

Rexford S. Ahima *Editor*

# Metabolic Basis of Obesity



Springer

# Metabolic Basis of Obesity



Rexford S. Ahima  
Editor

# Metabolic Basis of Obesity

 Springer

*Editor*

Rexford S. Ahima  
Division of Endocrinology,  
Diabetes and Metabolism,  
and the Institute for Diabetes,  
Obesity and Metabolism,  
University of Pennsylvania  
School of Medicine,  
415 Curie Boulevard,  
712A Clinical Research Building,  
Philadelphia, PA 19104,  
USA  
ahima@mail.med.upenn.edu

ISBN 978-1-4419-1606-8 e-ISBN 978-1-4419-1607-5

DOI 10.1007/978-1-4419-1607-5

Springer New York Dordrecht Heidelberg London

Library of Congress Control Number: 2010937648

© Springer Science+Business Media, LLC 2011

All rights reserved. This work may not be translated or copied in whole or in part without the written permission of the publisher (Springer Science+Business Media, LLC, 233 Spring Street, New York, NY 10013, USA), except for brief excerpts in connection with reviews or scholarly analysis. Use in connection with any form of information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed is forbidden.

The use in this publication of trade names, trademarks, service marks, and similar terms, even if they are not identified as such, is not to be taken as an expression of opinion as to whether or not they are subject to proprietary rights.

Printed on acid-free paper

Springer is part of Springer Science+Business Media ([www.springer.com](http://www.springer.com))

*This book is dedicated to my family, mentors, colleagues and trainees, who have all contributed to my professional and scientific development. I am especially thankful to Grace, FAS, OH, Dedaa, Osei, Opare and Patrick, for making this and other endeavors worthwhile.*



# Preface

Overweight, defined as body-mass index (BMI) greater than 25, and obesity, defined as BMI greater than 30, are characterized by excessive fat accumulation which poses adverse risks to health. Worldwide, there are more than one billion overweight and 300 million obese adults. Obesity is a major risk factor for diabetes, cardiovascular disease, sleep apnea, non-alcoholic fatty liver disease, arthritis, cancer and other diseases, and has been associated with enormous health costs, premature death, reduction in the quality of life and disability. The increasing global incidence of child obesity is also a major concern. The obesity epidemic is not just a problem for industrialized societies. Indeed, obesity rates have risen three times or more over the past two decades in some developing countries. Although the growing obesity epidemic undoubtedly reflects profound changes in diet and lifestyle over recent decades, genetic factors are important in determining a person's susceptibility to weight gain and adverse health consequences of obesity.

The goal of this book is to highlight the pathophysiology of obesity and associated diseases. While a completely comprehensive discussion of the metabolic basis of obesity is beyond the scope of this book, we present in-depth reviews of a wide range of topics, including energy homeostasis and intermediary metabolism, adipocyte biology, central neuronal pathways, adipokines, cytokines, classical hormones, abnormal glucose and lipid metabolism, and dysregulation of major organs, with an emphasis on human obesity. We believe these topics will be interesting and provide critical information on the metabolic basis of obesity to researchers, clinicians, students, and the public at large.

Philadelphia, Pennsylvania, USA

Rexford S. Ahima





# Contents

<b>1 Principles of Human Energy Metabolism.....</b>	<b>1</b>
Jose Galgani and Eric Ravussin	
<b>2 Intermediary Metabolism of Carbohydrate, Protein, and Fat.....</b>	<b>25</b>
Keith Tornheim and Neil B. Ruderman	
<b>3 Adipose Tissue Development, Structure and Function .....</b>	<b>53</b>
Jaswinder K. Sethi and Antonio J. Vidal-Puig	
<b>4 Adipokines in Health and Disease .....</b>	<b>69</b>
Rexford S. Ahima and Marcus D. Goncalves	
<b>5 Neural Control of Feeding and Energy Homeostasis.....</b>	<b>89</b>
Emilie Caron and Rexford S. Ahima	
<b>6 Gastrointestinal Hormones and Obesity.....</b>	<b>109</b>
Yan Wang and Efi Kokkotou	
<b>7 Genes and Human Obesity.....</b>	<b>127</b>
R. Arlen Price	
<b>8 Classical Hormones Linked to Obesity .....</b>	<b>139</b>
Hyeong-Kyu Park and Rexford S. Ahima	
<b>9 Inflammation and Adipose Dysfunction .....</b>	<b>155</b>
Rachana Shah and Muredach P. Reilly	
<b>10 Insulin Resistance in the Metabolic Syndrome .....</b>	<b>175</b>
Sudha B. Biddinger and Brice Emanuelli	

<b>11</b>	<b>Pancreatic Islet <math>\beta</math>-Cell Failure in Obesity .....</b>	<b>199</b>
	Tomoaki Morioka and Rohit N. Kulkarni	
<b>12</b>	<b>Non-Alcoholic Fatty Liver Disease and the Metabolic Syndrome .....</b>	<b>219</b>
	Sonia M. Najjar	
<b>13</b>	<b>Sleep, Circadian Rhythms and Metabolism .....</b>	<b>229</b>
	Eleonore Maury, Kathryn Moynihan Ramsey, and Joseph Bass	
<b>14</b>	<b>Obesity and Cardiac Dysfunction.....</b>	<b>257</b>
	Gary Sweeney, Sheldon E. Litwin, and E. Dale Abel	
<b>15</b>	<b>Atherogenic Lipid Metabolism in Obesity .....</b>	<b>293</b>
	Sue-Anne Toh, Michael Levin, and Daniel J. Rader	
<b>16</b>	<b>Gut Microbes, Immunity, and Metabolism .....</b>	<b>311</b>
	Evelyn Hsu and Gary Wu	
<b>17</b>	<b>Impact of Obesity on Female Reproductive Health.....</b>	<b>331</b>
	Moshood O. Olatinwo, Djana Harp, Winston Thompson, Hyeong-Kyu Park, and Roland Mathews	
<b>18</b>	<b>Lessons from HIV Lipodystrophy and Drug-Induced Metabolic Dysfunction .....</b>	<b>343</b>
	Steven Grinspoon	
<b>19</b>	<b>Principles of Obesity Therapy .....</b>	<b>359</b>
	Rexford S. Ahima	
	<b>Index.....</b>	<b>381</b>

# Contributors

**Evan Dale Abel, MD, PhD**

Division of Endocrinology, Metabolism and Diabetes,  
University of Utah School of Medicine, Salt Lake City, UT, USA

**Rexford S. Ahima, MD, PhD**

Division of Endocrinology, Diabetes and Metabolism, and  
the Institute for Diabetes, Obesity and Metabolism,  
University of Pennsylvania School of Medicine, 415 Curie Boulevard,  
712A Clinical Research Building, Philadelphia, PA 19104, USA

**Joseph Bass, MD, PhD**

Department of Medicine, Division of Endocrinology, Metabolism, and  
Molecular Medicine, Northwestern University, Feinberg School of Medicine,  
2200 Campus Drive, Evanston, Illinois 60208, USA  
and  
Department of Neurobiology and Physiology, Northwestern University,  
2200 Campus Drive, Evanston, Illinois 60208, USA

**Sudha B. Biddinger, MD, PhD**

Division of Endocrinology, Children's Hospital Boston, MA, Boston, USA

**Emilie Caron, PhD**

INSERM U995, Lille 2 University, Lille Cedex, France

**Brice Emanuelli, MD**

Joslin Diabetes Center, Harvard Medical School, Boston, MA, USA

**Jose E. Galgani, PhD**

Department of Nutrition, University of Chile, Santiago, Chile

**Marcus D. Goncalves, MSE**

Cell and Molecular Biology Graduate Program, University of Pennsylvania  
School of Medicine, Philadelphia, PA, USA

**Steven Grinspoon, MD**

Massachusetts General Hospital, Program in Nutritional Metabolism,  
Harvard Medical School, Boston, MA 02114, USA

**Djana Harp, MD**

Department of Obstetrics and Gynecology, Morehouse School of Medicine,  
Atlanta, GA, USA

**Evelyn Hsu, MD**

Division of Pediatric Gastroenterology, Hepatology and Nutrition,  
The Children's Hospital of Philadelphia, Philadelphia, PA, USA

**Efi Kokkotou, MD, PhD**

Department of Medicine, Gastroenterology Division, Beth Israel Deaconess  
Medical Center, Harvard Medical School, Boston, MA, USA

**Rohit Kulkarni, MD, PhD**

Division of Islet Cell Biology and Regenerative Medicine,  
Joslin Diabetes Center, and Department of Medicine,  
Brigham and Women's Hospital, Harvard Medical School,  
One Joslin Place, Boston MA 02215, USA

**Michael Levin**

University of Pennsylvania, School of Medicine, 654 Biomedical Research  
Building, 421 Curie Boulevard, Philadelphia, PA 19104-6160, USA

**Sheldon E. Litwin, MD**

University of Utah School of Medicine, Salt Lake City Veterans  
Affairs Medical Center, Salt Lake City, UT, USA

**Eleonore Maury, PhD**

Department of Medicine, Division of Endocrinology, Metabolism, and  
Molecular Medicine, Northwestern University, Feinberg School of Medicine,  
2200 Campus Drive, Evanston, Illinois 60208, USA  
and

Department of Neurobiology and Physiology, Northwestern University,  
2200 Campus Drive, Evanston, Illinois 60208, USA

**Roland Mathews, MD**

Department of Obstetrics and Gynecology, Morehouse School of Medicine,  
Atlanta, GA, USA

**Tomoaki Morioka, MD, PhD**

Division of Islet Cell Biology and Regenerative Medicine,  
Joslin Diabetes Center, and Department of Medicine,  
Brigham and Women's Hospital, Harvard Medical School,  
One Joslin Place, Boston MA 02215, USA  
and

Division of Metabolism, Endocrinology & Molecular Medicine,  
Osaka City University Graduate School of Medicine, Osaka, Japan

**Sonia M. Najjar, PhD**

Center for Diabetes and Endocrine Research,  
University of Toledo College of Medicine, Toledo, OH, USA

**Moshood O. Olatinwo, MD**

Department of Obstetrics and Gynecology, Morehouse School of Medicine,  
Atlanta, GA, USA

**Hyeong-Kyu Park, MD**

Department of Internal Medicine, Division of Endocrinology and Metabolism,  
Soonchunhyang University College of Medicine, Seoul, South Korea

**R. Arlen Price, PhD**

Pennsylvania Center for Neurobiology and Behavior,  
Translational Research Laboratories, 125 South 31st Street,  
Philadelphia, PA 19104, USA

**Daniel J. Rader, MD**

Cooper-McClure Professor of Medicine and Pharmacology,  
Director, Preventive Cardiovascular Medicine and Lipid Clinic  
Director, Clinical and Translational Research Center  
Associate Director, Institute for Translational Medicine and Therapeutics  
Director, Cardiovascular Metabolism Unit, Institute for Diabetes,  
Obesity, and Metabolism, University of Pennsylvania School of Medicine,  
654 Biomedical Research Building, 421 Curie Boulevard, Philadelphia,  
PA 19104-6160, USA

**Kathryn M. Ramsey, PhD**

Department of Medicine, Division of Endocrinology, Metabolism, and  
Molecular Medicine, Northwestern University, Feinberg School of Medicine,  
2200 Campus Drive, Evanston, Illinois 60208, USA  
and  
Department of Neurobiology and Physiology, Northwestern University,  
2200 Campus Drive, Evanston, Illinois 60208, USA

**Eric Ravussin, PhD**

Division of Health and Performance Enhancement, Pennington Biomedical  
Research Center, Baton Rouge, LA 70808, USA

**Muredach P. Reilly, MB BCh, MSCE**

Cardiovascular Institute, University of Pennsylvania School of Medicine,  
Philadelphia, PA, USA

**Neil B. Ruderman, MD, DPhil**

Diabetes Research Unit, Boston University School of Medicine,  
Boston Medical Center, Boston, MA, USA

**Jaswinder K. Sethi, PhD**

Department of Clinical Biochemistry, University of Cambridge,  
Cambridge, United Kingdom

**Rachana Shah, MD**

Division of Endocrinology, Children's Hospital of Philadelphia,  
University of Pennsylvania School of Medicine, Philadelphia, PA, USA

**Gary Sweeney, PhD**

York University, Toronto, Canada

**Winston Thompson, PhD**

Department of Obstetrics and Gynecology, Morehouse School of Medicine,  
Atlanta, GA, USA

**Sue-Anne Toh, MD**

Department of Medicine, National University of Singapore,  
5, Lower Kent Ridge Road, Singapore 119074

**Keith Tornheim, PhD**

Department of Biochemistry, Boston University School of Medicine,  
Boston, MA, USA

**Antonio J. Vidal-Puig, MD, PhD**

Department of Clinical Biochemistry, University of Cambridge,  
Cambridge, United Kingdom

**Yan Wang, MD**

Department of Medicine, Gastroenterology Division, Beth Israel Deaconess  
Medical Center, Harvard Medical School, Boston, MA, USA

**Gary D. Wu, MD**

Department of Medicine, Division of Gastroenterology,  
University of Pennsylvania School of Medicine, Center for Molecular Studies  
in Digestive and Liver Diseases, Philadelphia, PA, USA

# Chapter 1

## Principles of Human Energy Metabolism

Jose Galgani and Eric Ravussin

### Introduction

Energy is defined as the ability of a system to perform work. Energy is present in many forms, such as luminous energy coming from sun or kinetic energy obtained from wind and water. Humans obtain their energy from foods which is stored in the CH bonds of carbohydrates, lipids, proteins, and alcohol. To obtain the energy to live, grow, and reproduce, organisms must extract it in a usable form from plants and/or animal foods. This potential chemical energy is liberated inside cells through oxidative pathways that convert these CH bonds to energy-rich molecules such as creatine phosphate and adenosine triphosphate (ATP). The energy released from the breakdown of ATP is then used to power muscle activity, to synthesize many molecules necessary for cell structure and function, and create concentration gradients between the intra- and extra-cellular spaces (e.g.,  $\text{Na}^+$ ,  $\text{K}^+$ , or  $\text{Ca}^{2+}$  gradients). During these energy conversion processes, i.e., from foods to utilizable energy, and from utilizable energy to mechanical work, part of the energy is converted to heat. Thus, the energy utilized can be measured by the work generated or heat released. Work is measured in Joule (J), 1 J being the work necessary to give a mass of 1 kg an acceleration of 1 m/s traveling through a distance of 1 m. Heat is measured in calories, with 1 cal being the amount of heat required to raise the temperature of 1 g of water from 14.5 to 15.5°C. One calorie is equivalent to 4.184 J. Multiples of 1,000 (kilojoules (kJ) or kilocalories (kcal)) or one million (megajoules (MJ)) are used in human nutrition.

To obtain the energy from foods, mammals such as humans have evolved complex processes to maximize the energy supply [1]. Foods contain the energy in the form of carbohydrate, fat, and protein. These macromolecules need to be processed into small molecules before being absorbed from the gastrointestinal tract. The process of digestion is facilitated when foods are cooked, and then by

---

E. Ravussin (✉)

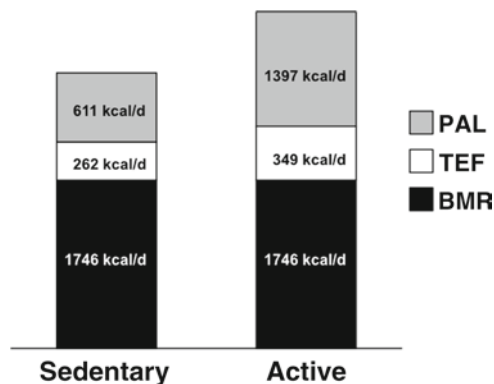
Division of Health and Performance Enhancement, Pennington Biomedical Research Center,  
Baton Rouge, LA 70808, USA  
e-mail: eric.ravussin@pbrc.edu



chewing and mixing with saliva and gastric movements, which ensure that the foods are properly mixed into a semifluid mass, known as chyme. Once the chyme gets to the duodenum and in contact with enzymes released from the gallbladder and pancreas, carbohydrate, fat, and protein are digested into smaller molecules, i.e., nutrients, which can be absorbed. From the bloodstream, the nutrients are taken up by cells to supply the energy required for cellular metabolism and survival. Within the cell, glucose, fatty acids, and amino acids are hydrolyzed in the presence of oxygen to release ATP, water, carbon dioxide, and heat [2]. Not all the energy contained in foods is available as utilizable energy, because some of the energy is lost during digestion and absorption [3]. Moreover, proteins are not completely oxidized to carbon dioxide and water; therefore, the intermediary metabolite of protein digestion still contains energy that is lost in the urine in the form of urea. The heat of combustion of exogenous nutrients, which is equivalent to their energy content measured using a bomb calorimeter is 4.2 kcal/g of carbohydrate, 9.4 kcal/g of fat, and 4.2–5.1 kcal/g of protein. However, after taking into account the intestinal absorption and urinary losses, the amount of energy available to be utilized by cells is approximately 4, 9, and 4 kcal for carbohydrate, fat, and protein, respectively [4].

## Energy Expenditure

Energy is expended in multiple processes occurring to sustain life. The major components of the human energy budget include the obligate energy required to keep us alive and that required to provide locomotion (Fig. 1). A description of these and other constituents of energy expenditure are discussed below.



**Fig. 1** Contribution of basal metabolic rate (BMR), thermic effect of food (TEF), and physical activity to total energy expenditure in a 70-kg young man having a sedentary or active physical activity level

*Basal and Resting Metabolic Rate*

The basal metabolic rate (BMR) is the energy expended by a subject under standard conditions that include being awake in the supine position after 10–12 h of fasting and 8 h of physical rest, and being in a state of mental relaxation in a room with environmental temperature that does not elicit heat-generating or heat-dissipating processes. The measurement of BMR requires specific conditions that are not always feasible. In contrast, resting metabolic rate (RMR) can be measured under less restricted conditions than BMR, and do not require that the subject spend the night sleeping in the testing facility prior to the measurement.

The BMR includes the cost of maintaining the integrated systems of the body at homeothermic temperature at rest. These processes are essential for life and include cation exchange to maintain gradient concentrations between the cellular compartments, muscle tone, protein synthesis and degradation, RNA and DNA turnover, cellular signaling, gluconeogenesis, synthesis of urea, fuel cycling, and many other biochemical processes [5]. In sedentary adults, these processes account for approximately 60–70% of daily energy expenditure [6]. The close correlation between BMR and body size has been known for many years and has formed the basis for the development of widely used equations to predict BMR from weight [7–9]. The equations derived from Schofield’s work [8] are conventionally accepted [10] (Table 1). The studies to derive these equations were mostly performed in Western Europe and North America. Almost half of the data used to generate the equations for adults were from studies carried out in the late 1930s and early 1940s on Italian men with relatively high BMR values, hence questions have been raised about the universal applicability of those equations [10].

Predictive equations derived from a database with broader geographical and ethnic representation have been evaluated [11]. The accuracy of the latter equations

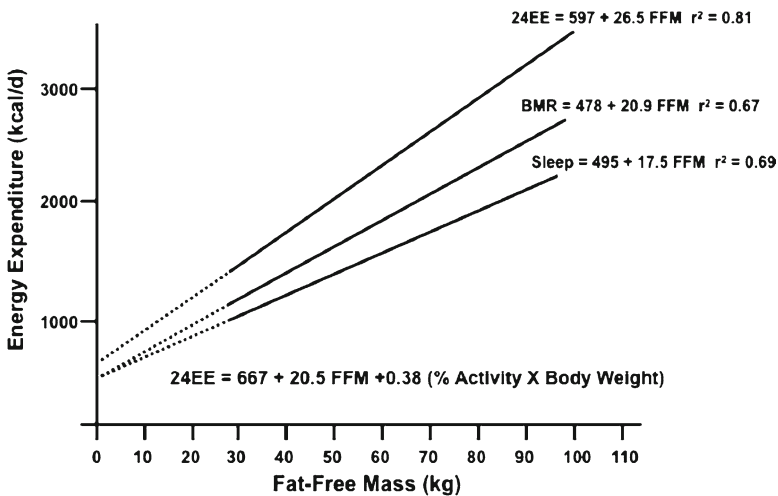
**Table 1** Equations for estimating BMR from body weight (10)

Age (years)	<i>n</i>	BMR (kcal/day)
<i>Males</i>		
<3	162	59.51 weight (kg) –30
3–10	338	22.71 weight (kg) + 504
10–18	734	17.69 weight (kg) + 658
18–30	2,879	15.06 weight (kg) + 692
30–60	646	11.47 weight (kg) + 873
>60	50	11.71 weight (kg) + 588
<i>Females</i>		
<3	137	58.32 weight (kg) –31
3–10	413	20.32 weight (kg) + 486
10–18	575	13.38 weight (kg) + 693
18–30	829	14.82 weight (kg) + 487
30–60	372	8.13 weight (kg) + 846
>60	38	9.08 weight (kg) + 659

and those from Schofield were compared with published measurements of BMR in adults from different parts of the world, which were not part of the databases used to generate the predictive equations [12]. Although the new equations had some merits, such as reductions in prediction error and overestimation bias among men, these were not robust enough to justify the replacement of Schofield's equations.

In our search for the possible mechanisms underlying the intersubject variability in BMR, we have explored the impact of body composition, gender, physical training, age, muscle metabolism, sympathetic nervous system (SNS) activity, and body temperature. Fat-free mass (FFM) accounts for two-third of the intersubject variance in BMR [6, 13] (Fig. 2). Additional predictors of BMR are fat mass and age. In combination, these three factors account for over 70% of the BMR variance in humans [13]. Keys et al. [14] investigated the effect of age and concluded that the decline in BMR with aging was less than 1–2% per decade from the second to the seventh decade of life. Subsequent work supported Keys' conclusion that the decrease in BMR seen in elderly people can be explained largely by decreases in FFM [15, 16].

The unexplained variance in BMR may be partially accounted for by the differences in organ size between subjects with similar FFM. Table 2 shows the relative contributions of various organs to BMR [5]. About half of FFM is skeletal muscle, however, this tissue accounts for only one-fifth of the BMR. In contrast, liver and brain constitute less than 5% of total body mass, but together account for two-fifth of BMR. New technologies, such as magnetic resonance imaging, are now being used to estimate organ sizes and better determine the differences in BMR among individuals [17, 18].



