrogen is a well-established risk factor for postmenopausal breast cancer. A nested case control study within European Prospective Investigation into Cancer and Nutrition (EPIC) study analyzed 677 postmenopausal breast cancer cases and 1,309 age-matched controls. Women in the highest quintile of free estradiol (active estrogen) levels had twice the risk of developing breast cancer compared to those in the lowest quintile [45]. Similar results were reported in a pooled analysis of nine prospective studies assessing the effect of endogenous estrogen on breast cancer risk in postmenopausal women ($n = 663$ cases and 1,765 controls) [46]. Of these nine studies, six were conducted in the US and the remaining three were in the UK, Italy, and Japan. The relative risk (RR) for breast cancer in highest quintile of estradiol levels was 2.6 (95 % CI = 1.8–3.9) compared to those in lowest quintile

and there was a significant dose response. A nested case control of postmenopausal participants in the New York University Women's Health Study ($N=297$ cases and 563 controls) collected blood samples at baseline and within 5 years following cancer diagnosis [47]. A significant association between elevated estrogen at baseline and breast cancer risk were found. Follow-up levels confirmed the baseline levels reflected pre-disease rather than tumor related hormone production. Postmenopausal participants ($N=418$ cases and 817 controls) with elevated baseline serum estrogen levels in the Nurse's Health Study Cohort also had greater risks for breast cancer which was independent of genetic and epigenetic factors included in other risk prediction models (Gail scores, Rosners models) [48].

Obesity Induced Estrogen Synthesis and Breast Cancer

Ovarian estrogen production decreases after menopause, however because excess adipose tissue continues to act as a primary source of estrogen, levels decline less in obese than in lean women [49]. Excess adiposity also decreases hepatic production of sex hormone binding protein (SHBG) [50, 51]. This hormone has high affinity for estrogen; thus, conditions that lower its production increase unbound estrogen levels. Cross sectional data of postmenopausal women ($n=267$) randomly selected from Women's Health Initiative Dietary Modification Trial found BMI was positively associated with estrogen and negatively associated with SHBG [52]. These results were corroborated in healthy women participating in the EPIC study ($n=1{,}217$ postmenopausal women) [53]. Linear regression in a subset of postmenopausal women ($n=456$) in the EPIC study demonstrated BMI was positively related with estradiol (active estrogen) and negatively related with SHBG in a dose response manner. Alcohol consumption, smoking, family history and reproductive history (all risk factors for breast cancer) did not diminish this finding [54]. A nested case control of all postmenopausal women included in the EPIC study ($n=613$ cases; 1,139 controls) round the elevated risk for breast cancer was diminished significantly after adjusting for elevated estrogen levels and decreased SHBG levels [55]. The pooled analysis of nine cohort studies ($n=624$ cases; 1,669 controls) found the increased risk for postmenopausal breast cancer associated with greater BMI was largely due to an increase in the bioavailable estradiol rather than any other hormone measurements [56].

Estrogen Synthesis in Normal and Malignant Breast Tissue

There are three main enzymes involved in synthesis of estrogen in peripheral tissue: (a) Aromatase, the key enzyme responsible for conversion of androgens into estrone; (b) Estrone sulfatase that catalyzes estrone from estrone sulfate; and (c) estradiol-17-β-hydroxysteroid dehydrogenase (17β-HSD) which is responsible for conversion of estrone to active estrogen, estradiol [57]. Presence of these three enzymes has been reported in both malignant as well as normal breast tissues [58]. In normal breast tissue aromatase expression is regulated primarily by IL-6 and TNF-α, whereas in malignant breast tissue prostaglandin E2 (PGE2) is the regulator. Cytokines such as IL-6 and TNF-α also enhance 17β-HSD and E1-STS [59]. Various animal and human studies support mammary adipose tissue as the primary site for aromatase expression and estrogen production [44, 60]. Morris et al. reported that breast tissue of obese women had inflammatory mediators that activate cyclooxygenase (COX)-2 derived Prostaglandin E2 (PGE2) which stimulates the cyclic AMP/ PKA signal transduction pathway that activates aromatase [43]. The paracrine interaction between estrogen produced by adipose tissue and breast epithelial cells have been hypothesized to predispose women to breast hyperplasia and cancer.