**Fig. 2** Relationship between BMR and fat-free mass (FFM) in humans. Relationships by simple regression analysis between 24-h energy expenditure (24EE), BMR, sleeping metabolic rate, and FFM in 177 subjects. The slopes are statistically different from each other whereas the intercepts are not different. By multiple regression analysis, the effect of percent activity  $\times$  weight on 24EE is given for 118 subjects

**Table 2** Contributions of oxygen-consuming organs to body mass and resting metabolic rate in humans

Organ	Body mass (%)	Whole-body oxygen consumption (%)
Liver	2	17
Gastrointestinal tract	2	10
Kidney	0.5	6
Lung	0.9	4
Heart	0.4	11
Brain	2	20
Skeletal muscle	42	20
Total	49.8	88

The activity of the SNS is involved in the regulation of BMR [19, 20]. Using a direct measurement of SNS activity via microneurography, we found that the variability in energy expenditure was related to the variability of muscle sympathetic nerve activity [21]. The variability in BMR after adjustment for differences in FFM, fat mass, and age was also related to the variability in body temperature, indicating that body temperature could be a marker for high or low relative metabolic rate [22]. Some of the remaining variance was explained by family membership, suggesting that BMR is at least partly determined by genetics [23, 24].

*Thermic Effect of Food*

The thermic effect of food (TEF) is the increase in energy expenditure observed after a meal. The TEF includes the energy required for ingestion and digestion of food, and for absorption, transport, interconversion, oxidation, and deposition of nutrients. The TEF has also been called “specific dynamic action” of food or “dietary-induced thermogenesis.” The TEF accounts for 5–15% of the BMR over 24 h [25, 26]. Many factors influence the TEF, including the meal size and composition, palatability of food, time of the meal, subject’s genetic background, age, physical fitness, and insulin sensitivity. Brundin et al. [27] showed that TEF is also a function of the heat leakage across the abdominal wall, which is inversely related to the thickness of the abdominal adipose tissue layer. These factors together with the techniques of energy measurement, e.g., the position of the subject and the duration of the measurement, make TEF the most difficult and least reproducible component of daily energy expenditure to assess [26].

*Physical Activity*

Physical activity, the most variable component of daily energy expenditure, can account for a significant amount of calories in very active people. However, sedentary adult individuals exhibit a range of physical activity, which represents only 20–30%

**Table 3** Examples of activities performed by sedentary, moderate, or vigorous lifestyle

Daily activities	Time (h)	Energy cost (PAR)	Time energy cost	PAL
<i>Sedentary or light active lifestyle</i>				
Sleeping	8	1.0	8.0	
Dressing, showering	1	2.3	2.3	
Eating	1	1.5	1.5	
Cooking	1	2.1	2.1	
Office work, tending shop	8	1.5	12.0	
General household work	1	2.8	2.8	
Driving car to/from work	1	2.0	2.0	
Walking at varying paces without a load	1	3.2	3.2	
Watching TV, chatting	2	1.4	2.8	
Total	24		36.7	36.7/24 = 1.53
<i>Active or moderately active lifestyle</i>				
Sleeping	8	1.0	8.0	
Dressing, showering	1	2.3	2.3	
Eating	1	1.5	1.5	
Standing, carrying light loads	8	2.2	17.6	
Commuting to/from work on the bus	1	1.2	1.2	
Walking at varying paces without a load	1	3.2	3.2	
Low intensity aerobic exercise	1	4.2	4.2	
Watching TV, chatting	3	1.4	4.2	
Total	24		42.2	42.2/24 = 1.76
<i>Vigorous or vigorously active lifestyle</i>				
Sleeping	8	1.0	8.0	
Dressing, showering	1	2.3	2.3	
Eating	1	1.4	1.4	
Cooking	1	2.1	2.1	
Nonmechanized agricultural work (planting, weeding, gathering)	6	4.1	24.6	
Collecting water/wood	1	4.4	4.4	
Nonmechanized domestic chores (sweeping, washing clothes and dishes by hand)	1	2.3	2.3	
Walking at varying paces without a load	1	3.2	3.2	
Miscellaneous light leisure activities	4	1.4	5.6	
Total	24		53.9	53.9/24 = 2.25

of total energy expenditure (TEE) (Fig. 1). Until the introduction of the doubly labeled water method for measuring 24-h energy expenditure in free-living conditions [28], there was no satisfactory method to assess the impact of physical activity on daily energy expenditure. Physical activity level (PAL) can be measured or estimated from the 24-h TEE to BMR ratio ( $PAL = TEE/BMR$ ). Multiplying the PAL by the BMR gives the actual daily energy requirements. For example, a male with PAL value of 1.75 and BMR value of 1,697 kcal/day would have an energy requirement of

$1.75 \times 1,697 = 2,970$  kcal/day. Schulz and Schoeller [29] have provided data on more than 200 subjects showing a wide variability in TEE and physical activity. In a review of 574 doubly labeled water measurements, Black et al. [30] compiled data to establish the limits of sustainable human energy expenditure, determine the average range of habitual energy expenditure in relation to age and sex, and evaluate the lifestyle and activity patterns associated with different levels of physical activity. From this review, Black et al. showed a modal value for PAL of 1.60 (range 1.55–1.65) for both men and women from affluent societies in developed countries, with a predominantly sedentary lifestyle. A joint FAO/WHO/UNU report on Human Energy Requirements [10] classified as sedentary or light activity lifestyle, individuals with a PAL value between 1.40 and 1.69. Active or moderately active lifestyle corresponds to PAL value between 1.70 and 1.99, and vigorously active lifestyle corresponds to PAL values between 2.00 and 2.40. The PAL values that can be sustained for a long period of time in free-living adult populations fall in the range of 1.40–2.40. It is thought that PAL values of 1.70 or higher will reduce the risk of becoming overweight and developing a variety of noncommunicable chronic diseases often associated with obesity. Table 3 shows typical activities performed under a sedentary, moderate, or vigorous lifestyle conditions. Table 4 shows the frequency, duration, and intensity of physical activity recommended by selected organizations.

**Table 4** Minimum frequency, duration, and intensity of physical activity recommended by different organizations

Organization	Recommendation
World Health Organization (2002)	30 min of moderate activity every day
World Cancer Research Fund/American Institute for Cancer Research (1997)	30 min of vigorous or 60 min of moderate activity daily, plus additional 30–60 min of vigorous activity once a week
Unites States Centers for Disease Control and Prevention (1996)	30 min of moderate activity on all or most days of the week
American College of Sports Medicine (1998)	For cardio-respiratory fitness and body composition: 20–60 min of continuous or intermittent (bouts of at least 10 min) aerobic activity at 55–90% maximum heart rate, or at 40–85% maximum oxygen uptake, 3–5 days/week For muscular strength and endurance, body composition and flexibility: one set of 8–10 exercises, with 8–12 repetitions of each exercise, 2–3 days/week
International Agency for Research on Cancer (2002)	To maintain healthy body weight: 60 min moderate activity on all or most days of the week For cancer prevention: Substitute moderate for vigorous activity several times per week
International Association for the Study of Obesity (2002)	To prevent weight regain in formerly obese individuals: 60–90 min of moderate daily activity or shorter periods of vigorous activity To prevent transition to overweight or obesity: 45–60 min of moderate activity daily or 1.7 PAL. For children, more activity time is recommended

## Measurement of Energy Expenditure

Energy expenditure can be measured by several methods; however, those with higher accuracy and precision are more expensive.

### *Direct Calorimetry*

The measurement of the energy expenditure was first made in the late eighteenth century by Lavoisier, who discovered that respiration was the basis of all life-sustaining processes, and that life was a form of chemical combustion [31]. Lavoisier measured the rate at which heat was lost from the body to the environment [31]. Heat loss from the body includes non-evaporative heat loss, and evaporative heat loss in the form of water vapor. Non-evaporative heat loss is determined from the temperature gradient across the walls of a well-insulated chamber. Evaporative heat loss is measured by determining the increase in water content in the air in the test chamber and calculating its latent heat of condensation. Heat loss is estimated from the sum of evaporative and non-evaporative loss. Although very accurate, the measurement of energy expenditure by direct calorimetry is not done often, because an easier estimation of the energy expenditure can be obtained from the chemical reactions that release heat.

### *Indirect Calorimetry*

The transformation of food nutrients to a usable source of energy requires oxygen. One liter of oxygen consumed generates approximately 5 kcal (~21 kJ). Given that there is proportionality between  $\text{VO}_2$  and ATP synthesis, and because each mole of ATP synthesized is accompanied by a given amount of heat, it is possible to calculate heat production from  $\text{VO}_2$  measurements alone. However, the heat produced by the utilization of 1 L of oxygen varies according to the proportion in the diet of carbohydrate, fat, and protein. By measuring carbon dioxide production, oxygen consumption, and urinary nitrogen excretion, it is possible to determine the proportion of the different nutrients that are oxidized, and the energy produced can be precisely calculated [6, 31]. This indirect calorimetry method takes into account the heat released by the oxidation of the three macronutrients. Three measurements must be carried out: oxygen consumption, carbon dioxide production, and protein oxidation. Gas exchange is measured using oxygen and carbon dioxide analyzers and flow, while protein oxidation is estimated from urinary nitrogen excretion rate. Indirect calorimetry predicts heat production (energy expenditure) from the rates of respiratory gas exchange and nitrogen excretion. The subject is kept in a sealed room or a canopy is placed over his head. The chamber or canopy is ventilated with a constant supply of fresh air. The subject's respiratory gas exchange is measured by comparing the composition of well-mixed air in the chamber with the composi-

tion of air entering the chamber, together with the flow rate of air through the system. After determining how much oxygen is consumed, carbon dioxide produced, and urinary nitrogen excreted, energy expenditure can be calculated using one of many equations. A difference less than 4% in energy expenditure is observed between equations due to slightly different constants for the amounts of oxygen consumed and carbon dioxide produced during oxidation of the three classes of nutrients, i.e., fat, carbohydrate, and protein [31].

$$\text{MR (kcal / day)} = 3.941 \text{VO}_2 \text{ (L / day)} + 1.106 \text{VCO}_2 \text{ (L / day)} - 2.17 \text{N}_{\text{urine}} \text{ (g / day)},$$

where MR is the metabolic rate;  $\text{VO}_2$ , oxygen consumption;  $\text{VCO}_2$ , carbon dioxide production; and  $\text{N}_{\text{urine}}$  is the nitrogen excreted in urine.

The correction for urinary nitrogen resulting from incomplete combustion of protein is small, and a value of 12 g/day (0.5 g/h) is often used for the calculation of metabolic rate.

The ratio between carbon dioxide and oxygen is known as the respiratory quotient (RQ) or respiratory exchange ratio. Each nutrient requires a given amount of oxygen to be oxidized and releases a given amount of carbon dioxide. The oxygen and carbon dioxide exchange in a system can then indicate the proportion of nutrients participating in the ATP production. Because proteins are not completely oxidized, the ratio between  $\text{VCO}_2$  and  $\text{VO}_2$  is calculated only for fat and carbohydrate, which is known as the nonprotein RQ. The RQ in healthy humans usually ranges between 0.7 and 1.0, and its variability depends on the availability of carbohydrate, fat, and protein for oxidation. When fat is the only nonprotein fuel available, this ratio approaches 0.7, whereas, when carbohydrate is solely oxidized, RQ is equal to 1.0. The proportion of energy coming from carbohydrate and fat, and the kilocalories expended per liter of oxygen consumed can be calculated using these equations [4]:

$$\text{CHO}\% = 504.7(\text{npRQ} - 0.707) / [5.047(\text{npRQ} - 0.707) + 4.686(1.00 - \text{npRQ})]$$

$$\text{Fat}\% = 468.6(1.00 - \text{npRQ}) / [5.047(\text{npRQ} - 0.707) + 4.686(1.00 - \text{npRQ})]$$

$$\text{kcal} = 4.686 + [(\text{npRQ} - 0.707) / 0.293] 0.361$$

where CHO % is the proportion of energy coming from carbohydrate; Fat %, proportion of energy coming from fat; kcal, kilocalories expended per liter of oxygen consumed; and npRQ, nonprotein respiratory quotient, nonprotein  $\text{VCO}_2$ /nonprotein  $\text{VO}_2$ .

Indirect calorimetry is largely used to measure BMR or RMR for short periods of time (minutes to hours). Using respiratory chambers, energy metabolism can be measured for longer periods, up to several days. However, subjects confined to a metabolic chamber have an energy expenditure which is usually not fully represen-



tative of the energy expenditure under free-living conditions, because physical activity is limited.

### ***Doubly Labeled Water***

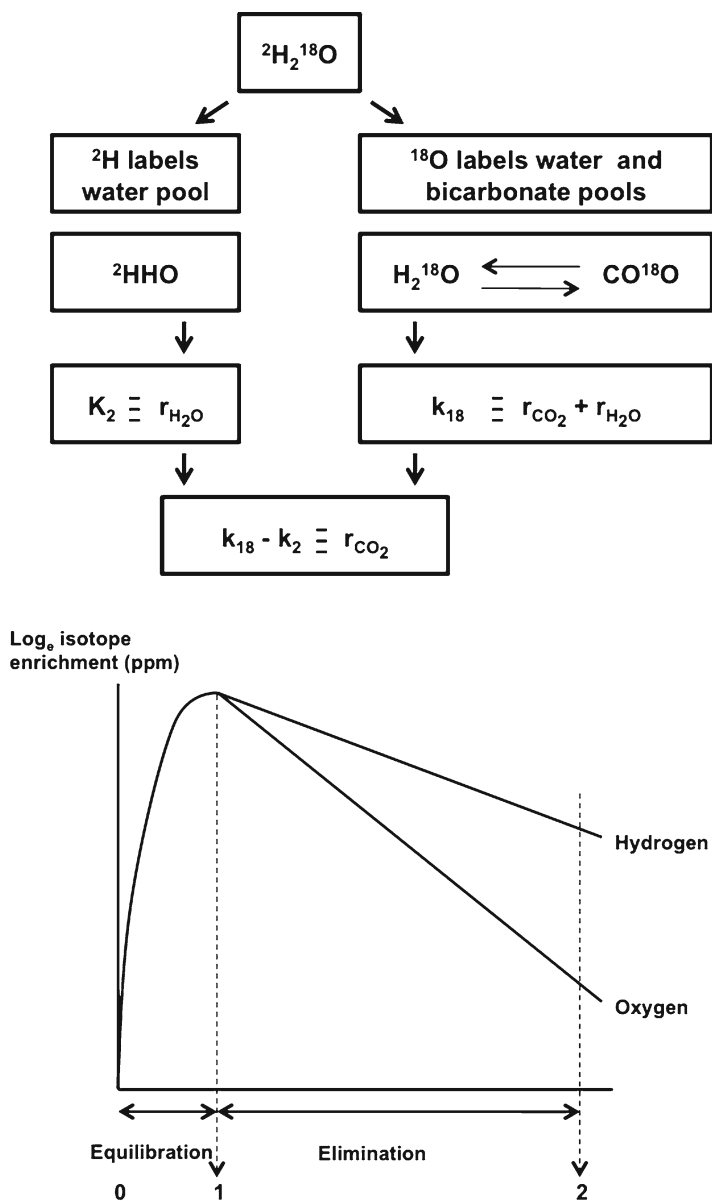
Free-living energy expenditure can be measured by doubly labeled water [28, 32]. The method consists of giving a single oral dose of water enriched in two stable isotopes: deuterium ( $^2\text{H}$ ) and  $^{18}\text{O}$  (Oxygen ( $^{18}\text{O}$ )). Because the natural abundance of these isotopes is very low, body water is labeled by both isotopes. After equilibrium is reached in 3–6 h, the loss of  $^{18}\text{O}$  occurs as  $\text{CO}^{18}\text{O}$  and  $\text{H}_2^{18}\text{O}$ , since a rapid exchange of  $^{18}\text{O}$  between water and carbon dioxide takes place via the carbonic anhydrase enzyme, whereas deuterium is lost only in water. Enrichments in  $^{18}\text{O}$  and  $^2\text{H}$  are performed by isotope ratio mass spectrometry usually in urine or saliva. The calculation of carbon dioxide production rate is based on the difference in turnover rates between the oxygen and hydrogen labels. Because oxygen has two routes of elimination (expired carbon dioxide and water in urine, saliva, sweating, etc.), the disappearance rate of  $^{18}\text{O}$  will be faster than of  $^2\text{H}$ , which is eliminated as water in urine, saliva, sweating, etc. After log-transformation of isotope disappearance rates, the difference between the slopes for  $^2\text{H}$  and  $^{18}\text{O}$  is proportional to the amount of carbon dioxide produced in a given time (Fig. 3). Assuming a 24-h RQ value of 0.85, oxygen consumption and hence the energy expenditure can be calculated. The doubly labeled water method is an excellent field technique, which has been validated by comparing results to those obtained in a metabolic chamber [33].

### ***Factorial Method***

When experimental data on TEE are not available, it can be estimated by factorial calculations based on the time allocated to activities that are habitually performed and the energy cost of those activities. Factorial calculations combine two or more components or “factors,” such as the sum of the energy spent while sleeping, resting, working, doing social or discretionary household activities, and in leisure. Energy spent in each of these components may in turn be calculated by knowing the time allocated to each activity and its corresponding energy cost.

## **Energy Balance and Implications for Obesity**

Energy homeostasis is disturbed when food supply is restricted or food intake is increased. In response to energy restriction, several compensatory mechanisms including increasing appetite, reduction of physical activity, and enhancement of



**Fig. 3** Doubly labeled water technique. Theoretical time course of enrichments of isotopes of oxygen and hydrogen in body water after administration at time zero. Over an equilibration period of several hours the isotope enrichments reach a peak. If the amount of isotope administered is known, the peak enrichment can be used to estimate the volume of dilution space. After equilibration, the isotopes are washed out of the body along an exponential curve that is linear when expressed as log of the enrichment above background. The oxygen isotope leaves the body faster than the hydrogen isotope because it is washed out of the body by water and carbon dioxide. Carbon dioxide production and energy expenditure are estimated based on the divergence in enrichments at times 1 and 2. During the 4–14 days of the study in normal adults, they are free to engage in usual activities without being confined in a calorimetry chamber

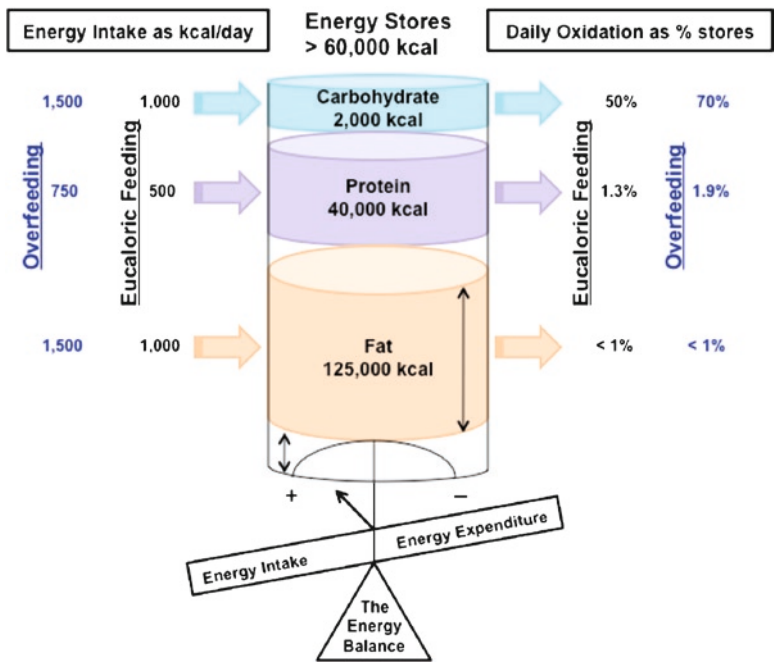
the coupling between oxidation and ATP synthesis are engaged. When negative energy balance is maintained for a longer period, the adaptation includes increasing energy efficiency, in order to prevent further weight loss [34, 35]. In contrast, overfeeding causes a suppression of appetite and an increase in energy expenditure, with probably a reduction in energy production efficiency [36]. However, such adaptation to energy surplus seems unable to prevent weight gain, in contrast to the ability of the metabolic response to energy deprivation to prevent weight loss.

During long-term energy balance, macronutrient oxidation has to eventually match macronutrient intake such that no macronutrients are stored or lost [37]. In other words, not only does 24-h energy expenditure have to be equal to 24-h energy intake, but 24-h RQ has to be equal to 24-h food quotient (FQ). The 24-h RQ corresponds to the mean proportion of macronutrient oxidized over a day whereas 24-h FQ represents the proportion of daily dietary macronutrients available for oxidation [4]. Many studies have shown that when people are in energy balance, 24-h RQ eventually matches 24-h FQ [38–42]. Both day-to-day variations in energy/macronutrient intake and day-to-day changes in energy expenditure lead to either slightly positive or negative energy balance. In response to these short-term variations in energy balance, carbohydrate and protein stores are closely regulated by an adjustment of oxidation to intake. Consequently, positive or negative energy balances are mostly buffered by changes in fat stores as evidenced by the tight correlation between fat storage and energy balance [43].

Obesity results from a chronic imbalance between energy intake and energy expenditure, resulting in weight gain, mostly as fat. If the origins of positive energy balance lies in a chronic imbalance of energy intake and expenditure, a key question is “How does this imbalance between intake and expenditure happen? An examination of the contribution of a particular nutrient to energy balance is only valid if that nutrient has a separate balance equation, implying a separate regulation (Fig. 4). Is each nutrient oxidized or stored in its own compartment (separate regulation), or does it get converted into another compartment for storage? This applies particularly to carbohydrate that can be converted to fat via *de novo* lipogenesis. The latter occurs only when large amounts of carbohydrate are ingested [44–47].

## ***Protein Balance***

Protein intake usually accounts 15% of total calories ingested, but protein stores in the body represent about one-third of the total stored calories in a 70-kg man. The daily protein intake amounts to a little over 1% of the total protein stores [48] (Fig. 4). The protein stores increase in response to growth hormone, androgens, and strength and weight bearing exercises, but do not increase simply from increased dietary protein. Protein stores are, therefore, tightly controlled and protein balance is maintained on a day-to-day basis [43]. It is doubtful that protein imbalance plays



**Fig. 4** Relationship between energy intake and expenditure, and the storage of carbohydrate, protein, and fat

a major role in obesity, however, as with the other nonfat nutrients, protein intake can affect the fat balance equation.

### *Carbohydrate Balance*

Carbohydrate is often the main source of dietary calories, yet the body stores of glycogen are very limited: 500–1000 g of carbohydrate in the form of glycogen [48]. The daily caloric intake of carbohydrate corresponds to about 50–100% of carbohydrate stores [49] (Fig. 4). Thus, the carbohydrate stores fluctuate markedly over hours and days, compared to protein and fat stores. However, as with protein stores, carbohydrate stores are tightly controlled even if the mechanisms are unknown [43]. Whether carbohydrate control is based on humoral and/or neural signals exchanged between the muscle and liver and the brain remains to be established. Dietary carbohydrate intake stimulates both glycogen storage and glucose oxidation and suppresses fat oxidation [37]. That which is not stored as glycogen, is oxidized (not converted to fat), and carbohydrate balance is achieved. Like other nonfat nutrients, chronic imbalance between carbohydrate intake and oxidation is unlikely to be an explanation for weight gain, because storage capacity is limited and controlled.

## ***Fat Balance***

In marked contrast to glycogen and protein stores, fat stores are large. Fat intake represents less than 1% of the total fat stores [48] (Fig. 4). Fat stores are the energy buffer for the body. The daily surplus or deficit in energy intake is translated into surplus or deficit in fat stores [43, 50]. Energy balance and diet macronutrient composition are the main determinants for fat oxidation [43, 51]. For example, a deficit of 200 kcal of energy over 24 h means 200 kcal comes from the fat stores, and the same holds true for an excess of 200 kcal of energy, which ends up in the fat stores. Additionally, diets with high fat-to-carbohydrate ratios result in a progressive increase in fat oxidation over periods of days [52].

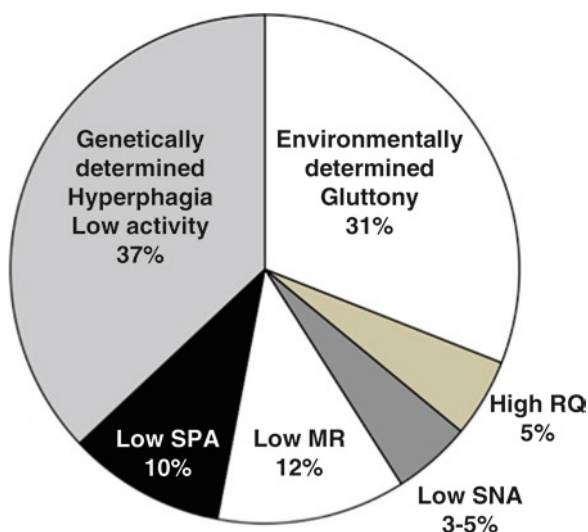
In summary, when energy balance in humans is considered under physiological conditions, fat is the main nutrient capable of sustaining a chronic imbalance between intake and oxidation, and thus contributing to an increase in adipose tissue. The other nutrients influence adiposity indirectly by their contribution to overall energy balance. The use of the fat balance equation, instead of the energy balance equation, offers a new framework for understanding the pathogenesis of obesity.

## **Metabolic Risk Factors for Weight Gain**

An understanding of the etiology of human obesity demands longitudinal studies. Cross-sectional studies have added little to our understanding of the physiological mechanisms predisposing to weight gain [53]. Cross-sectional studies can only provide associations, whereas longitudinal studies reveal predictors or risk factors. Several studies have examined such predictors in Pima Indians, a population prone to obesity [54] (Fig. 5). Other studies have been done in African-American population, another population prone to obesity [55, 56].

## ***Low Metabolic Rate***

The relation between metabolic rate and body size suggests that, at any given body size, individuals can have a “high,” “normal,” or “low” relative metabolic rate. From our own studies in adult nondiabetic Pima Indians, we found that a low relative metabolic rate adjusted for differences in FFM, fat-mass, age, and sex was a risk factor for body weight gain [57]. After 4 years of follow-up, the risk of gaining 10 kg was approximately eight times greater in subjects with the lowest RMR (lower tertile) compared with those with the highest RMR (higher tertile). According to a meta-analysis, formerly obese subjects have a 3–5% lower mean relative RMR than control subjects, which likely contributes to the high rate of weight regain in formerly obese persons [58]. Nevertheless, these data need to be



**Fig. 5** Genetic and nongenetic factors in body weight regulation. Studies of monozygotic twins reared apart indicate that approximately one-third of the variability in BMI is attributable to non-genetic factors and two-thirds to genetic factors. In this chart, the genetic contributions to the variability in BMI is broken down into metabolic (MR), respiratory quotient (RQ), spontaneous physical activity (SPA), and sympathetic nervous activity (SNA)

interpreted with caution because the variability of baseline energy expenditure accounted for only 16% of the variability of weight gain. Theoretical estimates suggest that only 30–40% of the increase in body energy stores in people who gained weight can be attributed to the baseline “deficit” in energy expenditure. Furthermore, relatively low energy expenditure does not seem to be a predictor of weight gain in other adult populations [59, 60]. Together, the results suggest that a low metabolic rate may not be only a direct risk factor for body weight gain but also a marker for inactivity or hyperphagia.

### ***Low Physical Activity***

Reduced physical activity, as a cause of obesity is an attractive hypothesis. The energy expended in physical activity is quite variable and the secular increase in obesity parallels the increase in sedentary lifestyles. Physical activity may have decreased in many populations over the past few decades with the increased number of cars per household and the increase in the numbers of hours spent in front of television sets or personal computers [61]. However, Westerterp and Speakman concluded that decreased energy expenditure via decreased physical activity is unlikely the cause of the obesity epidemic [62]. Using doubly labeled water data, they could not find major differences in physical activity in

Westernized populations as well as developing countries. Furthermore, they could not find a decrease in physical activity measures over the past 3–4 decades. Swinburn et al. used data from 963 children and 1,399 adults to calculate the energy flux for given body weights [63, 64]. Using such equations to estimate changes in energy intake in the United States from 1970s to the 2000s, it was determined that virtually all the weight gain in the United States appeared to be due to increased energy intake rather than decreased energy expenditure. Whether a low level of physical activity is a cause or a consequence of obesity can only be tested in prospective studies. Some studies indicate that patients engaging in considerable amount of physical activity up to 80 min/day are successful at maintaining weight loss [65].

Another component of 24-h energy expenditure is the energy cost of spontaneous physical activity, that accounts for 8–15% of the total daily expenditure [6]. Consistent with the cross-sectional observation of a decrease in spontaneous physical activity in obese subjects, our longitudinal studies showed that even in the confined environment of a respiratory chamber, spontaneous physical activity is a familial trait and that a low level of spontaneous physical activity is associated with subsequent weight gain in males, but not in females [66]. Other data show that resistance to the development of obesity may be due to spontaneous physical activity, also called non-exercise activity thermogenesis (NEAT) [67, 68]. An increase in NEAT by 200 kcal/day would be the equivalent to engaging in 2.5 h/day of activities such as fidgeting or strolling.

### ***Low Fat Oxidation***

The composition of nutrient intake has been shown to be an important factor in the pathogenesis of obesity. Apart from the effect of diet composition, the RQ is also influenced by recent energy balance (negative balance causing more fat oxidation), sex (females tend to have reduced fat oxidation), adiposity (higher fat mass leads to higher fat oxidation), and family membership, suggesting genetic determinants [51, 69].

In a longitudinal study in Pima Indians, a high 24-h RQ predicted weight gain [51]. Those in the ninetieth percentile for RQ (“low fat oxidizers”) had a 2.5 times larger risk of gaining 5 kg or more body weight than those in the tenth percentile (“high fat oxidizers”). This effect was independent of a relatively low or high 24-h metabolic rate. In support of this observation, others have demonstrated that weight-reduced obese volunteers have high RQs, i.e., low rates of fat oxidation [70, 71], and those who are able to maintain weight loss have lower RQs compared to those experiencing weight relapse [72]. A low fat oxidation could lead to higher carbohydrate oxidation, which would decrease carbohydrate store. The size of carbohydrate store has been inversely related to food intake in mice [73] and humans [74], and prospective weight gain in humans [75]. Mechanisms accounting for this relationship remain unclear.

## ***Low Thermogenesis***

The fact that weight gain is usually not directly proportional to the excess energy intake, and weight gain is quite variable among individuals suggests that overfeeding can induce different levels of thermogenesis [36]. This has been called “adaptive thermogenesis,” and refers to changes in energy expenditure not attributable to the changes in the size of the body or tissue composition in response to excess caloric intake. Hypothetically, this increase in energy expenditure would prevent further weight gain. This idea was supported by the findings of the Vermont studies in prisoners in whom almost 50% more energy intake was necessary to maintain their new body weights [76]. Bouchard et al. [77] undertook a controlled study to determine whether there are physiological differences in the responses among individuals to long-term overfeeding, and to assess the possibility that genotypes are involved in such differences. In response to 84 days of 1,000 kcal/day of overfeeding, 12 pairs of monozygotic twins gained on average 8.1 kg, but the range was from 4.3 to 13.3 kg. However, the similarity within each twin pair in the response to overfeeding was significant with respect to gain in body weight, percentage of fat, and total fat mass with about three times more variance among pairs than within pairs. The similarity in the adaptation to long-term overfeeding within the pairs of twins clearly indicated that genetic factors are involved in the partitioning between fat and lean mass deposition and in determining the energy expenditure response.

At present, the role of thermogenesis in body weight regulation is still a matter of debate [78, 79]. Dietary compliance is an important factor to explain such variability, but well-controlled studies have shown similar variability in weight gain [36]. Such differences in weight gain can first be explained by our inability to assess weight-maintenance energy requirements and therefore the actual energy excess. Differences in digestion and absorption may modify the amount of “bio-available” energy, thus affecting the actual positive energy balance. The composition of weight gain (i.e., fat mass and lean mass) makes a difference in extent of weight gain, because the energy cost of protein deposition is higher than that of adipose tissue. Furthermore, the dietary protein content may be a critical determinant of weight gain during overfeeding, by increasing the energy cost of increasing body weight [36]. Differences in mitochondrial energy efficiency may also represent an underlying cause of the variability in weight gain [78]. Rosenbaum et al. [80] showed that maintenance of reduced or elevated body weight results in respective decrease or increase in energy expended in physical activity. At reduced body weight, muscle work efficiency was increased significantly. In contrast, weight gain resulted in a decrease in muscle work efficiency. Longitudinal changes in energy expenditure and RQ have been associated with spontaneous long-term weight change in Pima Indians [81]. The results showed that metabolic adaptation did occur in response to spontaneous long-term weight change, but the magnitude of the adaptive changes were small and the interindividual variability were large.

Impaired TEF has been proposed as a contributing factor to weight gain. While a review of the literature found similar number of studies supporting or denying a role



of TEF on obesity, it was indicated that obesity may be associated with impaired TEF through insulin resistance [82]. A prospective study in more than 100 subjects did not find any relationship between the TEF and weight change [26]. Therefore, decreased TEF is an unlikely explanation for significant degrees of obesity.

### ***Low Sympathetic Nervous Activity***

The activity of the SNS is positively related to the three major components of energy expenditure: RMR [21], TEF [20], and spontaneous physical activity [83]. It is also negatively related to 24-h RQ [84]. Further indications of the possible role of SNS activity in the regulation of energy balance in humans come from a study showing that a low SNS activity is associated with a poor weight loss outcome in obese subjects treated with diet [85]. Furthermore, Pima Indians, who are prone to obesity, have low muscle sympathetic nerve activity compared to weight-matched Caucasians [21]. In a prospective study, we found that baseline urinary excretion rate of norepinephrine, a global index of SNS activity, was negatively correlated with body weight gain in male Pima Indians [86]. Thus, a low activity of the SNS is associated with the development of obesity.

## **Conclusion**

Obesity develops as a result of a small but constant imbalance in energy intake relative to energy expenditure. Better understanding of energy metabolism and novel technologies to measure it has allowed the identification of risk factors for obesity. More research is required to identify the physiological and molecular mechanisms involved in the propensity to develop obesity in our “obesogenic environment,” characterized by excess food consumption and sedentary lifestyle. Although only few genes have so far been associated to common obesity [87, 88], new and cheaper genetic tools are enabling large scale screening of individuals at risk of obesity. Epidemiological evidence has shown that intra-uterine and postnatal environment represent important factors in the development of adulthood obesity. We are now only scratching the surface of our understanding of the mechanisms underlying fetal and neonatal programming towards obesity [89, 90]. We also need to study the potential role of brown adipose tissue in body weight control in humans. The thermogenic role of brown adipose tissue in adult human energy balance had been neglected until recently [91]. Obesity, diabetes, and other metabolic diseases are major threats facing health systems worldwide, which require radical changes in preventive and treatment strategies.

## References

1. Spiller, R.C. (1994). Intestinal absorptive function. *Gut*, 35(1 Suppl), S5–S9.
2. Saraste, M. (1999). Oxidative phosphorylation at the fin de siècle. *Science*, 283(5407), 1488–1493.
3. Elia, M., & Cummings, J.H. (2007). Physiological aspects of energy metabolism and gastrointestinal effects of carbohydrates. *European Journal of Clinical Nutrition*, 61(Suppl 1), S40–S74.
4. Livesey, G., & Elia, M. (1988). Estimation of energy expenditure, net carbohydrate utilization, and net fat oxidation and synthesis by indirect calorimetry: Evaluation of errors with special reference to the detailed composition of fuels. *American Journal of Clinical Nutrition*, 47(4), 608–628.
5. Rolfe, D.F., & Brown, G.C. (1997). Cellular energy utilization and molecular origin of standard metabolic rate in mammals. *Physiology Review*, 77(3), 731–758.
6. Ravussin, E., Lillioja, S., Anderson, T.E., Christin, L., Bogardus, C. (1986). Determinants of 24-hour energy expenditure in man. Methods and results using a respiratory chamber. *Journal of Clinical Investigation*, 78(6), 1568–1578.
7. Roza, A.M., & Shizgal, H.M. (1984). The Harris Benedict equation reevaluated: Resting energy requirements and the body cell mass. *American Journal of Clinical Nutrition*, 40, 168–182.
8. Schofield, W.N. (1985). Predicting basal metabolic rate, new standards and review of previous work. *Human Nutrition – Clinical Nutrition*, 39(Suppl 1), 5–41.
9. Cunningham, J.J. (1991). Body composition as a determinant of energy expenditure: A synthetic review and a proposed general prediction equation. *American Journal of Clinical Nutrition*, 54(6), 963–969.
10. Human energy requirements: Report of a joint FAO/ WHO/UNU Expert Consultation. (2005). *Food Nutrition Bulletin*, 26(1), 166.
11. Henry, C.J. (2005). Basal metabolic rate studies in humans: Measurement and development of new equations. *Public Health Nutrition*, 8(7A), 1133–1152.
12. Ramirez-Zea, M. (2005). Validation of three predictive equations for basal metabolic rate in adults. *Public Health Nutrition*, 8(7A), 1213–1228.
13. Johnstone, A.M., Murison, S.D., Duncan, J.S., Rance, K.A., Speakman, J.R. (2005). Factors influencing variation in basal metabolic rate include fat-free mass, fat mass, age, and circulating thyroxine but not sex, circulating leptin, or triiodothyronine. *American Journal of Clinical Nutrition*, 82(5), 941–948.
14. Keys, A., Taylor, H.L., Grande, F. (1973). Basal metabolism and age of adult man. *Metabolism*, 22(4), 579–587.
15. Frisard, M.I., Fabre, J.M., Russell, R.D., et al. (2007). Physical activity level and physical functionality in nonagenarians compared to individuals aged 60–74 years. *Journal of Gerontology A: Biological Science Medicine Science*, 62(7), 783–788.
16. Bosy-Westphal, A., Eichhorn, C., Kutzner, D., Illner, K., Heller, M., Muller, M.J. (2003). The age-related decline in resting energy expenditure in humans is due to the loss of fat-free mass and to alterations in its metabolically active components. *Journal of Nutrition*, 133(7), 2356–2362.
17. Heymsfield, S.B., Gallagher, D., Kotler, D.P., Wang, Z., Allison, D.B., Heshka, S. (2002). Body-size dependence of resting energy expenditure can be attributed to nonenergetic homogeneity of fat-free mass. *American Journal of Physiology: Endocrinology Metabolism*, 282(1), E132–E138.
18. Bosy-Westphal, A., Reinecke, U., Schlorke, T., et al. (2004). Effect of organ and tissue masses on resting energy expenditure in underweight, normal weight and obese adults. *International Journal of Obesity Related Metabolic Disorder*, 28(1), 72–79.
19. Saad, M.F., Alger, S.A., Zurlo, F., Young, J.B., Bogardus, C., Ravussin, E. (1991). Ethnic differences in sympathetic nervous system-mediated energy expenditure. *American Journal of Physiology*, 261(6 Pt 1), E789–E794.
20. Schwartz, R.S., Jaeger, L.F., Veith, R.C. (1988). Effect of clonidine on the thermic effect of feeding in humans. *American Journal of Physiology*, 254(1 Pt 2), R90–R94.

21. Spraul, M., Ravussin, E., Fontvieille, A.M., Rising, R., Larson, D.E., Anderson, E.A. (1993). Reduced sympathetic nervous activity. A potential mechanism predisposing to body weight gain. *Journal of Clinical Investigation*, 92(4), 1730–1735.
22. Rising, R., Keys, A., Ravussin, E., Bogardus, C. (1992). Concomitant interindividual variation in body temperature and metabolic rate. *American Journal of Physiology*, 263(4 Pt 1), E730–E734.
23. Bogardus, C., Lillioja, S., Ravussin, E., et al. (1986). Familial dependence of the resting metabolic rate. *New England Journal of Medicine*, 315(2), 96–100.
24. Bouchard, C., Tremblay, A., Nadeau, A., et al. (1989). Genetic effect in resting and exercise metabolic rates. *Metabolism*, 38(4), 364–370.
25. Weststrate, J.A. (1993). Resting metabolic rate and diet-induced thermogenesis: A methodological reappraisal. *American Journal of Clinical Nutrition*, 58(5), 592–601.
26. Tataranni, P.A., Larson, D.E., Snitker, S., Ravussin, E. (1995). Thermic effect of food in humans: Methods and results from use of a respiratory chamber. *American Journal of Clinical Nutrition*, 61(5), 1013–1019.
27. Brundin, T., Thorne, A., Wahren, J. (1992). Heat leakage across the abdominal wall and meal-induced thermogenesis in normal-weight and obese subjects. *Metabolism*, 41(1), 49–55.
28. Speakman, J.R. (1998). The history and theory of the doubly labeled water technique. *American Journal of Clinical Nutrition*, 68(4), 932S–938S.
29. Schulz, L.O., & Schoeller, D.A. (1994). A compilation of total daily energy expenditures and body weights in healthy adults. *American Journal of Clinical Nutrition*, 60(5), 676–681.
30. Black, A.E., Coward, W.A., Cole, T.J., Prentice, A.M. (1996). Human energy expenditure in affluent societies: An analysis of 574 doubly-labelled water measurements. *European Journal of Clinical Nutrition*, 50(2), 72–92.
31. Jequier, E., Acheson, K., Schutz, Y. (1987). Assessment of energy expenditure and fuel utilization in man. *Annual Review of Nutrition*, 7, 187–208.
32. Schoeller, D.A. (1999). Recent advances from application of doubly labeled water to measurement of human energy expenditure. *Journal of Nutrition*, 129(10), 1765–1768.
33. Ravussin, E., Harper, I.T., Rising, R., Bogardus, C. (1991). Energy expenditure by doubly labeled water: Validation in lean and obese subjects. *American Journal of Physiology*, 261(3 Pt 1), E402–E409.
34. Redman, L.M., Heilbronn, L.K., Martin, C.K., et al. (2009). Metabolic and behavioral compensations in response to caloric restriction: Implications for the maintenance of weight loss. *PLoS ONE*, 4(2), e4377.
35. Rosenbaum, M., Goldsmith, R., Bloomfield, D., et al. (2005). Low-dose leptin reverses skeletal muscle, autonomic, and neuroendocrine adaptations to maintenance of reduced weight. *Journal of Clinical Investigation*, 115(12), 3579–3586.
36. Stock, M.J. (1999). Gluttony and thermogenesis revisited. *International Journal of Obesity Related Metabolic Disorder*, 23(11), 1105–1117.
37. Flatt, J.P., Ravussin, E., Acheson, K.J., Jequier, E. (1985). Effects of dietary fat on postprandial substrate oxidation and on carbohydrate and fat balances. *Journal of Clinical Investigation*, 76(3), 1019–1024.
38. Smith, S.R., de Jonge, L., Zachwieja, J.J., et al. (2000). Fat and carbohydrate balances during adaptation to a high-fat. *American Journal of Clinical Nutrition*, 71(2), 450–457.
39. Davy, K.P., Horton, T., Davy, B.M., Bessesen, D., Hill, J.O. (2001). Regulation of macronutrient balance in healthy young and older men. *International Journal of Obesity Related Metabolic Disorder*, 25(10), 1497–1502.
40. Hill, J.O., Peters, J.C., Reed, G.W., Schlundt, D.G., Sharp, T., Greene, H.L. (1991). Nutrient balance in humans: Effects of diet composition. *American Journal of Clinical Nutrition*, 54(1), 10–17.
41. Schrauwen, P., van Marken Lichtenbelt, W.D., Saris, W.H., Westerterp, K.R. (1997). The adaptation of nutrient oxidation to nutrient intake on a high-fat diet. *Zeitschrift Fur Ernährungswissenschaft*, 36(4), 306–309.
42. Shetty, P.S., Prentice, A.M., Goldberg, G.R., et al. (1994). Alterations in fuel selection and voluntary food intake in response to isoenergetic manipulation of glycogen stores in humans. *American Journal of Clinical Nutrition*, 60(4), 534–543.