Leptin and Adiponectin and Postmenopausal Breast Cancer

Leptin and adiponectin are adipokine hormones synthesized and secreted by adipocytes and have been recently studied for their influence on breast cancer. Biological activities of leptin and adiponectin and their effect on breast neoplastic cells are largely opposite to each other [61]. Plasma leptin levels increases, whereas adiponectin decreases proportionally to BMI [62]. Tessitore et al. reported that women with breast cancer have higher plasma leptin and expression of leptin mRNA in adipose tissue compared to healthy subjects [63]. A large prospective study of Swedish women ($n=561$ cases; 561 controls) found baseline BMI was positively correlated with leptin ($r=0.72$, $p=<0.01$) and negatively correlated with adiponectin ($r=-0.23$, $p=<0.01$) [64]. A nonsignificant positive association for baseline leptin levels in advanced breast cancer cases was found after 8-years of follow-up; no association with adiponectin occurred. A nested case control within National Surgical Adjuvant Breast and Bowel Protocol-P1 reported strong positive correlation between BMI and leptin in women at high risk for breast cancer based on Gail score ($n=231$ cases; 856 controls) [65]. After adjusting for baseline BMI, leptin was no longer associated with breast cancer development (crude OR for leptin = 1.3, $p=0.52$; adjusted OR = 1.09, $p=0.7$). Unfortunately results were not reported separately for postmenopausal women in either of these studies. Very recently a nested case control within the Multiethnic Cohort (MEC) study has analyzed the effect of serum leptin and adiponectin concentration on breast cancer risk in postmenopausal women ($n=706$ cases; 706 controls) [66]. Women in the highest quartile for baseline circulating leptin had almost twice the risk of developing breast cancer (OR = 1.9; 1.4–2.7) as women in the lowest quartile. The leptin–adiponectin ratio was also significantly associated with breast cancer risk (OR = 1.9; 1.4–2.7). No association was detected for adiponectin. The associations of leptin and the leptin–adiponectin ratio were dose dependent and remained significant after adjusting for BMI. Three case control studies reported similar findings supporting the leptin–breast cancer link [67–69]. Two out of these three studies also analyzed serum adiponectin and found a significant negative association with breast cancer risk [67, 69]. Miyoshi et al. reported women in lowest vs. highest tertile of serum adiponectin had almost four times the risk of developing breast cancer (OR = 3.9; 1.2–12.3) [70]. Similar inverse associations were reported among postmenopausal women independent of other breast cancer risk factors (BMI, leptin, IGF-1, sociodemographic variables) [62]. A nested case control study of women in the Nurses Health Study assessed the adiponectin-breast cancer risk in women with and without a history of hormone replacement therapy [71]. A significant negative association was found (RR = 0.73, 95 % CI = 0.55–0.98; p trend = 0.08) between the highest to the lowest quartile of adiponectin in women who never used hormone replacement.

Both leptin and adiponectin have been associated with poor prognosis of breast cancer. BMI and leptin were significantly associated with pathological tumor size and TNM stage in women with postmenopausal breast cancer ($n=98$) [72]. A case control study in 130 women ($n=80$ breast cancer cases; 50 controls) reported high serum leptin levels and low adiponectin levels were independent risk factors for cancer metastasis [69].

Mechanisms of Leptin and Adiponectin in Breast Cancer

Both endocrine and paracrine actions of leptin are responsible for inducing breast carcinogenesis. Invasive breast tumors have higher expression of leptin and leptin receptors compared to healthy mammary tissues [73]. Genetically obese leptin-deficient (MMTV-TGF-α/Ob-Ob-) female mice do not develop mammary tumors [74] supporting a significant role for leptin in obesity induced mammary carcinogenesis. Various in vitro experiments demonstrated that leptin influences secondary intracellular messengers (STAT3; PI3K/Akt; ERK2; MAPK) which are involved in cell proliferation,

differentiation, and survival. These messengers activated via leptin are also involved in cell migration and angiogenesis (as activates Vascular Endothelial Growth Factor (VEGF) gene transcription) [75].