43. Abbott, W.G., Howard, B.V., Christin, L., et al. (1988). Short-term energy balance: Relationship with protein, carbohydrate, and fat balances. *American Journal of Physiology*, 255(3 Pt 1), E332–E337.
44. Chascione, C., Elwyn, D.H., Davila, M., Gil, K.M., Askanazi, J., Kinney, J.M. (1987). Effect of carbohydrate intake on de novo lipogenesis in human adipose tissue. *American Journal of Physiology*, 253(6 Pt 1), E664–E669.
45. Acheson, K.J., Schutz, Y., Bessard, T., Anantharaman, K., Flatt, J.P., Jequier, E. (1988). Glycogen storage capacity and de novo lipogenesis during massive carbohydrate overfeeding in man. *American Journal of Clinical Nutrition*, 48(2), 240–247.
46. Aarsland, A., Chinkes, D., Wolfe, R.R. (1997). Hepatic and whole-body fat synthesis in humans during carbohydrate overfeeding. *American Journal of Clinical Nutrition*, 65(6), 1774–1782.
47. Hellerstein, M.K. (1999). De novo lipogenesis in humans: Metabolic and regulatory aspects. *European Journal of Clinical Nutrition*, 53(Suppl 1), S53–S65.
48. Bray, G.A. (1991). Treatment for obesity: A nutrient balance/nutrient partition approach. *Nutrition Reviews*, 49(2), 33–45.
49. Schutz, Y., Flatt, J.P., Jequier, E. (1989). Failure of dietary fat intake to promote fat oxidation: A factor favoring the development of obesity. *American Journal of Clinical Nutrition*, 50(2), 307–314.
50. Frayn, K.N. (2002). Adipose tissue as a buffer for daily lipid flux. *Diabetologia*, 45(9), 1201–1210.
51. Zurlo, F., Lillioja, S., Esposito-Del Puente, A., et al. (1990). Low ratio of fat to carbohydrate oxidation as predictor of weight gain: Study of 24-h RQ. *American Journal of Physiology*, 259(5 Pt 1), E650–E657.
52. Schrauwen, P., & Westerterp, K.R. (2000). The role of high-fat diets and physical activity in the regulation of body weight. *British Journal of Nutrition*, 84(4), 417–427.
53. Ravussin, E., & Swinburn, B.A. (1993). Metabolic predictors of obesity: Cross-sectional versus longitudinal data. *International Journal of Obesity Related Metabolic Disorder*, 17(Suppl 3), S28–S31; discussion S41–S22.
54. Knowler, W.C., Pettitt, D.J., Saad, M.F., et al. (1991). Obesity in the Pima Indians: Its magnitude and relationship with diabetes. *American Journal of Clinical Nutrition*, 53(6 Suppl), 1543S–1551S.
55. Weyer, C., Snitker, S., Bogardus, C., Ravussin, E. (1999). Energy metabolism in African Americans: Potential risk factors for obesity. *American Journal of Clinical Nutrition*, 70(1), 13–20.
56. Melby, C.L., Ho, R.C., Jeckel, K., Beal, L., Goran, M., Donahoo, W.T. (2000). Comparison of risk factors for obesity in young, nonobese African-American and Caucasian women. *International Journal of Obesity Related Metabolic Disorder*, 24(11), 1514–1522.
57. Ravussin, E., Lillioja, S., Knowler, W.C., et al. (1988). Reduced rate of energy expenditure as a risk factor for body-weight gain. *New England Journal of Medicine*, 318(8), 467–472.
58. Astrup, A., Gotzsche, P.C., van de Werken, K., et al. (1999). Meta-analysis of resting metabolic rate in formerly obese subjects. *American Journal of Clinical Nutrition*, 69(6), 1117–1122.
59. Amatruda, J.M., Statt, M.C., Welle, S.L. (1993). Total and resting energy expenditure in obese women reduced to ideal body weight. *Journal of Clinical Investigation*, 92(3), 1236–1242.
60. Weinsier, R.L., Nelson, K.M., Hensrud, D.D., Darnell, B.E., Hunter, G.R., Schutz, Y. (1995). Metabolic predictors of obesity. Contribution of resting energy expenditure, thermic effect of food, and fuel utilization to four-year weight gain of post-obese and never-obese women. *Journal of Clinical Investigation*, 95(3), 980–985.
61. Prentice, A.M., & Jebb, S.A. (1995). Obesity in Britain: Gluttony or sloth? *British Medical Journal*, 311(7002), 437–439.
62. Westerterp, K.R., & Speakman, J.R. (2008). Physical activity energy expenditure has not declined since the 1980s and matches energy expenditures of wild mammals. *International Journal of Obesity (London)*, 32(8), 1256–1263.
63. Swinburn, B.A., Jolley, D., Kremer, P.J., Salbe, A.D., Ravussin, E. (2006). Estimating the effects of energy imbalance on changes in body weight in children. *American Journal of Clinical Nutrition*, 83(4), 859–863.

64. Swinburn, B.A., Sacks, G., Lo, S.K., et al. (2009). Estimating the changes in energy flux that characterize the rise in obesity prevalence. *American Journal of Clinical Nutrition*, 89(6), 1723–1728.
65. Schoeller, D.A., Shay, K., Kushner, R.F. (1997). How much physical activity is needed to minimize weight gain in previously obese women? *American Journal of Clinical Nutrition*, 66(3), 551–556.
66. Zurlo, F., Ferraro, R.T., Fontvielle, A.M., Rising, R., Bogardus, C., Ravussin, E. (1992). Spontaneous physical activity and obesity: Cross-sectional and longitudinal studies in Pima Indians. *American Journal of Physiology*, 263(2 Pt 1), E296–E300.
67. Levine, J.A., Eberhardt, N.L., Jensen, M.D. (1999). Role of nonexercise activity thermogenesis in resistance to fat gain in humans. *Science* 283(5399), 212–214.
68. Johannsen, D.L., & Ravussin, E. (2008). Spontaneous physical activity: Relationship between fidgeting and body weight control. *Current Opinion in Endocrinology, Diabetes, Obesity*, 15(5), 409–415[AU1].
69. Toubro, S., Sorensen, T.I., Hindsberger, C., Christensen, N.J., Astrup, A. (1998). Twenty-four-hour respiratory quotient: The role of diet and familial resemblance. *Journal of Clinical Endocrinology and Metabolism*, 83(8), 2758–2764.
70. Astrup, A., Buemann, B., Christensen, N.J., Toubro, S. (1994). Failure to increase lipid oxidation in response to increasing dietary fat content in formerly obese women. *American Journal of Physiology*, 266(4 Pt 1), E592–E599.
71. Larson, D.E., Ferraro, R.T., Robertson, D.S., Ravussin, E. (1995). Energy metabolism in weight-stable postobese individuals. *American Journal of Clinical Nutrition*, 62(4), 735–739.
72. Froidevaux, F., Schutz, Y., Christin, L., Jequier, E. (1993). Energy expenditure in obese women before and during weight loss, after refeeding, and in the weight-relapse period. *American Journal of Clinical Nutrition*, 57(1), 35–42.
73. Flatt, J.P. (1987). Dietary fat, carbohydrate balance, and weight maintenance: Effects of exercise. *American Journal of Clinical Nutrition*, 45(1 Suppl), 296–306.
74. Pannacciulli, N., Salbe, A.D., Ortega, E., Venti, C.A., Bogardus, C., Krakoff, J. (2007). The 24-h carbohydrate oxidation rate in a human respiratory chamber predicts ad libitum food intake. *American Journal of Clinical Nutrition*, 86(3), 625–632.
75. Eckel, R.H., Hernandez, T.L., Bell, M.L., et al. (2006). Carbohydrate balance predicts weight and fat gain in adults. *American Journal of Clinical Nutrition*, 83(4), 803–808.
76. Sims, E.A., Danforth, E., Jr., Horton, E.S., Bray, G.A., Glennon, J.A., Salans, L.B. (1973). Endocrine and metabolic effects of experimental obesity in man. *Recent Progress in Hormone Research*, 29, 457–496.
77. Bouchard, C., Tremblay, A., Despres, J.P., et al. (1990). The response to long-term overfeeding in identical twins. *New England Journal of Medicine*, 322(21), 1477–1482.
78. Harper, M.E., Green, K., Brand, M.D. (2008). The efficiency of cellular energy transduction and its implications for obesity. *Annual Review of Nutrition*, 28, 13–33.
79. Lowell, B.B., & Spiegelman, B.M. (2000). Towards a molecular understanding of adaptive thermogenesis. *Nature*, 404(6778), 652–660.
80. Rosenbaum, M., Vandenborne, K., Goldsmith, R., et al. (2003). Effects of experimental weight perturbation on skeletal muscle work efficiency in human subjects. *American Journal of Physiology – Regulatory, Integrative and Comparative Physiology*, 285(1), R183–R192.
81. Weyer, C., Pratley, R.E., Salbe, A.D., Bogardus, C., Ravussin, E., Tataranni, P.A. (2000). Energy expenditure, fat oxidation, and body weight regulation: A study of metabolic adaptation to long-term weight change. *Journal of Clinical Endocrinology and Metabolism*, 85(3), 1087–1094.
82. de Jonge, L., & Bray, G.A. (1997). The thermic effect of food and obesity: A critical review. *Obesity Research*, 5(6), 622–631.
83. Christin, L., O’Connell, M., Bogardus, C., Danforth, E., Jr., Ravussin, E. (1993). Norepinephrine turnover and energy expenditure in Pima Indian and white men. *Metabolism*, 42(6), 723–729.
84. Snitker, S., Tataranni, P.A., Ravussin, E. (1998). Respiratory quotient is inversely associated with muscle sympathetic nerve activity. *Journal of Clinical Endocrinology and Metabolism*, 83(11), 3977–3979.

85. Astrup, A., Buemann, B., Gluud, C., Bennett, P., Tjur, T., Christensen, N. (1995). Prognostic markers for diet-induced weight loss in obese women. *International Journal of Obesity Related Metabolic Disorder*, 19(4), 275–278.
86. Tataranni, P.A., Young, J.B., Bogardus, C., Ravussin, E. (1997). A low sympathoadrenal activity is associated with body weight gain and development of central adiposity in Pima Indian men. *Obesity Research*, 5(4), 341–347.
87. Loos, R.J., & Bouchard, C. (2008). FTO: The first gene contributing to common forms of human obesity. *Obesity Review*, 9(3), 246–250.
88. Loos, R.J., Lindgren, C.M., Li, S., et al. (2008). Common variants near MC4R are associated with fat mass, weight and risk of obesity. *Nature Genetics*, 40(6), 768–775.
89. Cripps, R.L., Archer, Z.A., Mercer, J.G., Ozanne, S.E. (2007). Early life programming of energy balance. *Biochemical Society Transactions*, 35(Pt 5), 1203–1204.
90. Uauy, R., Kain, J., Mericq, V., Rojas, J., Corvalan, C. (2008). Nutrition, child growth, and chronic disease prevention. *Annals of Medicine*, 40(1), 11–20.
91. Seale, P., & Lazar, M.A. (2009). Brown fat in humans: Turning up the heat on obesity. *Diabetes*, 58(7), 1482–1484.



## Chapter 2

# Intermediary Metabolism of Carbohydrate, Protein, and Fat

Keith Tornheim and Neil B. Ruderman

### Introduction

The cause of obesity in an individual may involve many factors, both genetic and environmental, including fat cell production and development, appetite and energy regulation. However, the excessive accumulation of triglyceride (triacylglycerol) that characterizes obesity and its effects on the use and storage of various fuels (glucose, fatty acids, and amino acids) are clearly the result of abnormalities in metabolism. The three fatty acyl chains in a triglyceride molecule can be derived either from fats in the diet or de novo fatty acid biosynthesis from acetyl-CoA originating from carbohydrate or protein/amino acid metabolism (though de novo fatty acid synthesis is considerably less important in humans than in rodent models). The glycerol component of the triglyceride generally is derived from carbohydrate metabolism, though potentially it could also come from glucogenic amino acids. Triglyceride stores are also broken down as needed for energy production, depending in part on the availability of the other fuels; however, adipose tissue triglyceride is normally the largest energy reserve of the body. In addition, although fatty acids cannot be converted to glucose, the glycerol portion of triglyceride is an important gluconeogenic substrate during a prolonged fast, as it diminishes the need for breakdown of muscle protein for this purpose. Thus, triglyceride storage is intimately related to the whole of intermediary metabolism. The objective of this chapter is to present a brief description of the pathways of carbohydrate, protein, and fat metabolism and their interactions and regulatory mechanisms. The latter part of the chapter will focus on new insights that have been obtained from studies of genetic models with ablation or modification of particular enzymes or hormone receptors often in a tissue-specific manner. In addition, we will discuss the enzyme AMP-activated protein kinase (AMPK), which has recently been identified as a cellular mediator of many events in intermediary metabolism and whose dysregulation may be a cause of disorders associated with the metabolic syndrome and a target for their therapy.

---

K. Tornheim (✉)

Department of Biochemistry, Boston University School of Medicine, Silvio Conte Building,  
Office: K109, 72 E. Concord Street, Boston, MA 02118, USA  
e-mail: tornheim@bu.edu



## ***Principles of Metabolic Regulation***

Regulation of metabolism is ultimately regulation of the enzyme catalysts in pathways. There are various kinds of regulation to be considered, all of which are important and often interact in intermediary metabolism. First, the amount of an enzyme can be increased or decreased, by changing its rate of synthesis at the transcriptional, translational, or post-translational stage, or its rate of degradation. Second, changes in the concentration of the substrate (provided it is at or below the  $K_M$ ) can affect the rate of the reaction. Third, an enzyme can be regulated by metabolites that are inhibitors or activators binding to its catalytic or allosteric/regulatory sites. Fourth, an enzyme can be inhibited or activated by covalent modification, in particular by phosphorylation by protein kinases, some of which mediate hormonal actions. In addition, the importance of other types of covalent modification, such as acetylation, acylation, adenylation, and methylation, is increasingly recognized. Fifth, an enzyme can be inhibited or activated by protein–protein interactions with specific protein regulators. Sixth, an enzyme's functional activity can be affected by compartmentation within the cell and thus controlled by translocation from one area to another. Finally, different tissues may exhibit differences in metabolism despite identical or nearly identical pathways, because of the presence of isozymes, that is, enzymes that catalyze the same reaction but are different proteins and thus can have different kinetic and regulatory properties, due to differences in the catalytic site and in regulatory sites for noncovalent and covalent regulation.

Nutritional and hormonal states are intertwined in affecting intermediary metabolism. Food intake raises the level of the key peptide hormone insulin, which is synthesized in and secreted from the  $\beta$ -cells of the pancreatic islets primarily in response to glucose. However, fatty acids and some amino acids can potentiate the secretory response, as can certain gut hormones such as glucagon-like peptide (GLP)-1. Insulin is the primary regulator of whole body carbohydrate metabolism. Increases in its concentration activate glucose uptake in muscle and fat cells, inhibit glucose synthesis (gluconeogenesis) and glucose output by the liver, and stimulate glucose storage into glycogen, whereas decreases in its concentration have the opposite effect. In addition, insulin promotes other kinds of fuel storage, by stimulating triglyceride synthesis and inhibiting lipolysis (triglyceride breakdown) and by similar effects on protein synthesis and degradation. A number of counter-regulatory hormones oppose the action of insulin, including the peptide hormone glucagon, which is secreted from the  $\alpha$ -cells of the pancreatic islets in response to low blood glucose and promotes hepatic glycogen breakdown and gluconeogenesis as well as adipose tissue lipolysis, and the catecholamine epinephrine (adrenaline), which is secreted from the adrenal glands in response to various excitatory stimuli and promotes glycogen breakdown and lipolysis. In subjects with diabetes, a lack of insulin or resistance to its action leads to high blood glucose levels due to impaired glucose disposal (primarily into muscle glycogen) and unrestrained hepatic glucose output. Also contributing to these abnormalities are excessive lipolysis and hence circulating fatty acid levels and increased protein

breakdown. Obesity, which is often although not always associated with elevations in circulating free fatty acids as well as inflammatory cytokines, is thought to contribute to the development of diabetes by causing insulin resistance, thus increasing the amount of insulin necessary for glucose homeostasis.

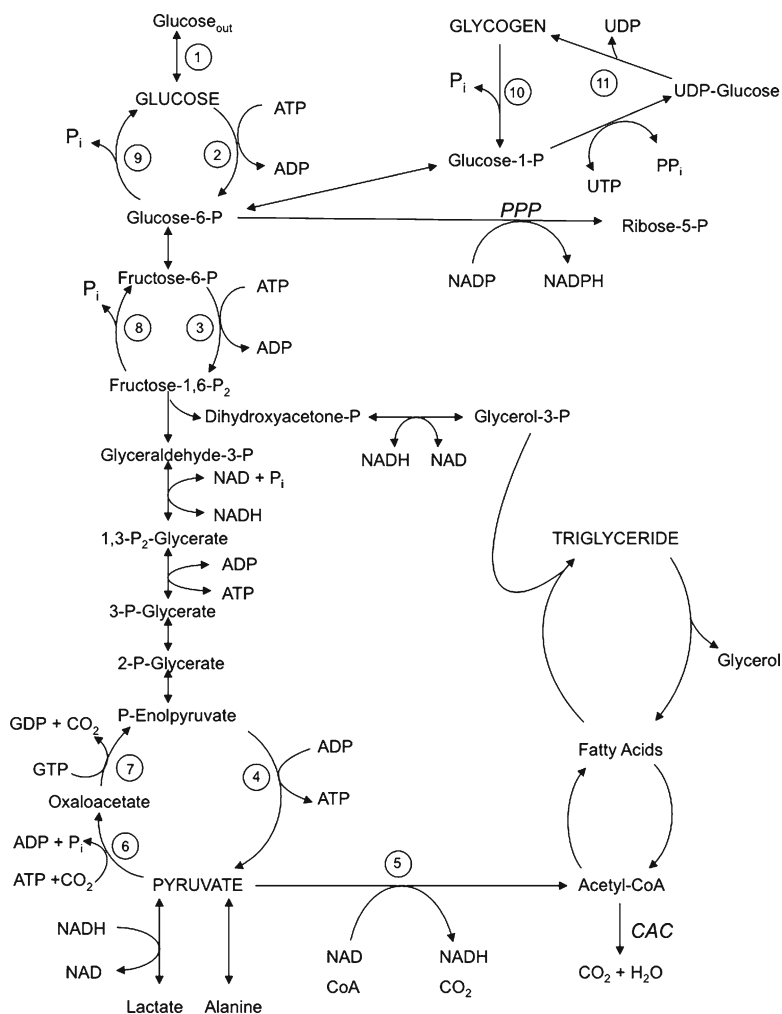
## Carbohydrate and Energy Metabolism

### *Glucose Transport and Phosphorylation*

Glucose enters most cells through glucose transporters that allow passive diffusion down the concentration gradient from the blood. Glut1 is present in most cells. Glut4 is the dominant transporter in muscle and fat cells. It is stimulated by insulin and also by anoxia or low energy state in a process that involves translocation of Glut4 in intracellular vesicles to the plasma membrane. In liver and pancreatic  $\beta$ -cells, the dominant isoform is Glut2, a high capacity transporter that essentially equilibrates glucose, so that its cytoplasmic concentration is close to that in plasma. Liver also releases glucose, derived from breakdown of the storage polymer glycogen and from synthesis by the pathway of gluconeogenesis. Thus, movement of glucose across the hepatocyte plasma membrane is functionally bidirectional. Energy-linked glucose transporters are found in intestinal and kidney cells where glucose must be taken up against its concentration gradient. For this purpose, these cells utilize the sodium gradient across the plasma membrane, which is established by expulsion of sodium by the sodium–potassium ATPase.

Once glucose enters the cell, it can be trapped by phosphorylation by hexokinase, an enzyme that uses ATP and produces glucose 6-phosphate and ADP. In liver, the dominant hexokinase isoform is glucokinase, or Type 4 hexokinase, which has a high  $K_M$  for glucose of about 10 mM, in comparison to basal blood (plasma) glucose levels of about 5 mM. This is of especial note because the portal vein brings nutrients directly to the liver from the intestine; therefore, a rise in portal vein glucose resulting from carbohydrate ingestion readily increases its metabolism in liver. A glucokinase variant is also the major hexokinase isozyme in pancreatic  $\beta$ -cells where it serves as the “glucose sensor” that promotes the increase in glucose metabolism that causes increased insulin release and synthesis [1].

Glucose 6-phosphate is a central branch point in carbohydrate metabolism (Fig. 1). It can be further metabolized in the glycolytic pathway to pyruvate, which in turn can be converted to lactate or alanine or oxidized to acetyl-CoA which can enter the citric acid cycle or be used for fatty acid synthesis. Glycolysis also supplies the glycerol portion of the triglyceride molecule via conversion of the glycolytic intermediate dihydroxyacetone phosphate to glycerol 3-phosphate. Alternatively, glucose 6-phosphate can be converted to glucose 1-phosphate for glycogen synthesis or metabolized in the pentose phosphate pathway, to generate the ribose 5-phosphate needed for nucleotide/nucleic acid synthesis and the NADPH needed for many



**Fig. 1** Glucose and glycogen metabolism and the connections to the pentose phosphate pathway (PPP), the citric acid cycle (CAC), and fatty acid and triglyceride metabolism. Important regulatory steps are 1, glucose transport, notably by insulin-stimulated Glut 4 in muscle and fat; 2, hexokinase (glucokinase in liver and pancreatic  $\beta$ -cells); 3, phosphofructokinase; 4, pyruvate kinase; 5, pyruvate dehydrogenase; 6, pyruvate carboxylase; 7, phosphoenolpyruvate carboxykinase; 8, fructose 1,6-bisphosphatase; 9, glucose 6-phosphatase; 10, phosphorylase; 11, glycogen synthase

purposes including the synthesis of fatty acids. In addition to its formation by the hexokinase/glucokinase reaction, glucose 6-phosphate can be produced via glucose 1-phosphate following glycogen breakdown and from pyruvate, lactate, alanine, or citric acid cycle intermediates by the process of gluconeogenesis. Glucose 6-phosphatase, present in significant amounts only in the fully gluconeogenic tissues of liver and kidney cortex, can hydrolyze glucose 6-phosphate to yield free glucose.