Elevated leptin has been linked with increased estrogen production. Higher leptin levels with increase plasma estradiol were found in a cross sectional study of postmenopausal women ($n=87$) with gynecological and breast cancer [76]. Serum leptin levels were also positively associated with increased expression of estrogen and progesterone receptors on the breast tumors. Leptin amplifies estrogen signaling by increasing aromatase gene expression [77]. Breast cancer cell lines when exposed to estrogen and/or leptin have much larger tumors than when exposed to estrogen alone [78]. Increased leptin production in breast tissue also results in tissue acquired macrophages (TAM) accumulation and enhanced expression of cadherin-1, an adhesive molecule implicated in cell proliferation and survival [78, 79].

Adiponectin's protective effect on breast cancer occurs via activation of the PPAR-ϒ metabolic pathways that enhance insulin sensitivity and DNA repair mechanisms. Generally adiponectin biosynthesis is inhibited with increasing adiposity. Thus, low adiponectin results in decreased PPAR-ϒ signaling with low BRCA1 at nuclear level which impairs DNA repair and increases breast cancer risk [80]. Adiponectin also inhibits proliferation of several cell types and negatively regulates angiogenesis [81]. Obese women with low adiponectin levels may have elevated breast cancer risk with aggressive phenotype and enhanced neoangiogenesis. Although most studies appear to indicate the role for low adiponectin in breast cancer risk in conjunction with high leptin levels, more work is needed in this area.

Postmenopausal Breast Cancer Summary

A strong, consistent association between obesity and increased risk for postmenopausal breast cancer has been reported. Centrally located fat and VAT are more predictive of risk than peripheral or subcutaneous fat. Although BMI and WC are less precise measures of adiposity, risk predictions based on these parameters are similar to those obtained from DXA measurements. Thus, while DXA and CT scans provide mechanistic insights for the influence of adiposity they are not required for detecting the increased risks from obesity for postmenopausal breast cancer.

The mechanistic summaries presented are brief overviews for each of the individual risk factors. These factors are highly interrelated as demonstrated by the interconnecting arrows in Fig. 22.2. Thus, therapies/treatments that alter one pathway will ultimately influence others. Research is needed to disentangle and clarify the exact mechanisms of obesity and postmenopausal breast cancer.

Obesity and Colon Cancer

Colorectal Cancer (CRC) is third in incidence and mortality of all cancers in the US [82]. Worldwide incidence rates parallel economic development and risk factors associated with obesity and Western lifestyles (e.g., physical inactivity, excessive dietary fat consumption, and disproportionate energy intakes.) [83–85] Other known risk factors for CRC include older age, male gender, family history of CRC, history of colorectal polyps, inflammatory bowel disease, type 2 diabetes, excessive alcohol use, smoking, and diets high in red and processed meats [86]. The lifetime risk of having a diagnosis of CRC is 5 % in the US [86]. Overall, CRC mortality rates began declining in the 1980s for men and 1950s for women, possibly as a result of screening initiatives focused on earlier detection and removal of precancerous polyps [86, 87]. Recently, incidence rates have been increasing in those younger than 50 years of age, possibly reflecting the impact of obesity on CRC risk. Data from 2003 to 2007 from the North American Association of Central Cancer Registries and National Center for

Health Statistics indicate African American (AA) men compared to Caucasian men have the highest age-adjusted incidence rate (68.3/100,00 vs. 56.8/100,000) and age-adjusted mortality rate (30.5/100,000 vs. 20.9/100,000) for CRC [86]. Additionally, AA are younger and have more advanced CRC stage at diagnosis than Caucasians [88].

Obesity and CRC Risk

Colorectal cancer is a slowly progressing disease that develops over 10–15 years and is characterized by the accumulation of mutations that arise from hereditary causes and/or spontaneous mutations of genes controlling cell proliferation, differentiation, apoptosis, and DNA repair in the colonic mucosa [89]. Only a small fraction of CRC results from hereditary causes, the approximately 90 % are sporadic and nonhereditary [85, 90].

For over 20 years, obesity has been identified as a significant risk factor for CRC [91]. Large prospective cohort studies have consistently demonstrated positive associations between obesity and CRC [3, 92–95] exhibiting a dose–response relationship with BMI, WC and WHR [93, 96]. Campbell et al. reported a 5 kg/m^2 increase in BMI increments was positively associated with greater odds in women (OR = 1.20; 95 % CI = 1.10–1.32) and men (OR = 1.24; 95 % CI = 1.15–1.34) compared to sex-matched siblings [97]. Findings from a meta-analysis of 31 studies concluded that a 5-unit increase in BMI (men RR = 1.30 95 % CI = 1.25–1.35 and women RR = 1.12 95 % CI = 1.07–1.18) and a 10 cm increase in waist circumference (men RR = 1.33; 95 % CI = 1.19–1.49 and women RR = 1.16; 95 % CI = 1.09–1.2) significantly increases CRC risk in both genders [98]. A nationally representative US sample (Cancer Prevention Study II, 1982–1998) found the RR for CRC in women with a BMI ≥ 40 was 1.46 (95 % CI = 0.94–2.24) and in men with a BMI 35–39.9 was 1.84 (1.39–2.41) compared to those with a normal BMI (18.5–24.9) [3]. A large prospective Norwegian study found increased risk of CRC in men with a BMI ≥ 25 and a ≥10 kg weight gain from baseline; no association was found in postmenopausal women [99]. Women have increased risk of CRC when high BMI is accompanied by low physical activity [100].

Central adiposity is associated with increased morbidity and mortality in CRC [101, 102]. The higher risks in men are thought to reflect their greater abdominal circumference [103]. Risks for CRC increase by 33 % per 10 cm increase in WC and 43 % per 0.1 unit increase in WHR [98]. Risks for proximal and distal colon cancers have been observed with increasing WC in both genders [103]. WC has been shown to significantly and independently predict the presence of diabetes (HR 1.56) and hypertension (HR 1.7) in patients with CRC [16] and has been associated with colorectal adenomas in men but not in women [104].

Abdominal Adipose Depots and CRC

Three studies (predominantly international investigators) have explored the influence of abdominal adipose depots (SAT and VAT) on risk for CRC [105–107] and five examined the relationship with adenoma risks [104, 108–111]. Erarslan et al. [105] found no difference in VAT area in Turkish adults with CRC ($n = 23$) compared to controls ($n = 50$), however BMI and adiponectin were significantly lower in cases. They speculated this may have reflected weight loss prior to CRC diagnosis. Significantly greater VAT area has been reported in Japanese adults with CRC ($n = 22$), however SAT, insulin resistance, total fat and BMI were similar to controls ($n = 66$) [106]. Kang et al. found every 10 cm increase in VAT area was an independent risk factor (OR 3.09, 95 % CI = 2.19–4.36) for adenomas after adjusting for smoking, alcohol consumption, family history of CRC, use of aspirin or

nonsteroidal anti-inflammatory drugs (NSAIDS) [108]. Most, but not all, studies have shown VAT is a significant predictor for colorectal adenoma risk. Japanese adults with colorectal adenomas had more VAT than controls [110], independent of BMI [109]. In contrast Sass et al. did not find differences in VAT area in adults with adenomas compared to controls [111].

Obesity Prognosis and Survival

Obese adults with CRC have worse prognosis and lower survival rates than normal weight counterparts [112, 113]. Analysis of a large nationally representative sample (NHANES 1971–1975) determined that mortality from CRC was 0.39, 0.68, and 0.96/1,000 person-years for normal weight (BMI = 18.5–24.9), overweight (BMI = 25–29.9), and obese (≥30) respectively (p value for log-rank trend test < 0.001) [112]. Postmenopausal obese women with CRC have increased risk of CRC death compared to normal weight postmenopausal women, independent of hormone use; however, the impact of physical activity and other preexisting comorbidities were not evaluated [114]. It has been observed that very lean patients (lowest BMI quartile) or obese patients (highest BMI quartiles) of both genders present with advanced stage cancer and lymph node involvement contributing to worse prognoses compared to patients between these two quartiles [115, 116]. Obese patients with CRC have significantly longer surgeries, higher blood loss, and a trend towards more infections than non-obese CRC patients [117]. Significant linear trends of age-adjusted death rates from CRC in both genders occur with increasing BMI [3, 118].