## ***Glycolysis***

The further metabolism of glucose 6-phosphate in the glycolytic pathway begins with its conversion to fructose 6-phosphate by phosphoglucose isomerase (Fig. 1). Phosphofructokinase then catalyzes the phosphorylation of fructose 6-phosphate to fructose 1,6-bisphosphate. Fructose 1,6-bisphosphate is cleaved by aldolase into the two triose phosphates glyceraldehyde 3-phosphate and dihydroxyacetone phosphate. The latter are interconverted by triose phosphate isomerase. Glycolysis continues from glyceraldehyde 3-phosphate with its conversion to the high energy phosphate donor 1,3-bisphosphoglycerate by glyceraldehyde-3-phosphate dehydrogenase, using NAD and  $P_i$ . 1,3-Bisphosphoglycerate is then used to phosphorylate ADP to ATP by phosphoglycerate kinase (named for the reverse reaction). The resulting 3-phosphoglycerate is converted to 2-phosphoglycerate by phosphoglycerate mutase and then to the second high energy phosphate donor phosphoenolpyruvate by enolase. Phosphoenolpyruvate is then used to phosphorylate ADP to ATP by pyruvate kinase (also named for the reverse reaction). This makes for a net of 2 ATP produced in glycolysis per glucose molecule, since 1 ATP is used in the hexokinase reaction and 1 in the phosphofructokinase reaction, but four are synthesized in the lower glycolytic pathway from the two triose phosphates. Pyruvate is converted to lactate in the lactate dehydrogenase reaction, if it must be used to reoxidize the NADH produced in the glyceraldehyde-3-phosphate dehydrogenase reaction; however, if the cytosolic NADH can be reoxidized by shuttles transferring the reducing equivalents to the mitochondrial electron transport chain, then pyruvate is available to be further oxidized in the pyruvate dehydrogenase (PDH) reaction.

Hexokinase is usually considered the first enzyme in glycolysis. However, as indicated above, its product glucose 6-phosphate can be used in other pathways, notably glycogen synthesis and the pentose phosphate pathway. Therefore, phosphofructokinase is the first non-equilibrium step that is purely glycolytic; it is an important control point, regulated by a number of metabolites that reflect the fuel and energy state of the cell. Phosphofructokinase is inhibited by ATP and citrate and activated by ADP, AMP,  $P_i$ , fructose 1,6-bisphosphate, and fructose 2,6-bisphosphate. ATP is a substrate for phosphofructokinase because the enzyme is a kinase; however, ATP is also an allosteric inhibitor binding at a separate regulatory site. This is an example of classic feedback inhibition of an early step in a pathway by an ultimate end product, as one of the major functions of glycolysis is to produce ATP. Muscular contraction hydrolyzes ATP to ADP and  $P_i$ ; thus these rise and activate as ATP falls and inhibits less. ADP is a more sensitive indicator of ATP usage than ATP itself, as ATP levels in muscle are ten times that of ADP. Thus if 10% of ATP is used, the concentration of ADP doubles. AMP is an even more sensitive indicator of ATP usage, since the AMP concentration varies as the square of the ADP concentration because of equilibration of the adenine nucleotides in the myokinase (or adenylate kinase) reaction ( $AMP + ATP \leftrightarrow 2 ADP$ ), and the principal emphasis has been on AMP as the indicator of the energy state. AMP is also a major regulator of other pathways via AMPK, in particular fatty acid oxidation (see below).

Citrate inhibition of phosphofructokinase is rationalized as mediating the effect of an alternative fuel, fatty acids or ketone bodies, to spare glucose usage, as part of a glucose-fatty acid cycle. This was originally proposed by Randle in heart but may also function in some circumstances in skeletal muscle and brain [2].  $\beta$ -Oxidation of fatty acids produces acetyl-CoA inside the mitochondrion, where it is converted to citrate, which can then be transported out to the cytoplasm to inhibit phosphofructokinase.

The phosphofructokinase activator, fructose 2,6-bisphosphate is particularly important in regulation of liver glycolysis/gluconeogenesis. It is made, and also degraded, by the bifunctional enzyme phosphofructo-2-kinase/fructose-2,6-bisphosphatase (PFKFB). The liver isoform is regulated by phosphorylation at a single site that inhibits the kinase activity and activates the phosphatase activity. This phosphorylation, by the cyclic-AMP dependent protein kinase (PKA) in response to glucagon, thus causes a decrease in fructose 2,6-bisphosphate and hence decreased activity of the glycolytic enzyme phosphofructokinase, as well as decreased inhibition of the opposing gluconeogenic enzyme fructose 1,6-bisphosphatase, thus promoting net gluconeogenesis. The muscle isoform is a splice variant lacking the phosphorylation site and therefore is not inhibited when PKA is activated to promote glycogenolysis, say in response to epinephrine. In contrast, the heart isoform is activated by phosphorylation, in response to insulin or by AMPK. Recent work has proposed an important role for the inducible isoform PFKFB3 in fat cells, to promote glycerol 3-phosphate production for triglyceride synthesis [3, 4]. Like the heart isoform, PFKFB3 is activated by insulin.

Fructose 1,6-bisphosphate was early recognized as an activator of phosphofructokinase, a somewhat puzzling property because it is a product of the reaction. Once the more potent fructose 2,6-bisphosphate was discovered, it was thought that fructose 1,6-bisphosphate activation was not so important, that perhaps the hexose bisphosphate binding site just could not be made that specific. However, whereas there is only about tenfold difference in sensitivity for muscle type phosphofructokinase, there is a 1,000-fold difference or more for the other phosphofructokinase isoforms. This suggests that the product activation of muscle type phosphofructokinase, which can lead to oscillatory behavior of glycolysis, might have some special role. It has been suggested that this may underlie the normal oscillatory secretion of insulin in the pancreatic  $\beta$ -cell [5]. More recently, it has been found that phosphofructokinase-M deficient mice have greatly reduced fat stores, despite the presence of the other two isoforms in fat, suggesting the possible importance of glycolytic oscillations for glycerol 3-phosphate generation [6].

Hexokinase can follow the lead of phosphofructokinase, because hexokinase is inhibited by glucose 6-phosphate. This is not product inhibition at the active site, but rather is mediated by binding to a separate regulatory site, apparently created by gene duplication. (Glucokinase is not sensitive to glucose 6-phosphate inhibition and is half the size of hexokinase, because it lacks this duplicated portion.) This allows hexokinase to be responsive to the demand for glucose 6-phosphate. Thus, if phosphofructokinase is inhibited, then the concentration of glucose 6-phosphate (in equilibrium with fructose 6-phosphate) will rise and inhibit hexokinase; on the

other hand, if phosphofructokinase is activated, such as by muscular contraction, and uses fructose 6-phosphate, then the concentration of glucose 6-phosphate will also drop and hexokinase will be deinhibited. Use of glucose 6-phosphate for glycogen synthesis could also deinhibit hexokinase.

## ***Fructose***

Fructose metabolism is of increasing interest because of the now widespread incorporation of high fructose corn syrup in beverages and other foodstuffs. The advantage to the food industry is that free fructose is even more potent as a sweetener than common sugar (sucrose, a glucose–fructose disaccharide). Fructose is largely metabolized in the liver, where it bypasses the limiting glycolytic steps of glucokinase/hexokinase and phosphofructokinase. It is phosphorylated by a specific fructokinase to fructose 1-phosphate, which is then cleaved by liver aldolase to dihydroxyacetone phosphate and glyceraldehyde. (Muscle aldolase is relatively specific for fructose 1,6-bisphosphate, in contrast to the liver isoform.) The glyceraldehyde is then phosphorylated by triokinase to enter the glycolytic pathway as well.

Fructose does not compete with glucose for metabolism, but rather fructose increases glucose metabolism in liver. This surprising effect has been explained as follows: There is a glucokinase inhibitory protein that binds to and sequesters glucokinase in the nucleus. Fructose 1-phosphate prevents that binding and promotes glucokinase translocation to the cytoplasm. Fructose 6-phosphate counters the action of fructose 1-phosphate.

Whether fructose consumption is contributory to obesity and the metabolic syndrome and associated diseases as a result of its distinct metabolism or simply by increasing caloric consumption is not established. Under some conditions fructose metabolism can cause excessive ATP use, leading to purine degradation and elevated uric acid levels. This has recently been proposed to be responsible for increases in hypertension and other facets of metabolic syndrome [7].

Fructose not removed from the blood stream by the liver could potentially be readily taken up and metabolized in adipose tissue, by its own transporter Glut5 and then presumably via hexokinase and phosphofructokinase. Hence, it could serve as another source of glycerol 3-phosphate for triglyceride synthesis, in addition to glucose passing through the insulin-regulated Glut4.

## ***Pyruvate Dehydrogenase and the Citric Acid Cycle***

Pyruvate dehydrogenase is an enzyme complex that oxidizes pyruvate to acetyl-CoA, using NAD and CoA and producing NADH and CO<sub>2</sub>. Regulation of this step is very important, because although acetyl-CoA can be incorporated into fatty acids

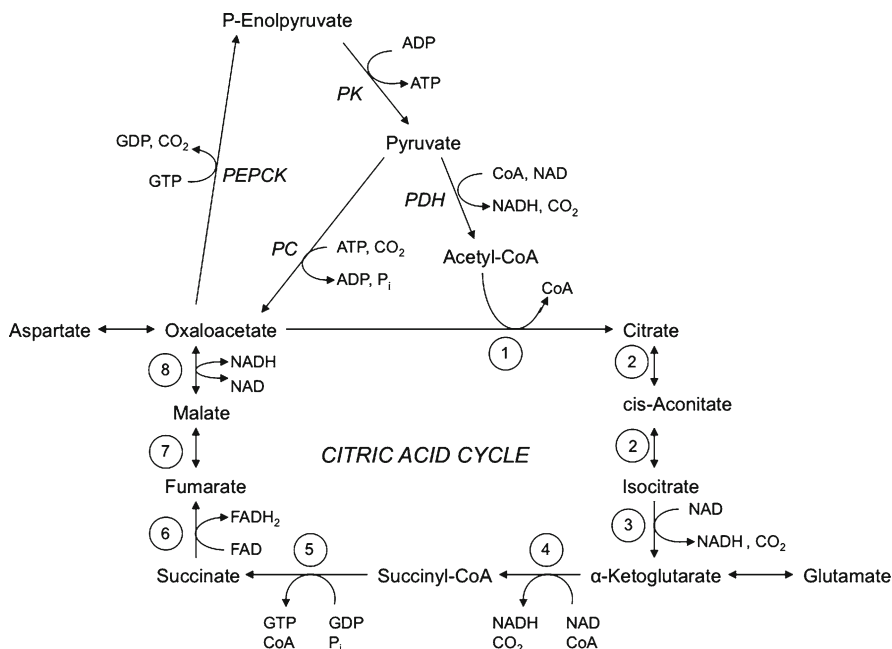
or made from fatty acids, carbon at that stage cannot be converted back to glucose. The regulation of PDH is on two levels. First, acetyl-CoA and NADH inhibit the enzyme as products at the active sites, and this is competed by the respective substrates CoA and NAD, so the acetyl-CoA/CoA and NADH/NAD ratios are the important inhibitory parameters. Second, the complex is inactivated by phosphorylation by PDH kinase (also bound in the complex), where the kinase is activated by high acetyl-CoA/CoA, NADH/NAD, and ATP/ADP ratios. The phosphorylation is reversed by a specific PDH phosphatase, also bound in the complex, which is activated by high pyruvate concentrations and by insulin. Thus, PDH is inhibited if there is already plenty of acetyl-CoA for the citric acid cycle, or NADH for the electron transport chain and oxidative phosphorylation, or ATP, the end product itself, so that pyruvate is spared for possible need for gluconeogenesis. On the other hand, if there is abundant pyruvate or high glucose, as indicated by high insulin levels, then there is no need to spare the pyruvate.

Acetyl-CoA can be completely oxidized to  $\text{CO}_2$  in the reactions of the citric acid cycle, with the reducing equivalents captured in the form of NADH and  $\text{FADH}_2$  for transfer to the electron transport chain (Fig. 2). In the first reaction (citrate synthase), acetyl-CoA is combined with oxaloacetate (four carbons) to form citrate (six carbons). Citrate is then sequentially converted to cis-aconitate and to isocitrate by aconitase. Isocitrate dehydrogenase produces  $\alpha$ -ketoglutarate (five carbons), NADH, and  $\text{CO}_2$ .  $\alpha$ -Ketoglutarate dehydrogenase, an enzyme complex analogous to PDH, produces succinyl-CoA (four carbons), NADH, and  $\text{CO}_2$ . Succinyl-CoA synthetase then converts succinyl-CoA to succinate and CoA, coupled with the synthesis of GTP from GDP and  $\text{P}_i$  or of ATP from ADP and  $\text{P}_i$ , depending on the isoform; the GTP-producing isoform is dominant in liver and may provide a connection to a GTP-requiring enzyme in gluconeogenesis, whereas the ATP-producing isoform is dominant in skeletal muscle. Succinate dehydrogenase produces fumarate and  $\text{FADH}_2$ . Fumarate equilibrates to malate through the fumarase reaction. Finally, malate dehydrogenase produces a third equivalent of NADH and regenerates oxaloacetate for another turn of the cycle. Note that acetyl-CoA for the citric acid cycle in some tissues can also come from  $\beta$ -oxidation of fatty acids, from metabolism of ketone bodies ( $\beta$ -hydroxybutyrate and acetoacetate), and from ketogenic amino acids, as well as from carbohydrate via pyruvate.

## *Gluconeogenesis*

Another major pathway using pyruvate is gluconeogenesis. Gluconeogenesis occurs principally in the liver and to a lesser extent in the kidney, and it is an important source of glucose for the brain and nervous system during brief and sustained fasting and prolonged exercise. Gluconeogenesis uses many of the same reactions as glycolysis in reverse. However, the hexokinase/glucokinase, phosphofructokinase, and pyruvate kinase reactions involve large changes in free energy and are not reversible under cellular conditions; therefore these three steps are reversed by specific gluconeogenic enzymes





**Fig. 2** The citric acid cycle and its connections to pyruvate carboxylase (PC), phosphoenolpyruvate carboxykinase (PEPCK), pyruvate kinase (PK), and pyruvate dehydrogenase (PDH). 1, citrate synthase; 2, aconitase; 3, isocitrate dehydrogenase; 4,  $\alpha$ -ketoglutarate dehydrogenase; 5, succinyl-CoA synthetase; 6, succinate dehydrogenase; 7, fumarase; 8, malate dehydrogenase. Aspartate equilibrates with oxaloacetate via the glutamate-oxaloacetate transaminase reaction. Glutamate equilibrates with  $\alpha$ -ketoglutarate via various transaminases and the glutamate dehydrogenase reaction

(see Fig. 1). The synthesis of the high energy compound phosphoenolpyruvate from pyruvate involves two steps. First, pyruvate carboxylase converts pyruvate to the citric acid cycle intermediate oxaloacetate, using CO<sub>2</sub> and the energy of ATP hydrolysis. Then phosphoenolpyruvate carboxykinase (PEPCK) converts oxaloacetate to phosphoenolpyruvate with the release of CO<sub>2</sub> and more energy input via use of GTP. The reactions from phosphoenolpyruvate to fructose 1,6-bisphosphate are readily reversible in liver. Then fructose 1,6-bisphosphate is cleaved to fructose 6-phosphate and P<sub>i</sub> by fructose-1,6-bisphosphatase. Fructose 6-phosphate equilibrates to glucose 6-phosphate, and finally glucose 6-phosphate is cleaved to glucose and P<sub>i</sub> by glucose 6-phosphatase.

The synthesis of a glucose molecule requires the use of six high energy phosphate bonds: two each at pyruvate carboxylase, PEPCK and phosphoglycerate kinase (for the two triose phosphates that are combined to form glucose). The energy for this obviously cannot come from glycolysis. Pyruvate carboxylase has a required activator, acetyl-CoA, indicating that another source of fuel is available, in particular  $\beta$ -oxidation of fatty acids. The requirement of PEPCK for its substrate GTP, produced in turn by succinyl-CoA synthetase in the citric acid cycle, suggests a regulatory link between gluconeogenesis and adequate flux through the citric acid cycle.



Control of net gluconeogenesis involves regulation of the key glycolytic and the opposing gluconeogenic enzymes. Fructose-1,6-bisphosphatase is inhibited by AMP and fructose 2,6-bisphosphate, whereas these compounds activate phosphofructokinase. Glucagon, a signal of low glucose, causes phosphorylation and inhibition of liver PFKFB and thus a decrease in fructose 2,6-bisphosphate, which promotes net gluconeogenesis. There are several mechanisms for inhibiting liver pyruvate kinase to prevent conversion of phosphoenolpyruvate back to pyruvate: the liver isoform is allosterically inhibited by ATP and by alanine (an important pyruvate/gluconeogenic precursor; see description of the alanine cycle below). It is dependent on activation by fructose 1,6-bisphosphate (and so will follow changes in phosphofructokinase activity) and also inhibited by phosphorylation by PKA in response to glucagon. Finally, the amounts of these key enzymes are adaptive, that is, affected by the nutritional and hormonal state, so the key glycolytic enzymes are increased by a high carbohydrate diet, whereas the key gluconeogenic enzymes are increased by a low carbohydrate diet or starvation. This involves regulation by insulin phosphorylation cascades and also carbohydrate responsive transcription factors. In recent years, there has been increasing emphasis on transcriptional control of PEPCK and glucose 6-phosphatase [8, 9].

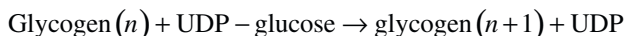
Note that the citric acid cycle is a central station or reservoir in metabolism. Pyruvate carboxylase is one way of filling up the cycle (anapleurosis), and PEPCK one way of depleting it. In addition, there is flow in and out through various other reactions such as those involving the amino acids glutamate and aspartate and their citric acid cycle counterparts  $\alpha$ -ketoglutarate and oxaloacetate, respectively (see Fig. 2). In skeletal muscle, which lacks pyruvate carboxylase, the citric acid cycle can be replenished by the conversion of aspartate to fumarate in the reactions of the purine nucleotide cycle [10].

The Cori cycle is an inter-organ cycle involving muscle glycolysis and liver gluconeogenesis. In muscle, pyruvate from glycolysis is converted to lactate in the lactate dehydrogenase reaction, together with the conversion of glycolytically generated NADH back to NAD. The lactate then moves through the blood to the liver, where it is converted back to pyruvate in the lactate dehydrogenase reaction, with the production of NADH. Gluconeogenesis in the liver then utilizes both the pyruvate and the NADH (the latter in the reversal of the glyceraldehyde 3-phosphate dehydrogenase reaction). The glucose so formed can then be exported through the blood to the muscle to complete the cycle. The alanine cycle is similar, with pyruvate being converted to alanine by transamination (see “Protein and Amino Acid Metabolism” section).

Glycerol released by fat cells during lipolysis is another gluconeogenic substrate and is especially important during prolonged starvation. The fat cell lacks glycerol kinase and so cannot recycle glycerol back into glycerol lipids. However, the released glycerol can be phosphorylated to glycerol 3-phosphate in liver and then converted to the glycolytic/gluconeogenic intermediate dihydroxyacetone phosphate by glycerol 3-phosphate dehydrogenase. This explains the ability of very obese individuals to survive a prolonged fast of many weeks despite the need for glucose for the brain.

## ***Glycogen Metabolism***

Glycogen is a branched polymer of glucose. Most glucose residues are connected by  $\alpha$ -1,4-glycosidic bonds, but branches are created by  $\alpha$ -1,6-glycosidic bonds roughly every ten residues. The branching allows for a more compact molecule and also greatly multiplies the concentration of the “nonreducing” ends that serve as substrate for addition and removal of glucosyl residues. Synthesis of glycogen by glycogen synthase uses UDP-glucose as an activated donor molecule:



UDP-glucose in turn is made by UDP-glucose pyrophosphorylase (named for the reverse reaction):



The reaction is pulled to the right by cleavage of  $\text{PP}_i$  by pyrophosphatase. Glucose 1-phosphate comes from glucose 6-phosphate through the phosphoglucomutase equilibrium reaction (Fig. 1). The UDP is phosphorylated back to UTP with ATP in the nucleoside diphosphate kinase reaction. Glycogen synthase only makes  $\alpha$ -1,4-glycosidic bonds. Branches are formed by branching enzyme taking a terminal chain of seven residues and transferring it further down, making the  $\alpha$ -1,6-glycosidic linkage.

The major stores of glycogen are in liver and muscle. Liver can have a greater concentration of glycogen per gram, but the total muscle glycogen is greater because of the much larger muscle mass. The purpose of liver glycogen is a reserve to be broken down to supply glucose to other tissues, in particular the brain, in time of need; thus liver glycogenolysis is stimulated by glucagon or epinephrine, along with gluconeogenesis. The purpose of muscle glycogen is for local glycolytic fuel for muscular contraction, and its breakdown is stimulated by epinephrine. (Note: there are no glucagon receptors on muscle.)

Most of the breakdown of glycogen is catalyzed by phosphorylase, which breaks  $\alpha$ -1,4-glycosidic bonds with phosphate, not water, so that the product is glucose 1-phosphate, together with a shortened glycogen. (There are other phosphorylase enzymes besides glycogen phosphorylase, but this one was discovered first and so is commonly called just phosphorylase.) The glucose 1-phosphate produced in glycogenolysis is converted to glucose 6-phosphate in the phosphoglucomutase reaction and so joins the glycolytic pathway in muscle and glucose production in liver.

Phosphorylase cannot break the  $\alpha$ -1,6-glycosidic bonds; in fact it cannot get within four residues of a branch point. So first a transferase enzyme takes three of the last four residues in a branch and transfers them to the end of another chain, and then the last residue is cleaved off as free glucose by  $\alpha$ -1,6-glucosidase. Phosphorylase can then proceed further down the chain. The fact that phosphorylase generates a phosphorylated sugar without expenditure of ATP means that three molecules of ATP can be generated in glycolysis per glucose residue from glycogen, rather than the two from free glucose, so in a sense glycogen is a more efficient fuel



phosphorylase *b* kinase without phosphorylation. I-strain mice, which lack phosphorylase *b* kinase and so cannot make phosphorylase *a*, nevertheless breakdown muscle glycogen during exercise; this presumably is due to allosteric activation of phosphorylase *b* by AMP (or its deamination product IMP).