Mechanisms Linking Obesity and CRC

The majority of colon adenocarcinomas develop from adenomas or polyps. The well accepted model by Vogelstein [119] for the development of CRC from normal mucosa to premalignant adenomas involves several key genes: the *APC* gene, a tumor suppressor gene, is one of the first genes that may become mutated and is usually expressed in 80 % of adenomas and adenocarcinomas [120, 121]; the *K-ras* gene, an oncogene responsible for cell proliferation is also mutated in CRC; and *p53* gene, a tumor suppressor gene associated with apoptosis, is believed to be the gene mutation responsible for the conversion of an adenoma to adenocarcinoma [121]. This transformation is a long-term process characterized by an accumulation of mutations that disrupt the normal processes of cell proliferation, differentiation, apoptosis, and DNA repair. The reason for the loss of control is unclear; however, as depicted in Fig. 22.3 chronic low-grade inflammation, alterations in insulin sensitivity, insulin-like growth factor (IGF), leptin, and adiponectin that accompany obesity have been hypothesized to play central roles [89]. Chronic inflammation stimulates CRC initiation and promotion by the production of the pro-inflammatory mediators TNF-α, IL-6, transcription factors (NFκB, STAT3) [122] and upregulating oncogenes (e.g., *COX-2, Mdm*). The PI3K/Akt (phosphotidylinositol-3-kinase/protein kinase B) signaling pathway is a central mechanistic pathway linking the obesity-related low-grade inflammation associated with CRC [123, 124].

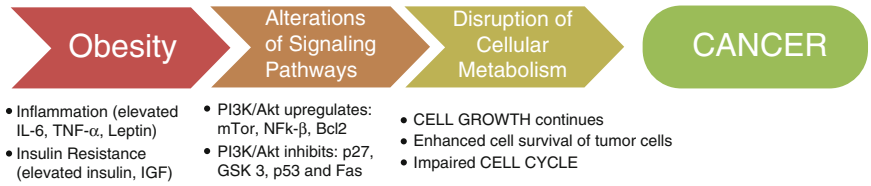

Fig. 22.3 Postulated role of obesity in CRC

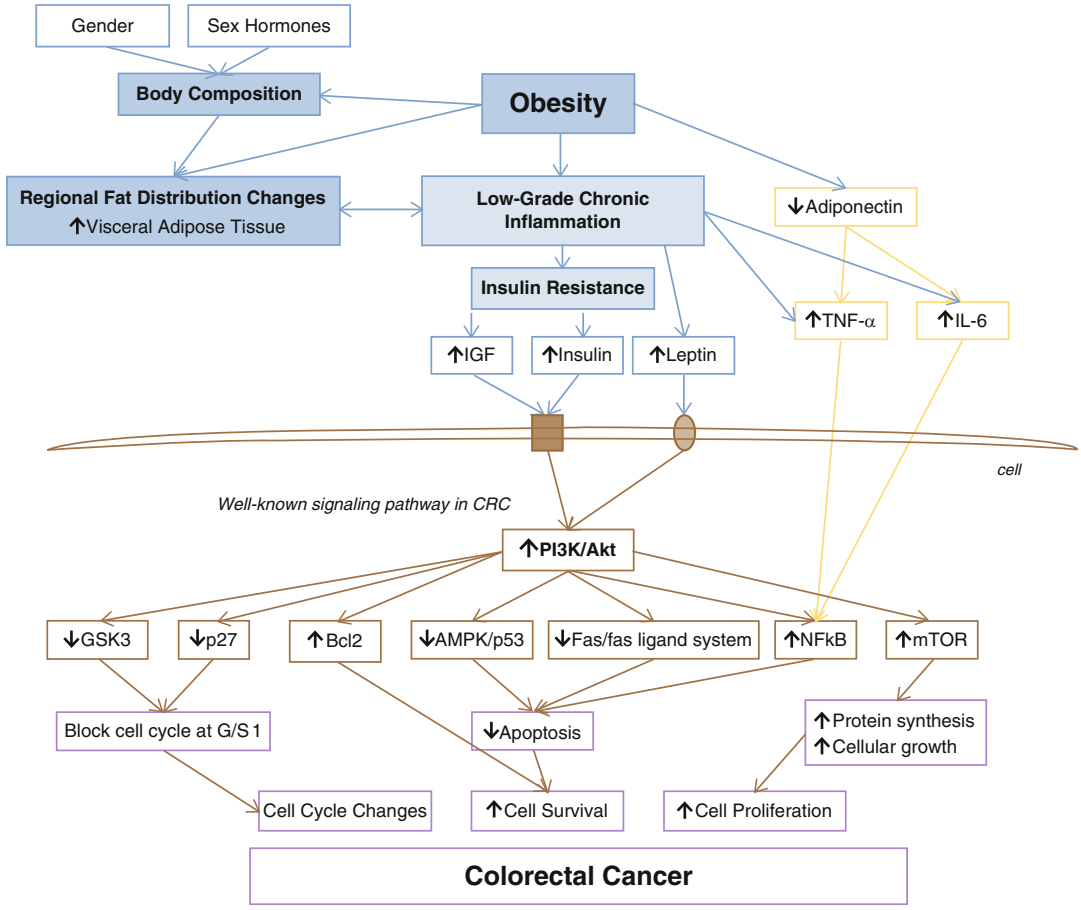

Fig. 22.4 Overview of signaling pathways leading to CRC involving PI3K/Akt. Adapted from Huang, X.F. and J.Z. Chen, *Obesity, the PI3K/Akt signal pathway and colon cancer.* Obesity Reviews: an official journal of the International Association for the Study of Obesity, 2009. **10**(6): p. 610–616

Inflammation and CRC

Alterations in transduction pathways intrinsically involved in colorectal carcinogenesis, specifically cell survival, cell cycle, cell growth, angiogenesis, and metastasis occur with obesity (Fig. 22.4) [122–126] Adipocyte production of adiponectin is down regulated in obesity which impairs suppression of TNF-α and IL-6. Elevated levels of these cytokines coupled with the obesity-induced increased concentrations of insulin, insulin like growth factor (IGF), and leptin activates the PI3K/Akt pathway which blocks pro-apoptosis proteins, p53 (a tumor suppressor gene) and Fas (fas/fas-ligand system) and activates cellular transcription factor, NFkB (nuclear factor kappa B) and Bcl2 (B-cell lymphoma 2) decreasing cellular apoptosis and thus initiating downstream cell survival cascade (Fig. 22.4). Similarly, PI3K/Akt deactivates p27 (a cyclin-dependent kinase inhibitor which blocks cell cycle at G1/S transition) and GSK3 (glycogen synthase kinase-3) leading to changes in the cell cycle and cell proliferation. Finally, PI3K/Akt stimulates the mTOR (mammalian target of rapamycin) sequence which promotes protein synthesis and cellular growth.

Known regulators of inflammation signaling pathways of tumor progression and metastases include (a) inhibitor of kappa B kinase (IKK)/NFkB, (b) signal transducer and activator of transcription 3 (STAT3) [127], and (c) cyclooxygenase-2/prostaglandin E_2 (COX-2/PGE2) [128].