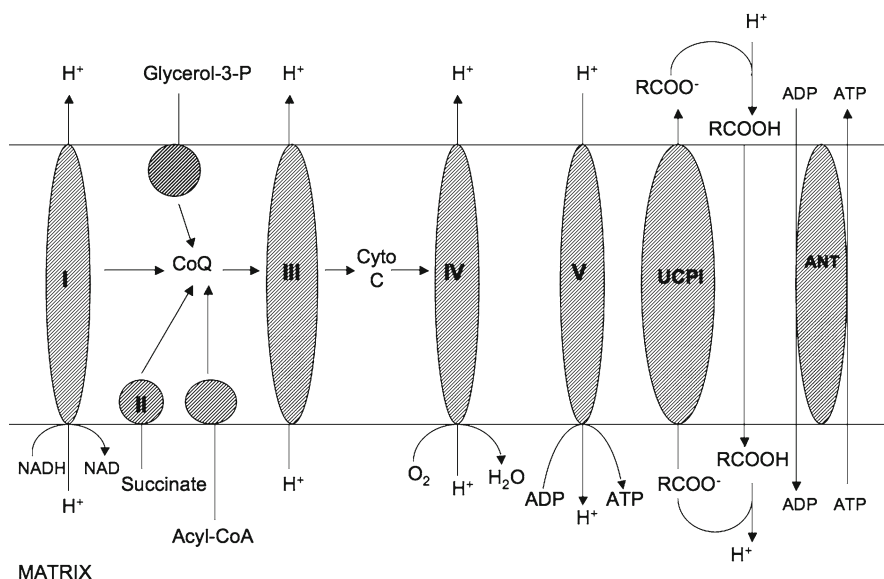
The cAMP cascade also operates to inhibit glycogen synthesis, since PKA phosphorylates and thereby inhibits glycogen synthase, converting it from the active, nonphosphorylated form (named glycogen synthase *a*) to the phosphorylated form (named glycogen synthase *b*) that requires high glucose 6-phosphate for activity. In contrast to phosphorylase, which has only one phosphorylation site, glycogen synthase has at least ten phosphorylation sites and can be phosphorylated by at least nine different protein kinases whose importance/hierarchy is still being studied. In addition to PKA, others of importance are glycogen synthase kinase 3 and AMPK.

Reversal of the signals in the cascade involves hydrolysis of cAMP to AMP by phosphodiesterase and removal of phosphates on enzymes by phosphoprotein phosphatases. The phosphodiesterase is inhibited by methylxanthines such as caffeine, theophylline, and theobromine, accounting in part for the stimulatory effects of the popular drinks coffee, tea, and cocoa. The phosphatases are also subject to regulation, by phosphorylation and dephosphorylation, by protein inhibitors whose activity is regulated by phosphorylation and dephosphorylation, and by allosteric effects inducing conformational changes in their substrates. For example, binding of AMP to phosphorylase *a* causes the phosphate to be tucked in where the phosphatase cannot reach it. Glucose, on the other hand, causes the phosphate to become accessible.

Insulin stimulates glycogen synthesis in three ways: First, it activates glycogen synthase by causing phosphorylation and inhibition of glycogen synthase kinase 3. Second, it reduces cAMP by activating phosphodiesterase. Third, in muscle, it increases glucose transport by activating Glut4. The latter may be the dominant action and the one impaired in diabetes, though impairment of glycogen synthase is also of importance [11, 12]. Interestingly, in liver, where glucose transport is not limiting, a relatively high proportion of glucosyl residues incorporated into glycogen appears to come via an indirect pathway of gluconeogenesis after peripheral catabolism of a glucose load [13].

## ***Oxidative Phosphorylation***

The bulk of ATP generation in most cells occurs via electron transport coupled to mitochondrial ATP synthase on the inner mitochondrial membrane, a process known as oxidative phosphorylation. The electron transport chain takes reducing equivalents from NADH and passes them in a series of steps to molecular oxygen (see Fig. 4). The classical sequence of the carriers of the electron transport chain is NADH to complex I (NADH:Q reductase, which contains bound flavin mononucleotide, FMN, and nonheme iron) to CoQ (ubiquinone) to complex III (QH<sub>2</sub>:cytochrome *c* reductase, which contains cytochromes *b* and *c*<sub>1</sub>) to cytochrome *c* to complex IV (cytochrome oxidase, which contains cytochromes *a* + *a*<sub>3</sub>) to O<sub>2</sub>, reducing the



**Fig. 4** The electron transport chain and oxidative phosphorylation. Complexes I, III, and IV are proton pumps. Complex II is succinate dehydrogenase; other flavin proteins transfer reducing equivalents from glycerol 3-phosphate and acyl-CoA to CoQ. The proton gradient is then used to drive ATP synthesis by complex V. Uncoupling protein I (UCPI) in brown fat uncouples by transporting fatty acid anions out, such that the proton gradient can be consumed while bypassing complex V, thereby generating heat. The adenine nucleotide translocase (ANT) carries ATP out in exchange for ADP

oxygen to  $H_2O$ . Complexes I, III, and IV are proton pumps that move protons out of the matrix and establish a proton gradient and membrane potential across the inner mitochondrial membrane. According to the chemiosmotic hypothesis of Peter Mitchell, this provides the driving force for protons moving back through a channel in the  $F_0$  component of the ATP synthase (complex V) to drive a molecular motor, the  $F_1$  component, that synthesizes ATP from ADP and  $P_i$ . Complex II of the electron transport chain is succinate dehydrogenase of the citric acid cycle, a flavin enzyme that passes reducing equivalents to CoQ. Two other flavin enzymes, mitochondrial glycerol 3-phosphate dehydrogenase of the glycerol phosphate shuttle and acyl-CoA dehydrogenase of fatty acid  $\beta$ -oxidation, also feed into the electron transport chain at CoQ. Classically it was thought that complexes I, III, and IV each generated the energy for the synthesis of 1 ATP, such that the yield would be 3 ATP for NADH and 2 ATP for succinate and other substrates feeding in at CoQ. However, it is now considered that the yield is 2.5 ATP for NADH and 1.5 for succinate. Part of the reason for the decreased yield is that the ATP synthesized by complex V is in the matrix, and that when  $ATP^{4-}$  is transported out to the cytoplasm by the adenine nucleotide translocase in exchange for  $ADP^{3-}$ , the charge difference consumes part of the proton gradient or membrane potential.

Because the site for NADH on complex I faces the matrix of the mitochondria and there is no carrier for NADH to cross the inner mitochondrial membrane, metabolic shuttles are used to carry the reducing equivalents from NADH produced

in the cytoplasm by glycolysis. In the glycerol phosphate shuttle, the glycolytic intermediate dihydroxyacetone phosphate is used as the initial acceptor, being reduced by NADH to glycerol 3-phosphate by the cytosolic, NAD-linked glycerol-3-phosphate dehydrogenase. Then the glycerol 3-phosphate is oxidized back to dihydroxyacetone phosphate by the mitochondrial, FAD-linked glycerol-3-phosphate dehydrogenase, with the reducing equivalents being subsequently transferred from  $\text{FADH}_2$  to CoQ. The site for glycerol 3-phosphate on the latter, inner-membrane-bound dehydrogenase faces out, so glycerol 3-phosphate does not need to be transported into the matrix. In the malate–aspartate shuttle, the initial acceptor is cytosolic oxaloacetate, which is reduced by NADH to malate by malate dehydrogenase present in the cytoplasm. Malate crosses the inner mitochondrial membrane via a dicarboxylic acid transporter and is oxidized back to oxaloacetate by malate dehydrogenase in the matrix, with the conversion of NAD to NADH. There is no carrier for oxaloacetate to go back out, so instead oxaloacetate is converted to aspartate by glutamate-oxaloacetate transaminase, using glutamate and producing  $\alpha$ -ketoglutarate. The  $\alpha$ -ketoglutarate exits (in exchange for malate), as does the aspartate, and the transamination is reversed in the cytoplasm, regenerating oxaloacetate and glutamate. Glutamate reenters the mitochondria (in exchange for aspartate).

Electron transport, and therefore oxygen consumption or respiration, is normally coupled to ATP synthesis or phosphorylation. If there is limited ADP available for complex V because of low ATP usage by the cell, then there will be limited consumption of the proton gradient, and the high gradient will inhibit the proton-pumping electron transport chain. Uncouplers, such as 2,4-dinitrophenol and FCCP, are weak acids that are lipid soluble in both the protonated and unprotonated states. Thus, they can catalyze transport of protons across the inner mitochondrial membrane, collapsing the proton gradient and the membrane potential. This will stimulate respiration but without ATP synthesis. Ionophores, such as valinomycin which carries  $\text{K}^+$  across membranes, can collapse the membrane potential and therefore have a partial uncoupling action. Classical inhibitors of electron transport are the site 1 inhibitors rotenone and amytal (inhibit complex I), the site 2 inhibitor antimycin A (inhibits complex III), and the site 3 inhibitors cyanide, azide, carbon monoxide, and hydrogen sulfide (analogs of  $\text{O}_2$  that block complex IV). Oligomycin is the classical inhibitor of phosphorylation (complex V); it blocks the  $\text{F}_0$  channel, preventing the flow of protons that powers the ATP synthase. Atractyloside and bongrekic acid inhibit the adenine nucleotide translocase. Inhibition of phosphorylation or the translocase will indirectly inhibit respiration of coupled oxidative phosphorylation.

### ***Brown Fat and Uncoupling Proteins***

Dinitrophenol is a poison. However, there is physiological uncoupling of mitochondria that does reduce ATP production and instead produces heat. The clearest example is in brown fat, so-called because the cells are indeed colored from the high amount of mitochondria with their colored cytochromes and the multiple small lipid droplets, in contrast to the large lipid droplet of the mature standard or white

fat cell. Rodents use brown fat for heat production when placed in a cold environment. Adult humans do have small amounts of brown fat, perhaps to warm certain critical areas, but in a cold environment largely use muscular nonproductive thermogenesis, that is, shivering. Nevertheless, it has recently been suggested that brown fat may consume significant energy in an adult human under some conditions [14, 15]. There is a thought that obesity could be treated by increasing energy expenditure by increasing brown fat (or even converting white fat to brown fat), though whether elevated body temperature would be a problem remains to be seen. Uncoupling in brown fat occurs because of the presence of uncoupling protein 1 (UCP1), which transports fatty acid anions across the inner mitochondrial membrane (see Fig. 4). Protonated, that is, uncharged fatty acids can easily dissolve in and flip across the lipid bilayer membrane, thus carrying in protons down the gradient, but normally the anion cannot flip back to repeat the process. UCP1 allows the fatty acid anion to go back and pick up another proton and thus catalyze consumption of the proton gradient. Thus, the uncoupling action of UCP1 is dependent on fatty acids. Physiologically its activity is initiated by neuronal adrenergic ( $\beta 3$ ) stimulation of lipolysis in the brown fat cell. Analogous proteins (by sequence homology), UCP2 and UCP3, exist in other tissues (notably UCP2 in muscle), but their mechanism of action has not been established.

## Triglyceride and Fatty Acid Metabolism

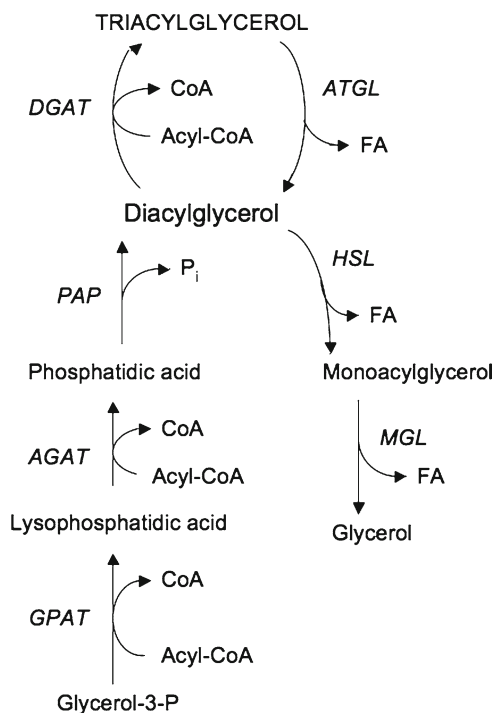
Triglyceride (or triacylglycerol) consists of a glycerol with three esterified fatty acids. Fatty acid simply means long chain carboxylic acid, normally 14–20 carbons. The fatty acids at the end positions tend to be saturated (palmitate, C16; stearate, C18), whereas the fatty acid at the two position tends to be unsaturated (oleate, 18:1) or polyunsaturated (linoleate, 18:2; linolenate, 18:3; arachidonate, 20:4). Myristate (saturated C14) is not normally important in triglyceride, but is important in post-translational modification of some proteins for targeting them to membranes. Triglyceride stores in fat tissue are the major energy reserve of the body, though other tissues may have triglyceride for internal usage. Excessive triglyceride in nonadipose tissues (notably fatty liver) can cause insulin resistance and loss of metabolic function.

### *Lipolysis*

Triglyceride breakdown in fat cells was originally thought to begin with hormone sensitive lipase (HSL), activated by phosphorylation by PKA in response to epinephrine or glucagon. Additionally, PKA phosphorylation of the lipid droplet protein perilipin allows movement of HSL to the lipid droplet. However, knockout of HSL did not greatly reduce such stimulated lipolysis. It was then discovered that

the major triglyceride lipase in fat cells was another protein, named adipose triglyceride lipase (ATGL). (This, too, is a misnomer, because ATGL is likely the dominant triglyceride lipase in other tissues as well.) The current view is that phosphorylation of perilipin also releases CGI-58, a protein activator of ATGL. The ATGL then hydrolyzes triacylglycerol to diacylglycerol. The HSL, which actually has a preference for diacylglycerol, then hydrolyzes diacylglycerol to monoacylglycerol, and a monoglyceride lipase finally hydrolyzes monoacylglycerol to free glycerol (see Fig. 5). Fat cells lack glycerokinase, so the release of glycerol is commonly used as a measure of lipolysis. The glycerol can be used for gluconeogenesis in liver, which is why very obese people can survive many weeks of starvation. The released fatty acids may be re-esterified into triglyceride, carried to other organs bound to albumin in the circulation or oxidized in the mitochondria. Oxidation is a relatively minor fate of fatty acids in fat cells, whereas heart and muscle are major consumers of fatty acids for fuel.

Insulin inhibition of lipolysis in part involves stimulation of phosphodiesterase 3B, the primary enzyme for degradation of cAMP in fat cells. More recently, a second important mechanism has been proposed, whereby insulin stimulates



**Fig. 5** Triglyceride synthesis and hydrolysis. *GPAT*, glycerophosphate acyl transferase; *AGAT*, acylglycerophosphate acyltransferase; *PAP*, phosphatidic acid phosphohydrolase (lipin); *DGAT*, diacylglycerol acyltransferase; *ATGL*, adipose triglyceride lipase; *HSL*, hormone-sensitive lipase; *MGL*, monoglyceride lipase; *FA*, free fatty acid

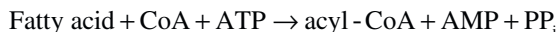


glucose uptake (Glut4) and metabolism to lactate, and the lactate then acts in an autocrine manner on the orphan receptor GPR81, which in turn acts via  $G_i$  G-protein to inhibit adenylyl cyclase, in contrast to the  $G_s$ -mediated action of epinephrine and glucagon to activate adenylyl cyclase [16]. The inhibitory action on lipolysis of some other compounds, such as adenosine,  $\beta$ -hydroxybutyrate, and nicotinic acid, is also due to their binding to receptors linked to  $G_i$ . Note that insulin also stimulates triglyceride synthesis, in part by the stimulation of glucose uptake and thus the synthesis of the precursor molecule glycerol 3-phosphate.

## ***Fatty Acid Oxidation***

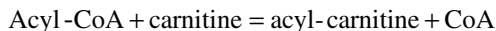
There are three steps in fatty acid oxidation, to be described in detail below. First, the fatty acid is activated to the CoA ester by acyl-CoA synthetase. Second, the acyl group is transported into the mitochondrial matrix attached to carnitine. Third, the regenerated acyl-CoA undergoes multiple cycles of  $\beta$ -oxidation, leading to cleavage into two-carbon fragments in the form of acetyl-CoA, which can then be fully oxidized in the citric acid cycle.

*Activation.* Nearly all enzymatic reactions of fatty acids utilize the CoA ester form (with the exception of prostaglandin and leukotriene synthesis from arachidonate). Fatty acids are activated to the CoA ester in the acyl-CoA synthetase reaction:



The thio ester bond of acyl-CoA has the same energy as an ATP phosphate anhydride bond. Therefore, the acyl-CoA synthetase reaction is pulled to the right by hydrolysis of the pyrophosphate ( $\text{PP}_i$ ) by pyrophosphatase.

*Translocation.* Acyl-CoA synthetase for fatty acids is located in the cytoplasm (or on membranes facing the cytoplasm), and there is no carrier for acyl-CoA itself to cross the inner mitochondrial membrane for  $\beta$ -oxidation in the matrix. Therefore, the acyl group is transferred to carnitine in a reaction catalyzed by carnitine acyl transferase I (or CPT-I, for carnitine palmitoyl transferase I, after its principal substrate, since the abbreviation CAT was already in use for the reporter enzyme chloramphenicol acetyltransferase):



A translocase then carries the acyl-carnitine across the inner mitochondrial membrane. In the matrix the acyl group is transferred back to CoA by the isoform CPT-II, regenerating acyl-CoA and carnitine. The carnitine is then transported back out by the translocase.

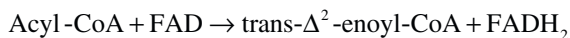
The purpose of this complex transport system is to provide regulation of fatty acid oxidation, since the free fatty acids could diffuse across the membrane but cannot be activated in the matrix. CPT-I is inhibited by malonyl-CoA, which is

made by the highly regulated enzyme acetyl-CoA carboxylase. Acetyl-CoA carboxylase is activated by citrate, the precursor for cytosolic acetyl-CoA, and inhibited by phosphorylation by AMPK. A drop in cellular energy state and hence rise in AMP activates AMPK, which phosphorylates a number of targets to increase energy production and decrease energy usage [17]. One important target is acetyl-CoA carboxylase, decreasing malonyl-CoA, and thus deinhibiting CPT-1 and promoting fatty acid oxidation. Also, malonyl-CoA decarboxylase, which degrades malonyl-CoA, is phosphorylated but activated by AMPK.

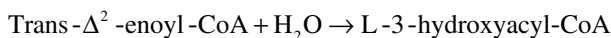
Malonyl-CoA is also the substrate for fatty acid synthesis (see below), so a rise in malonyl-CoA, say by high glucose which would promote the formation of citrate and reduce AMP and AMPK activity, would provide a shift from fatty acid oxidation to fatty acid synthesis. In tissues that do not do much fatty acid synthesis, the inhibition of fatty acid oxidation may shift the use of acyl-CoA from oxidation to complex lipid formation. This may also serve a signaling function, in that diacylglycerol is an activator of protein kinase C; furthermore, acyl-CoA itself is an allosteric regulator of a number of enzymes as well as the substrate for protein acylation. Such a role for malonyl-CoA, cytosolic acyl-CoA, and diacylglycerol has been proposed to be part of the stimulation of insulin secretion in pancreatic  $\beta$ -cells by glucose metabolism and its amplification by fatty acid metabolism [18].

Interestingly, medium and short chain acyl-CoA synthetases, including acetyl-CoA synthetase, are located in the mitochondrial matrix, and therefore for them transport can bypass the carnitine translocase system, and their  $\beta$ -oxidation is unregulated.

*$\beta$ -Oxidation.*  $\beta$ -Oxidation is so named because it involves oxidation of the  $\beta$  carbon, that is, the second carbon from the carboxylic acid group of the fatty acid. One round of  $\beta$ -oxidation involves the following sequence of steps. First, the carbon-carbon single bond between the  $\alpha$  and  $\beta$  carbons is oxidized to a double bond by acyl-CoA dehydrogenase:



Second, water is added across the double bond by enoyl-CoA hydratase to form an alcohol:



Third, the alcohol is oxidized to a carbonyl by L-3-hydroxyacyl-CoA dehydrogenase:



Finally, the  $\beta$  carbonyl is attacked by CoA in the  $\beta$ -ketothiolase reaction:



The process is repeated with the shortened acyl-CoA, until it is completely cleaved to acetyl-CoA fragments. Thus, palmitoyl-CoA (C16) would undergo seven cycles of  $\beta$ -oxidation to generate 8 acetyl-CoA, plus 7  $\text{FADH}_2$  and 7 NADH to enter the electron transport pathway.

Most fatty acids have an even number of carbons, because the synthetic pathway involves the addition of carbons two at a time. The occasional odd chain fatty acid (say from bacterial sources) is degraded by  $\beta$ -oxidation down to propionyl-CoA, which is then carboxylated to methylmalonyl-CoA and converted to the citric acid cycle intermediate succinyl-CoA.

## ***Ketone Bodies***

Excess fatty acid catabolism in the liver, generating more acetyl-CoA than can be oxidized in the citric acid cycle, may lead to the formation of the ketone bodies acetoacetate and  $\beta$ -hydroxybutyrate. These can be carried in the blood to other organs and converted back to acetyl-CoA. The acidity that can be caused by diabetic ketoacidosis is a serious concern. On the other hand, ketone body production during prolonged starvation is an advantage, in that they can be used as fuel by the brain, in contrast to fatty acids, thus reducing the demand for gluconeogenesis.