NFkβ regulates the expression of genes involved with invasion, angiogenesis, and metastasis including vascular endothelial growth factor (*VEGF*) [126] after it is released from the IKK complex as a result of activation by TNF-α and IL-1 (interleukin-1, another pro-inflammatory cytokine) [127]. VEGF is a cytokine necessary for epithelial cell growth and angiogenesis [124] and is associated in CRC with poor prognosis, metastases and relapse [124, 126]. STAT3 signaling cascade is triggered by binding of many factors such as IL-6, IL-11 (interleukin-11, pro-inflammatory cytokine), VEGF, epidermal growth factor (EGF), and IGF to cellular receptors activating the Janus kinases (JAK) or similar tyrosine kinases phosphorylating the STAT proteins and allowing their translocation into the nucleus [127, 129]. Specifically, TNF-α and IL-6 down regulate adiponectin and bind to receptors on colonic tissue activating the Jak and Akt enzymes [122, 130]. The Jak enzyme phosphorylates STAT3 allowing it to translocate into the nucleus [131]. The Akt enzyme phosphorylates and degrades the cytoplasmic IκB complex (an inhibitor of NFκB) and liberates NFkB allowing it to enter the nucleus where it reduces apoptosis and mediates tumor enhancing processes (cell proliferation, transformation, metastasis, invasion, and angiogenesis) [125, 130, 131]. When activated STAT3 translational programming up-regulates the expression of a host of pro-inflammatory genes (*COX-2, IL-6, BCL2*) further driving a cancer promoting inflammatory environment and blocks anti-tumor immune responses [129]. Excessive *COX-2* gene expression leads to enhanced secretion of PGE-2, the main metabolic product of COX-2, producing a pro-oxidative environment that further causes DNA damage or DNA repair system malfunction [124, 128]. *COX-2* overexpression in tumor cells is related to poor prognosis and lymph node metastasis in CRC patients [126].

Insulin Resistance, CRC, and Adenomas

Elevated homeostasis model assessment insulin resistance (HOMA-IR) is a strong independent risk factor of CRC [132, 133] and adenomas [108]. Limburg et al. reported age-adjusted risk models comparing highest versus lowest quartiles for insulin (HR, 1.84; 95 % CI = 1.03–3.30) and HOMA-IR (HR, 1.85; 95 % CI = 1.06–3.24) were associated with increased CRC risk [133]. One small study of Scandinavian patients with CRC compared to patients with adenoma or without CRC patients ($n = 40$/group) found no association between CRC risk and HOMA-IR [134]. Otake et al. reported HOMA-IR was directly associated with colorectal adenomas in patients with ($n = 51$) and without adenomas ($n = 52$) matched for BMI and oral glucose tolerance status [110]. A gender and age-matched case control study in Korean adults ($n = 3{,}585$) with and without adenomas found fasting glucose, insulin, HOMA-IR and triglycerides were significantly higher in those with adenomas [108]. Fasting glucose was significantly higher in patients with adenomas in a smaller study ($n = 200$); however, significance was lost in multivariate analyses [107]. A large prospective study ($n = 1{,}093$) found participants in the top quartiles for HOMA-IR were 63 % more likely (OR 1.63; 95 % CI = 1.09–4.22) to have adenomas compared to those in lowest quartile, however when stratified by gender, the relationship remained statistically significant only for men [135].

Obesity, IGF, and CRC

Recent animal and human studies have provided insights on the mechanisms involving insulin, IGF-1 and insulin-growth-factor 2 (IGF-2) in CRC [136]. IGF is involved in the growth and maintenance of tissues. Under normal conditions, insulin-like growth factor binding proteins 1 and 2 (IGFBPs 1, 2) bind IGF and inactivate its effect [137]. Obesity induced adipocyte hypertrophy stimulates inflammatory adipokines and insulin resistance altering expression of insulin receptors and of intracellular insulin signaling pathways [136–139]. Elevated insulin levels lead to increased IGF-1 and IGF-2 levels and downregulation of IGFBP-1 and IGFBP-2 proteins leading to increased

bioavailability of IGF [118, 140, 141]. Overexpression of IGF alters downstream metabolic pathways involved in proliferation, apoptosis, angiogenesis, cell adhesion, migration and wound healing [137]. Evidence from human and animal studies confirms that high levels of insulin and IGF acting via the insulin-IGF axis promote CRC [137].