## ***Triglyceride Synthesis***

Triglyceride synthesis begins with glycerol 3-phosphate, made by reduction of the glycolytic intermediate dihydroxyacetone phosphate with NADH by glycerol 3-phosphate dehydrogenase (see Figs. 1 and 5). Two acyl chains are added from acyl-CoA in the glycerol-phosphate acyltransferase (GPAT) reaction, followed by the acylglycerol-phosphate acyltransferase (AGAT) reaction. Then the phosphate is cleaved off in the phosphatidic acid phosphohydrolase reaction, to form diacylglycerol. Finally, the third acyl chain is added in the diacylglycerol acyltransferase (DGAT) reaction, forming triglyceride. Insulin promotion of triglyceride synthesis in the fat cell occurs at several steps: translocation of Glut4 to the plasma membrane to increase input into glycolysis and hence glycerol 3-phosphate synthesis; phosphorylation and activation of PFKFB3 to raise fructose 2,6-bisphosphate and thereby activate PFK; increased amount of GPAT.

However, the simple idea that triglyceride synthesis is largely controlled by glycolytic generation of glycerol 3-phosphate, especially via insulin regulation of Glut4, has been complicated by recent work showing that much of the triglyceride glycerol portion comes from glyceroneogenesis, that is, the reactions of gluconeogenesis from pyruvate up to dihydroxyacetone phosphate [19]. (Fat cells, like most cell types other than liver or kidney cortex, lack glucose 6-phosphatase and so cannot synthesize free glucose.) It should be recognized that triglyceride synthesis/lipolysis is a dynamic process, with much of the released fatty acids being re-esterified. This cycling must occur even during times of relative insulin lack. Perhaps this is the optimal way to provide fuel for the rest of the body as needed, without flooding the body with free

fatty acids that could lead to deleterious triglyceride accumulations in nonadipose tissue. Free fatty acids are just siphoned off by albumin in the blood as needed. The recycling, although energy consuming, is not that expensive. Much of the energy needs of the fat cell may be provided by glucose, and only a small amount of fatty acid is oxidized, compared to that exported or re-esterified. Yet the oxidation of a single fatty acid would provide the energy for the re-esterification of 50 fatty acid molecules, or over 30 even if the cost of glyceroneogenesis is included.

### ***Fatty Acid Synthesis***

Fatty acids are synthesized from acetyl-CoA. However, the synthesis occurs in the cytosol, whereas the acetyl-CoA from carbohydrate metabolism is generated in the mitochondrion in the PDH reaction. Because there is no carrier for acetyl-CoA to cross the mitochondrial membrane, it is first converted to citrate, which has a carrier. Citrate can exit the mitochondrion and be cleaved back to acetyl-CoA and oxaloacetate by ATP-citrate lyase. Much of the acetyl-CoA to be used is then converted to malonyl-CoA by acetyl-CoA carboxylase. As mentioned above, acetyl-CoA carboxylase is activated by citrate, the precursor for cytosolic acetyl-CoA, and inhibited by phosphorylation by AMPK.

The fatty acid synthase actually starts with an acetyl group transferred to an acyl carrier protein. Then successive malonyl groups are reacted, also bound to an acyl carrier protein, adding two carbons at a time, with the reaction driven by the decarboxylation. A cycle on the synthase includes reduction of the carbonyl of the adduct to a  $\beta$ -hydroxyl, followed by dehydration, and then reduction of the resulting double bond, in steps analogous to reversing the process seen in fatty acid  $\beta$ -oxidation. However, the reducing steps use NADPH, generated in the pentose phosphate pathway. Alternatively, NADPH can be generated in a malate-pyruvate cycle, whereby the cytosolic oxaloacetate from the citrate lyase reaction is converted to malate, the malate is converted to pyruvate in the malic enzyme reaction (with NADP conversion to NADPH), and the pyruvate is converted to oxaloacetate back in the mitochondria by pyruvate carboxylase. Insulin stimulates the synthesis of fatty acid synthase and acetyl-CoA carboxylase.

In humans most fatty acids for triglyceride synthesis are obtained from the diet or recycled from lipolysis. However, some de novo fatty acid synthesis may still occur. The rate is more substantial in rodent models; hence, triglyceride synthesis from labeled glucose may include incorporation of label into the fatty acid components as well as the glycerol component, but a distinction can be made by saponification of the sample.

## **Protein and Amino Acid Metabolism**

Amino acids differ from fatty acids and sugars as fuels in that their storage forms, proteins, all have other functions as enzymes or transporters, contractile or structural elements. Muscle protein constitutes the major reserve. Although there is

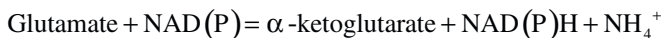
constant turnover of proteins, gross proteolysis only occurs during prolonged starvation or wasting diseases. Pathways for protein synthesis and degradation and the synthesis and degradation of many individual amino acids will not be considered in detail here, and the reader is referred to a standard biochemistry textbook (e.g., Stryer [20]). Focus instead will be on connections of amino acid metabolism to that of glucose and lipid. Insulin, as a general storage signal, promotes protein synthesis as well as glycogen and triglyceride synthesis.

Amino acids can be divided into two classes: “ketogenic,” that is, those that are metabolized to form ketones or acetyl-CoA and therefore can be oxidized in the citric acid cycle or in theory used for fatty acid biosynthesis, but cannot be converted to glucose; and “glucogenic,” that is, those that can be used for gluconeogenesis. Ketogenic amino acids include, for example, the branched chain amino acids (leucine, isoleucine, and valine). Important glucogenic amino acids include alanine, aspartate, glutamate, and glutamine.

The first step in the metabolism of most amino acids is removal of the amino group by transamination or deamination. In transamination reactions, the amino group is transferred to a keto acid, generating a second keto acid (corresponding to the first amino acid) and a second amino acid (corresponding to the first keto acid). One amino-acid/keto-acid pair is generally glutamate/ $\alpha$ -ketoglutarate. Thus, the glutamate-pyruvate transaminase (also called alanine amino transferase) reaction is:



Other transaminases include glutamate-oxaloacetate transaminase (aspartate amino transferase) and the branched chain amino transferase. An important connection between  $\alpha$ -amino groups and free ammonia, for both synthesis and degradation of amino acids, is provided by the reversible glutamate dehydrogenase reaction:



The major amino acid put out by muscle is alanine, not because muscle protein has inordinate amounts of alanine, but rather because alanine is produced by transamination of glycolytically generated pyruvate in the glutamate-pyruvate transaminase reaction; the glutamate in turn comes from transamination of other amino acids, such as the branched chain amino acids, or from the glutamate dehydrogenase reaction. The alanine can then go via the blood to the liver, where it is converted back to pyruvate by transamination and the carbon chain then used for gluconeogenesis, with the glucose then cycling back to the muscle. This interorgan cycle of muscle glycolysis and liver gluconeogenesis is known as the alanine cycle and is analogous to the Cori cycle. This is also the reason that liver pyruvate kinase is inhibited by alanine as a signal of substrate for gluconeogenesis. The alanine cycle is also important for bringing the excess amino nitrogen from muscle catabolism of amino acids to the liver for the synthesis of urea ( $\text{NH}_2\text{CONH}_2$ ), the mammalian excretion product. In the liver, the amino group of alanine is first transferred to glutamate and then to aspartate (glutamate-oxaloacetate transaminase) and to free ammonia (glutamate dehydrogenase) to provide the substrates for the urea cycle.

Glutamine is the major amino acid put out by other peripheral tissues to carry excess amino nitrogen to the liver for the synthesis of urea. Glutamine is synthesized from glutamate and free ammonia by glutamine synthetase, and it is converted back to glutamate and free ammonia by glutaminase. Because free ammonia is toxic to the brain, the efficient operation of the urea cycle is very important. The complete urea cycle only occurs in liver, and therefore liver disease can lead to a serious rise in blood ammonia levels. Kidney cortex can also use glutamine, putting out ammonium ion in the urine in response to metabolic acidosis; the carbon chain is then used in these cells for gluconeogenesis. Glutamine is a favored substrate for many cells in culture, including fat cells, in addition to glucose, and is often added to tissue culture media. Whether glutamine can contribute substantially to glyceroneogenesis in fat cells remains to be determined.

## **AMP-Activated Protein Kinase: An Integrated Modulator of Cellular Metabolism**

Until recently, cellular metabolism in the intact organism has been viewed as primarily under the control of insulin and counter insulin hormones (including glucagon, epinephrine, norepinephrine, and the glucocorticoids). On the other hand, it has long been appreciated that some form of regulation by energy state must also occur. Early evidence for this included the inhibition of the key glycolytic enzyme phosphofructokinase by ATP and its activation by AMP and the activation of glycogen phosphorylase by AMP. In the last 15 years, it has become apparent that changes in cellular metabolism to a considerable extent are regulated by the enzyme AMPK. As originally described [21], AMPK is a fuel sensing enzyme present in all eukaryotic cells that senses and responds to a decrease in a cell's energy state as reflected by an increase in the AMP/ATP ratio. For instance, its activation in skeletal muscle and other tissues during exercise both increases the activity of multiple processes that generate ATP (fatty acid oxidation, glucose transport in skeletal and cardiac muscle, and glycolysis in heart) and inhibits others that require ATP but can be downregulated temporarily without jeopardizing the cell (e.g., protein, triglyceride, and cholesterol synthesis) [22]. Conversely, a decrease in AMPK activity appears to have opposite effects [23].

Recently, it has become apparent that AMPK plays an even more fundamental role in metabolic regulation. Thus, upregulation of its activity by a wide variety of hormones (e.g., adiponectin, catecholamines), drugs (metformin and thiazolidinediones,  $\alpha$ -lipoic acid, statins), and lack of fuels (e.g., glucose deprivation) as well as its downregulation by other hormones and paracrine factors (e.g., glucocorticoids and endocannabinoids) and by a fuel excess (e.g., hyperglycemia) has been demonstrated. In addition, upstream molecules that phosphorylate and activate AMPK such as LKB1, a tumor suppressor, and  $\text{Ca}^{2+}$ -dependent CaMKKs have been identified. Thus, it has become increasingly evident that AMPK is a mediator of metabolic events within the cell in response to a wide variety of stimuli including

at least some that act in the apparent absence of a change in energy state. Perhaps most intriguing of all is that decreased AMPK activity has been associated with a metabolic syndrome phenotype (obesity, diabetes, insulin resistance, predisposition to atherosclerosis) in many experimental animals, whereas pharmacological agents and other therapies (e.g., exercise, diet) that activate AMPK have shown benefit in their treatment both in humans and experimental animals [17, 24]. An understanding of how AMPK exerts its many effects and whether its apparent clinical efficacy is related to its actions on intermediary metabolism are exciting questions that will be the object of intense interest in the foreseeable future.

LKB-1, an upstream kinase for AMPK, in turn can be activated by deacetylation by SIRT1, an NAD-dependent deacetylase. SIRT1 is sensitive to the redox state of the NAD/NADH couple. AMPK increases the production of NAD. Therefore, AMPK and SIRT1 may form an integrated system that is sensitive to both the adenine nucleotide energy state and the redox state as well as other factors [24, 25].

As mentioned above, AMPK is important in regulating fatty acid oxidation by phosphorylation and inhibition of acetyl-CoA carboxylase, and activation of malonyl-CoA decarboxylase, thus decreasing malonyl-CoA levels and deactivating CPT1. AMPK also phosphorylates and inhibits GPAT, a key enzyme in triglyceride synthesis, and decreases the levels of fatty acid synthase, GPAT and DGAT. Furthermore, SIRT1 causes the activation of mitochondrial and lipid oxidation genes. Therefore, activation of the AMPK-SIRT1 system should promote lipid consumption. This may be part of the beneficial effect of exercise.

Food intake in excess of energy usage leads to the storage of triglyceride in adipose tissue and eventual obesity. Although in humans most fatty acids come from the diet rather than *de novo* synthesis, preferential oxidation of carbohydrate rather than fat would leave fatty acids available for triglyceride synthesis. Interestingly, some studies indicate that obesity prone individuals have an increased respiratory quotient ( $RQ = \text{CO}_2 \text{ expelled divided by } \text{O}_2 \text{ consumed}$ ), implying greater usage of carbohydrate than fat compared with lean individuals [26–28]. Whether obese or obese-prone individuals have dysregulation of the AMPK-SIRT1 system, leading to inappropriate sparing of fatty acids from oxidation, is a tempting hypothesis that requires further study.

The subjects of whole-body regulation and dysregulation of food intake and the involvement of satiety factors and hormones, such as leptin and adiponectin, will be discussed in other chapters.

## Transgenic Models: Some Answers and More Questions

An increasingly important approach in metabolic research is the use of transgenic mice or cells, where a gene of interest is knocked out, reduced in expression, or overexpressed, sometimes in a tissue specific manner. Mention has already been made of how the knockout of HSL indicated that the primary triglyceride lipase was in fact a different enzyme, since identified as ATGL. Some other long-held theories have recently received clarification or revision from such experiments.



Knockout of the insulin receptor in muscle (MIRKO) had little effect on blood glucose or insulin levels, and the mice remained normally glucose tolerant [29]. Muscle insulin resistance could be seen in a hyperinsulinemic-euglycemic clamp, and there were effects on protein metabolism, indicated by decreased muscle mass. This calls into question the primary importance of insulin-stimulated glucose disposal in muscle for normal glucose homeostasis and the contribution of muscle insulin resistance to the development of diabetes. On the other hand, perhaps this represents a difference between mice and humans. Interestingly, adipose insulin-stimulated glucose uptake was substantially increased in the MIRKO mice, as was adipose tissue mass, but the enhancement was not seen in isolated adipocytes, suggesting a stimulatory factor released from MIRKO muscle. Knockout of the insulin receptor in liver (LIRKO) led to hyperglycemia and hyperinsulinemia, indicating the importance of the normal suppression by insulin of hepatic glucose output. Levels of the gluconeogenic enzymes PEPCK and G6Pase were elevated; they are normally suppressed by insulin. Hyperinsulinemia leads to insulin resistance in other tissues, which may have exacerbated the situation. Nevertheless, circulating levels of fatty acids and triglycerides were decreased, due to suppression of lipolysis in fat cells by the high insulin levels. On the other hand, knockout of the insulin receptor in fat (FIRKO) perhaps surprisingly led to improved glucose homeostasis and increased insulin sensitivity in the whole animal. It has been suggested that this may be due to alteration in the levels of adipokines (fat secreted signaling molecules), in particular increased adiponectin and leptin. Knockout of the insulin receptor in pancreatic  $\beta$ -cells ( $\beta$ -IRKO) led to the development of abnormal glucose tolerance, smaller islets, and reduced insulin content, indicating that insulin signaling is important in this cell type, too; this presumably is related to the role of insulin as a growth factor rather than as a metabolic regulator.

A major effect of insulin is to cause translocation of Glut4 and hence stimulation of glucose transport in muscle and fat. Knockout of Glut4 in muscle (and heart) (MG4KO) led to hyperglycemia, glucose intolerance, and insulin resistance [30]. The severity of these effects, in contrast to the relatively benign effects of MIRKO, perhaps argues for other/backup mechanisms of activating Glut4 besides insulin. The hyperglycemia in MG4KO also leads to insulin resistance of liver and adipose tissue as well. Knockout of Glut4 in adipose tissue (AG4KO) also led to glucose intolerance and insulin resistance in the whole animal, presumably by altered adipokine communication to other tissues. Adipose mass and adipocyte size were normal, in contrast to the 50% decrease in adipose mass and bimodal distribution of cell size reported for the FIRKO mouse; this may relate to the difference in effects on whole body metabolism and presumably adipokine production in the two knockout models.

One of the key downstream kinases in the insulin signaling cascade is Akt (protein kinase B). Overexpression of Akt1 in skeletal muscle increased the muscle mass through growth of type IIb fibers, which are glycolytic (MyoMouse). This resulted in decreased fat mass accumulation on a high fat/high sucrose diet. The mice did not eat less or exercise more, but burned more fat. This was due to enhanced fatty acid oxidation by the liver, not by the muscle, suggesting a role for muscle-derived myokines [31].



Unexpected connections between glycolytic enzymes and fat metabolism have been published recently. Haller et al. [32] used a photodynamic selection technique to generate a population of Chinese hamster ovary cells deficient in glycerolipid biosynthesis, where the lesion involved a reduction in phosphatidic acid phosphatase activity and downstream glycerolipids and increased  $\alpha$ -glycerophosphate; however, the DNA mutation turned out to be a point mutation in the gene for phosphoglucose isomerase. Getty et al. [6] reported that mice deficient in phosphofructokinase-M had greatly decreased fat stores, even though fat contains the other two isoforms of phosphofructokinase, suggesting the possible importance of intrinsic metabolic oscillations for triglyceride synthesis.

Gross obesity is readily apparent, and therefore spontaneous mutations in rodent colonies led to the establishment of such lines even before the development of targeted genetic techniques. The ob/ob (obese) mouse lacks leptin, a satiety hormone produced by fat cells that acts on the hypothalamus. It therefore has hyperphagia, develops obesity, and consequent insulin resistance and diabetes. Interestingly, the ob/ob mouse can outgrow the diabetes, through massive hyperplasia of the insulin-producing  $\beta$ -cells in pancreatic islets. The db/db (diabetic) mouse, which lacks the leptin receptor, has a somewhat more severe phenotype. Zucker diabetic and fa/fa rats also have mutations in the leptin receptor. These rodent models have been frequently used in obesity/diabetes research.

## References

1. Matschinsky, F., Liang, Y., Kesavan, P., et al. (1993). Glucokinase as pancreatic  $\beta$  cell glucose sensor and diabetes gene. *Journal of Clinical Investigation*, 92, 2092–2098.
2. Zorzano, A., Balon, T.W., Brady, L.J., et al. (1985). Effects of starvation and exercise on concentrations of citrate, hexose phosphates and glycogen in skeletal muscle and heart. Evidence for selective operation of the glucose-fatty acid cycle. *Biochemical Journal*, 232, 585–591.
3. Atsumi, T., Nishio, T., Niwa, H., et al. (2005). Expression of inducible 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase/PFKFB3 isoforms in adipocytes and their potential role in glycolytic regulation. *Diabetes*, 54, 3349–3357.
4. Huo, Y., Guo, X., Honggui, L., et al. (2010). Disruption of inducible 6-phosphofructo-2-kinase ameliorates diet-induced adiposity but exacerbates systemic insulin resistance and adipose tissue inflammatory response. *Journal of Biological Chemistry*, 285, 3713–3721.
5. Tornheim, K. (1997). Are metabolic oscillations responsible for normal oscillatory insulin secretion? *Diabetes*, 46, 1375–1380.
6. Getty-Kaushik, L., Viereck, J.C., Goodman, J.M., et al. (2010). Mice deficient in phosphofructokinase-M have greatly decreased fat stores. *Obesity*, 18, 434–440.
7. Johnson, R.J., Perez-Pozo, S.E., Sautin, Y.Y., et al. (2009). Hypothesis: could excessive fructose intake and uric acid cause type 2 diabetes? *Endocrine Review*, 30, 96–116.
8. Matsumoto, M., & Accili, D. (2006). The tangled path to glucose production. *Nature Medicine*, 12, 33–34.
9. Vidal-Puig, A., & O'Rahilly, S. (2001). Controlling the glucose factory. *Nature*, 413, 125–126.
10. Aragón, J.J., & Lowenstein, J.M. (1980). The purine-nucleotide cycle: Comparison of the levels of citric acid cycle intermediates with the operation of the purine nucleotide cycle in rat skeletal muscle during exercise and recovery from exercise. *European Journal of Biochemistry*, 110, 371–377.

11. Rothman, D.L., Magnusson, I., Cline, G., et al. (1995). Decreased muscle glucose transport/phosphorylation is an early defect in the pathogenesis of non-insulin-dependent diabetes mellitus. *Proceedings of the National Academy of Science USA*, 92, 983–987.
12. Rossetti, L., & Giaccari, A. (1990). Relative contribution of glycogen synthesis and glycolysis to insulin-mediated glucose uptake: A dose-response euglycemic clamp study in normal and diabetic rats. *Journal of Clinical Investigation*, 85, 1785–1792.
13. Bollen, M., Keppens, S., Stalmans, W. (1998). Specific features of glycogen metabolism in the liver. *Biochemical Journal*, 336, 19–31.
14. Yoneshiro, T., Aita, S., Matsushita, M., et al. (2010). Brown adipose tissue, whole-body energy expenditure, and thermogenesis in healthy adult men. *Obesity* [Epub ahead of print].
15. Ravussin, E. (2010). The presence and role of brown fat in adult humans. *Current Diabetic Reports*, 10, 90–92.
16. Ahmed, K., Tunaru, S., Tang, C., et al. (2010). An autocrine lactate loop mediates insulin-dependent inhibition of lipolysis through GPR81. *Cell Metabolism*, 11, 311–319.
17. Richter, E.A., & Ruderman, N.B. (2009). AMPK and the biochemistry of exercise: Implications for human health and disease. *Biochemical Journal*, 418, 261–275.
18. Yaney, G.C., & Corkey, B.E. (2003). Fatty acid metabolism and insulin secretion in pancreatic beta cells. *Diabetologia*, 46, 1297–1312.
19. Nye, C.K., Hanson, R.W., Kalhan, S.C. (2008). Glyceroneogenesis is the dominant pathway for triglyceride glycerol synthesis in vivo in the rat. *Journal of Biological Chemistry*, 283, 27565–27574.
20. Berg, J.M., Tymoczko, J.L., Stryer, L. (2006). *Biochemistry* (6th ed.). New York: WH Freeman.
21. Hardie, D.G., & Carling, D. (1997). The AMP-activated protein kinase – fuel gauge of the mammalian cell? *European Journal of Biochemistry*, 246, 259–273.
22. Towler, M.C., & Hardie, D.G. (2007). AMP-activated protein kinase in metabolic control and insulin signaling. *Circulation Research*, 100, 328–341.
23. Ruderman, N., & Prentki, M. (2004). AMP kinase and malonyl-CoA: Targets for therapy of the metabolic syndrome. *Nature Reviews Drug Discovery*, 3, 340–351.
24. Ruderman, N.B., Xu, X.J., Nelson, L., et al. (2010). AMPK and SIRT1: A long-standing partnership? *American Journal of Physiology: Endocrinology Metabolism*, 298, 751–760.
25. Cantó, C., Jiang, L.Q., Deshmukh, A.S., et al. (2010). Interdependence of AMPK and SIRT1 for metabolic adaptation to fasting and exercise in skeletal muscle. *Cell Metabolism*, 11, 213–219.
26. Filozof, C., & Gonzalez, C. (2000). Predictors of weight gain: The biological-behavioural debate. *Obesity Review*, 1, 21–26.
27. Ellis, A.C., Hyatt, T.C., Hunter, G.R., Gower, B.A. (May 6, 2010). Respiratory quotient predicts fat mass gain in premenopausal women. *Obesity* [Epub ahead of print].
28. Ruderman, N.B., Saha, A.K., Kraegen, E.W. (2003). Minireview: Malonyl CoA, AMP-activated protein kinase, and adiposity. *Endocrinology*, 144, S166–S171.
29. Biddinger, S.B., & Kahn, C.R. (2006). From mice to men: Insights into the insulin resistance syndromes. *Annual Review of Physiology*, 68, 123–158.
30. Herman, M.A., & Kahn, B.B. (2006). Glucose transport and sensing in the maintenance of glucose homeostasis and metabolic harmony. *Journal of Clinical Investigation*, 116, 1767–1775.
31. Walsh, K. (2009). Adipokines, myokines and cardiovascular disease. *Circulatory Journal*, 73, 13–18.
32. Haller, J.F., Smith, C., Liu, D., et al. (2010). Isolation of novel animal cell lines defective in glycerolipid biosynthesis reveals mutations in glucose-6-phosphate isomerase. *Journal of Biological Chemistry*, 285, 866–877.