Adiponectin, Obesity, and CRC

Adiponectin receptors are expressed on many cancer cell types, and patients with CRC have lower levels of adiponectin compared to controls [142]. Additionally, colonic tumors have higher expression of adiponectin receptors than non-involved tissue in patients with CRC [143, 144]. In obesity adiponectin is down-regulated, possibly by TNF-α [145]. Normally adiponectin activates the AMPK/mTOR pathway suppressing cell growth and proliferation by inhibiting the production of enzymes needed in protein regulation (mTOR) [146] and reducing the expression of a major transcriptional regulator, sterol regulatory element binding protein (SREBP) [147]. These changes suppress CRC cell growth and may also inhibit other tumor growth inhibitor enzymes downstream of AMPK [146, 147]. Adiponectin also upregulates p53 and p21, important proteins involved in growth arrest and apoptosis [147].

Estrogen and CRC

Some studies have found estrogen is protective for CRC in females taking hormone replacement therapy or oral contraceptives [148, 149]. In contrast a recent study from the Women's Health Initiative group did not find reduced CRC risks in women taking estrogen and progestin combined therapy [150]. A meta-analysis evaluating estrogen therapy (ET) or estrogen + progestin therapy (EPT) on CRC risk reported a decreased risk for ever users of ET(RR 0.79, 95 % CI = 0.69–0.91) or EPT (RR 0.74, 95 % CI = 0.68–0.81) [151].

Estrogen binds two separate receptors that act as antagonists: estrogen receptor-α (ER-α) and estrogen receptor-β (ER-β) [152]. Within the colonic mucosa only ER-β is expressed [152, 153] [154] and thought to be important in maintaining its health [155]. Under normal conditions ER-β expression increases growth, cell regeneration, repair of damaged DNA and apoptosis, inhibits cell proliferation and microsatellite instability, downregulates IL-6, improves insulin sensitivity, and regulates body fat distribution [153, 154, 156–158]. Human and animals studies demonstrate that the development of CRC reduces expression of ER-β and increases that of ER-α, leading to cellular proliferation and decreases differentiation [152, 153, 157]. Reduced expression of ER-β in CRC cells is associated with advanced CRC and poor prognosis [153, 157].

Circulating estrogen levels increase as BMI increases [153, 159]. Data from the Nurses' Health Study, the Women's Health Study, the Health Professionals study, and the Physician's Study II was used to assess the relationship between estradiol, testosterone, and estradiol–testosterone ratio in CRC cases and controls. Men with higher levels of total testosterone and estradiol–testosterone ratio were associated with decreased CRC risk which remained after adjusting for various factors (BMI, age at blood draw, smoking, current alcohol use, and family history) [160]. Only the estradiol–testosterone ratio was associated with an inverse relationship for CRC in women.

Testosterone and CRC

A few studies have explored the association between CRC, obesity and testosterone in men. Available evidence indicates circulating testosterone decreases with increasing adiposity (visceral obesity)

[161–163] and higher levels of testosterone are associated with decreased CRC risk in men with similar BMI after multi-variable adjustment (age at blood draw, fasting status, smoking etc.) [160]. Testosterone injections in middle-aged obese men improved insulin resistance, and this has been proposed as one mechanism for its influence [164].

Conclusions and Summary

We are amid an obesity epidemic and a concurrent rise in obesity-associated diseases, particularly cardiovascular disease and diabetes. The definitive role of obesity in cancer development and recurrence has yet to be determined; however, the majority of men with colon cancer and women with breast cancer are overweight or obese at the time of diagnosis. This chapter highlights epidemiologic studies showing the associations between cancer risk and obesity, with a specific focus on BMI, WC, and WHR. We propose several current mechanisms between carcinogenesis and obesity. Specifically, the summary models focus on adverse abnormalities in inflammation, adipokines, and hormones, all of which are initiated by obesity, predominantly abdominal obesity. While these mechanisms are far from understood and science is constantly progressing, weight control and weight loss are the undisputed cornerstones of cancer prevention. Future studies should explore and prioritize body composition methodologies to help us more precisely elucidate how specific adipose depots impact occurrence, recurrence, prognosis, and overall mortality.

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