# Chapter 3

## Adipose Tissue Development, Structure and Function

Jaswinder K. Sethi and Antonio J. Vidal-Puig

### Multiple Functions of Adipose Tissue

One of the earliest reports of adipose tissue was made by the Swiss naturalist Conrad Gessner in 1551 (as translated by Cannon and Nedergaard [1]). However, the notion that adipose tissue was composed of living lipid-laden cells was hotly debated [2]. The past decades have seen a remarkable increase in our understanding of adipose biology and obesity (Fig. 1). This trend is undoubtedly driven by the global epidemic of obesity and associated diseases. Adipose tissue is designed to function as the main long-term fuel-handling organ, and actively controls energy homeostasis. Adipose tissue stores excess fuel in the form of triglycerides and relinquishes these reserves during periods of nutritional deprivation. In homeotherms, adipose tissue also plays equally important roles in thermoregulation through both its insulatory properties and ability to generate heat via non-shivering thermogenesis. In addition to these energetically important functions, the mechanical properties of adipose tissue allow it to protect various organs from injury. To perform these multiple tasks, adipose tissue depots have developed characteristics that can be variable, adaptable and complex.

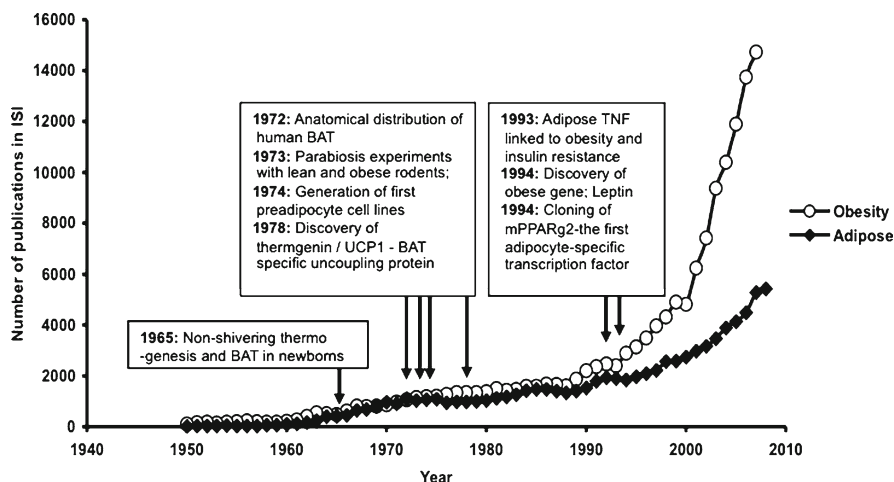
### Biochemical Properties of Adipocytes

As the major functional component of adipose tissues, adipocytes express the cellular machinery that enables their biochemical functions. Adipocytes can take up free fatty acids (FFAs) (through specific cell surface transporters and intracellular fatty acid binding proteins), and synthesize FFAs via *de novo* lipogenesis. The FFAs are then esterified with glycerol to form triacylglycerols (TGs), which are then stored

---

J.K. Sethi (✉)

Institute of Metabolic Science – Metabolic Research Laboratories, and Department of Clinical Biochemistry, University of Cambridge, Addenbrooke's Hospital, Cambridge, UK  
e-mail: jks30@cam.ac.uk



**Fig. 1** Chart showing the dramatic recent increase in the annual number of publications containing the words “Obesity” or “Adipose” (from ISI for 1950–2008). Similar trends were identified when searching PUBMED and Scopus. Also indicated is the timing of some seminal discoveries that have influenced the field of adipose tissue biology

in lipid droplets. Associated with these lipid droplets is a biochemical machinery that facilitates the break down of TGs into glycerol and fatty acid. FFA derived from adipose lipolysis are released into the circulation, and used for fatty acid oxidation in the liver, muscle and other organs. Adipocytes are sensitive to hormonal stimulation, and respond to both anabolic and catabolic hormones, such as insulin, IGF, glucagon and catecholamines. On the other hand, adipocytes synthesize and secrete numerous proteins that impact with potent local and systemic actions.

## Adipose Tissue is Connected to Other Physiological Systems

The functions of adipose tissue can vary depending on the type of adipose tissue and the anatomical location. The specific adipose tissue types and depots are discussed in greater detail below. Another fundamental aspect of adipose tissue is that its function is intricately linked with whole-body metabolism and nutritional status. Adipose tissue is not only capable of responding to neural, hormonal and nutritional signals, but can also secrete paracrine and endocrine signals. In so doing, adipose tissue has a major impact on appetite regulation, thermoregulation, immunity, reproduction, cardiovascular system, bone biology, wound repair, respiratory system and sleep.

Dysregulation of adipose tissue has been associated with a variety of pathological states, the metabolic syndrome, type 2 diabetes, atherosclerotic cardiovascular diseases, neurodegenerative diseases, non-alcoholic fatty liver disease, cancer,

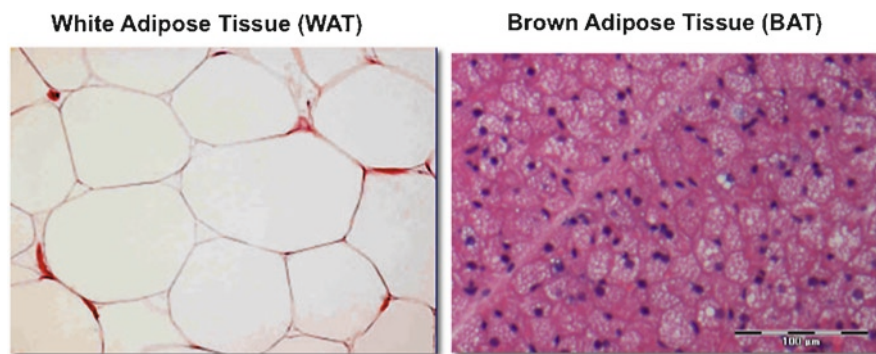
polycystic ovary syndrome, and sleep apnoea. The recognition of links between adipose tissue and disparate pathologies has ignited the current interest in adipose tissue and adipocyte biology.

The connection of adipose tissue with other physiological systems is mediated by the ability of adipose tissue to regulate energy availability and by its ability to communicate with other organs. Adipose-secreted proteins, known as “adipokines”, include hormones, proinflammatory cytokines, growth factors, complement factors, matrix metalloproteins and several types of binding proteins (e.g. lipocalins, IGFBPs, SFRPs). Adipose tissue also produces non-protein species, such as fatty acids, steroid hormones, prostaglandins and retinoids [3].

## Anatomical Distribution and Structure of Adipose Tissue

At first glance adipose tissue can be mistaken for an amorphous collection of lipid droplets loosely held together by connective tissue. However, adipose structure is more complex. In mammals, adipose tissue develops in many sites throughout the body, occurring in areas of loose connective tissue, such as subcutaneous layers between muscle and dermis. Adipose tissue also forms around internal organs, such as the heart, kidneys and pancreas. Adipose tissue is also found in the bone marrow. These disparate locations suggest that the ontogeny of the adipose tissue may vary, and that each depot may be functionally distinct.

The most popular classification of adipose tissues used in homeotherms divides adipose tissue into “white” and “brown”, based on appearance (Fig. 2). White adipose tissue (WAT) is visually more distinct, and is the predominant site of lipid storage and FFA release via lipolysis. In contrast, brown adipose tissue (BAT) is denser and highly vascularized, hence, its brownish coloration. The primary function of BAT is in energy dissipation through non-shivering thermoregulation. Both types of adipose tissues are further sub-classified based on their anatomical location. In humans WAT is spread throughout the body, with major intra-abdominal/visceral



**Fig. 2** Haematoxylin–eosin stained sections of white and brown adipose tissues

depots around the omentum, intestines, perirenal areas, as well as in subcutaneous depots in the buttocks, thighs and abdomen. WAT can additionally be found in areas as diverse as the face and extremities, in the retro-orbital space and in the bone marrow. In contrast, BAT depots are typically located in the paraaortic region, mediastinum, neck, perirenal and interscapular regions. Studies have also compared WAT depots and shown that despite their relatively conserved morphology, different WAT depots exert distinct metabolic features. For example, excessive accumulation of visceral WAT is associated with insulin resistance, diabetes, dyslipidaemia and higher risk of atherosclerosis [4].

The histology of WAT has received much attention, particularly in the last few years, following the demonstration that WAT has its own population of resident adipose tissue macrophages (ATMs). The extent of macrophage infiltration of WAT and levels of chemokines and pro-inflammatory cytokines correlates strongly with obesity and glucose intolerance [5, 6]. By far the largest amount of WAT in a lean people is composed of unilocular adipocytes with diameters ranging from 20 to 200  $\mu\text{m}$ . In larger white adipocytes, the unilocular lipid droplets occupy ~90% of the cell volume, thus compressing the nucleus and cytoplasmic organelles into the periphery of the adipocyte. Smaller (<20  $\mu\text{m}$ ) adipocytes are less visible in histological sections, but have been reported to cluster near sites of angiogenesis in WAT, and appear as immature multilocular adipocytes.

WAT comprising mostly of smaller adipocytes is associated with improved insulin sensitivity. However, the quality of types of lipids stored within adipocytes, irrespective of size, may also be an important indicator of adipose tissue function [7]. Typically, unilocular WAT adipocytes are composed of neutral TGs derived from oleic and palmitic acids. However, diacylglycerols, phospholipids, unesterified fatty acids and cholesterol are also detectable. WAT contains non-adipocyte cells that make up the stromovascular fraction (SVF). Among these are vascular endothelial cells, immune cells and vascular smooth muscle cells. Some of these cells have been implicated in the paracrine signalling events that control adipose tissue expansion [8, 9].

The kinds of immune cells being discovered in WAT is increasing as better tools for identification and isolation become available. In particular, the proinflammatory ATMs have been the subject of intense research. However, the ATM population is more diverse and at least three subtypes have been reported: the resident macrophages, pro-inflammatory (M1) and pro-fibrotic (M2) [10, 11]. Collectively, the ATMs play a significant role in the endocrinology and proper function of adipose tissue [12]. Indeed, the relative proportions present in a given WAT depot correlate with the degree of obesity and insulin resistance. That being said, additional immune cell types are also recruited into WAT, and while the full complement remains to be established, it does include monocytes [13, 14] and T lymphocytes [15].

WAT is innervated by sympathetic nerves which are present in the SVF. An increase in sympathetic activity stimulates lipolysis and FFA release. Another important non-adipose component of the SVF is the connective tissue and extracellular matrix (ECM). Collagen and elastic fibres and resident fibroblasts maintain

ECM integrity. On the whole, the ECM provides a structured mesh which binds the cellular components of adipose tissue, and creates a defined tissue mass, thereby defining the boundaries of individual adipose tissue depots, but allowing a close association with neighbouring organs.

Among the SVF cell types, arguably the most interesting are the progenitors, known as mesenchymal stem cells (MSCs), and sometimes referred to as adipose stem cells, mural cells or pericytes. MSCs can differentiate into mature adipocytes, and thus serve as an important source for new preadipocytes required for adipose tissue expansion. However, MSCs are also pluripotent and have the potential to develop into chondrocytes, osteoblasts, myoblasts, hepatocytes [16], neural cells [17], endothelial cells [18], macrophages [19] and megakaryocytes [20]. This aspect continues to fuel an ongoing debate regarding the similarities between macrophage and preadipocytes, and whether they belong to a common lineage or exhibit convergent functions [21–24]. Nonetheless, taking a broader perspective, it is clear that the pluripotency of adipose-derived stem cells hold the key for determining the potential of adipose tissue expansion. Not only are MSCs a source of new adipocytes, but MSCs have the potential to regenerate new blood vessels that may have been lost as a result of adipose tissue death. MSCs are attracting much attention because of their potential use in regenerative medicine [25, 26].

## **In Vivo Regulation of WAT Expansion**

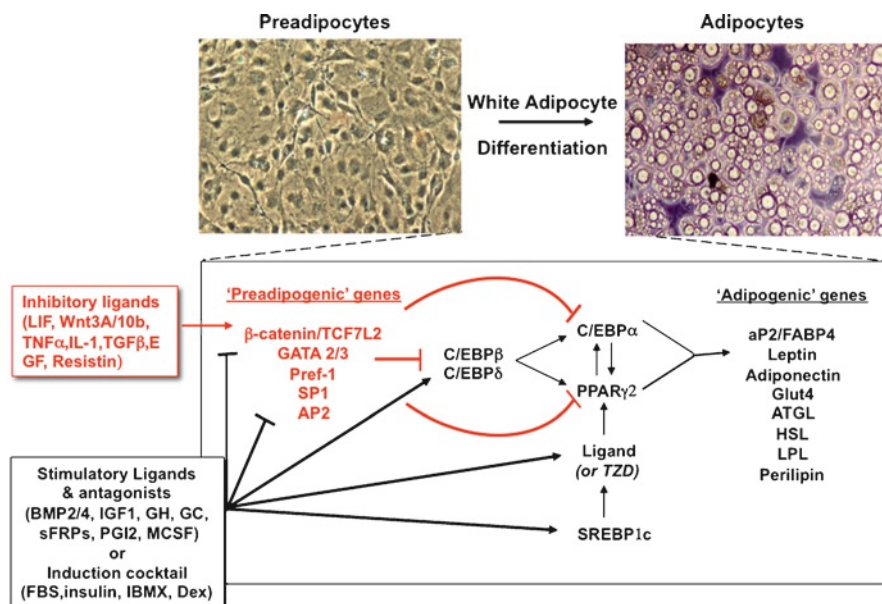
WAT expansion occurs as the combined result of two processes: enlargement of existing adipocytes (hypertrophy) and formation of new adipocytes (hyperplasia). However, the fundamental prerequisite for adipose tissue expansion is existence of an energy surplus and hence the need storage. Adiposity can be affected by genetic and environmental factors. The best characterized environmental factors are the overconsumption of energy-dense foods and sedentary lifestyle. Some drugs, notably thiazolidinediones, insulin glucocorticoids, oestrogen, atypical antipsychotics, antidepressants, and anticonvulsants, can increase body fat. Ageing, gender and ethnicity also influence adiposity but the molecular basis is unclear. Genome-wide association studies have discovered genes that alter appetite and adiposity [27, 28]. Epigenetic factors can also affect the development of adiposity [29], and some studies have linked in utero and early life events to obesity and metabolic syndrome [30, 31].

While these studies may identify new factors associated with increased risk of obesity, there are critical questions to be addressed. How does adipose tissue sense nutritional surplus, and how is this information transduced into adipose expansion? What are the molecular determinants of adipose expansion in obesity? Do specific signalling molecules facilitate the recruitment of new adipocytes during tissue hyperplasia? These fundamental aspects of the adipose tissue biology may hold the key to unveiling the association between obesity and the metabolic syndrome.



## Regulation of White Adipocyte Differentiation

Adipocyte differentiation is an important component of adipose tissue hyperplasia. The programme of adipocyte differentiation or adipogenesis is much more than an enhanced process of lipid accumulation (i.e. lipogenesis). Adipogenesis represents the orchestrated differentiation of proliferating fibroblast-like preadipocytes into non-proliferating, lipid laden, hormonally responsive and functional adipocytes (Fig. 3). Much of what we know about the molecular regulation of adipogenesis comes from *in vitro* studies that utilize either immortalized cell lines or primary cultures of freshly isolated MSCs from adipose tissue. The vast majority of these precursor cells require induction with a chemically defined adipogenic cocktail. These *in vitro* models cannot recapitulate *in vivo* adipose tissue expansion *per se*; nonetheless, they have allowed the manipulation from mechanisms underlying adipocyte differentiation, thereby increasing our understanding of the molecular basis of this developmental programme. Indeed, the need for specific adipogenic induction reagents is consistent with the notion that adipogenesis is not a spontaneous process, but one that requires a tightly regulated hormonal milieu [32]. Another advantage of *in vitro* models is that the homogeneous preadipocytes are amenable to the study of the temporal aspects of adipocyte differentiation, some of which are transient or cell autonomous.



**Fig. 3** Differentiation programme for white adipocytes

This is best detected in synchronized and homogenous cell populations [33–37]. PPAR $\gamma$  and C/EBP $\alpha$  are the key adipogenic transcription factors needed to drive the expression of genes to convert preadipocytes into non-proliferating, lipid-laden adipocytes [37, 38].

However, the *in vitro* models do not enlighten us about how adipocyte recruitment is regulated in heterogeneous tissue. It is clear that *in vivo*, adipogenesis is not a synchronous phenomenon, recruiting all progenitors into the adipogenic programme during a defined developmental stage. Rather adipose tissue retains a population of stem cells which replace dying adipocytes. Indeed, it has been suggested that ~10% of the body's adipose cells are regenerated each year [39]. However, the nature of the signals that prevent all MSCs from being recruited and control the extent of adipocyte differentiation despite excessive nutritional stimulation are unknown. An attractive hypothesis is that adipogenesis is carefully titrated by specific local paracrine and autocrine signals that are both cell-specific and regulated by physiological and nutritional cues. A possible candidate is the Wnt signalling network, which comprises of a host of ligands, antagonists and receptors that are secreted in a cell-specific manner. They often act in a paracrine/autocrine manner and have been implicated not only in both titrated developmental programmes but also in adult tissue remodelling. Until recently, Wnt/ $\beta$  catenin signalling has been implicated in lineage determination of MSCs, promoting bone and muscle development while inhibiting adipogenesis [40, 41]. We have identified Dapper1 (DACT1) as a preadipocyte gene that is required for adipogenesis. DACT1 is an intracellular scaffold protein whose cellular levels appear to modulate Wnt/ $\beta$ -catenin signals. During adipogenesis, DACT1 inhibits Wnt/ $\beta$ -catenin signalling primarily through paracrine and autocrine mechanisms, by controlling the production of key Wnt ligands and antagonists. Importantly, the relative expression of DACT1, Wnt ligands (Wnt10b and Wnt3A) and Wnt antagonists (sFRP1–sFRP5) are both cell-type specific and also regulated *in vivo* by nutritional status, pharmacological stimulation and during the development of dietary and genetic obesity [42]. A biochemical pathway that may link cellular glucose sensing and the Wnt/ $\beta$ -catenin signalling has also recently been reported in macrophages [43]. Furthermore, the paracrine actions of endothelial-derived factors have been shown to inhibit adipogenesis in part via induction of Wnt ligand expression in adipose stromal cells [9]. Taken together, a picture is now emerging to suggest that the Wnt/ $\beta$ -catenin signalling network may hold the molecular key to the physiological regulation of adipose tissue expansion *in vivo*.

Several lines of evidence now suggest that obesity-associated metabolic dysregulation is mediated by inflammation, at least partly, by limiting adipose tissue expansion [44–48]. Given that a number of pro-inflammatory cytokines, including TNF- $\alpha$  and IL-1 $\beta$  are potent inhibitors of adipogenesis, it has been proposed that these signals may interact with Wnt/ $\beta$ -catenin [49, 50]. By limiting the production of new and smaller adipocytes and adipose tissue expansion, pro-inflammatory cytokines may accelerate the progression of insulin resistance and prediabetes to overt diabetes [51].

## Identification of White Adipocyte Progenitors

The non-synchronous nature of adipose differentiation *in vivo* has made it difficult to dissect out homogeneous regions of specific precursor cells from embryos or adult tissues and study these *ex vivo*. Instead, many studies have used mixed populations of MSCs derived from adipose SVF or pluripotent murine embryonic fibroblasts to study adipogenesis. A few groups have used immortalized and/or subcloned adipogenic cell lines [36]. However, Rodeheffer and colleagues have recently used serial fluorescence-activated cell sorting to deplete SVF of cells from endothelial and haematopoietic lineages (using CD31, CD45, Ter119) followed by positive selection for three stem cell antigens (CD29+:CD34+:Sca-1+). In so doing, they isolated a subpopulation of SVF cells that exhibited enhanced lipogenic potential *in vitro*, formed unilocular adipocytes and represented 53.5% of SVF cells. A final selection for CD24-positive cells isolated a much smaller population (0.08% of total SVF cell number) and these were also capable of forming functional adipose depots *in vivo* [52]. It is noteworthy that the enhanced adipogenic capacity of Lin<sup>-</sup>:CD29+:CD34+:Sca-1+ cells in comparison to SVF is consistent with the notion that *in vivo* negative regulation of adipogenesis is mediated via paracrine signals between heterogeneous cell populations. This is further supported by the observations that like 3T3-L1 preadipocytes, these enriched primary adipocyte precursors do not differentiate when implanted into wild-type mice but require a proadipogenic environment [52]. Although the molecular basis for this is not understood, it is likely that such an environment requires not only a nutritional surplus but appropriate proadipogenic cues derived from endocrine and paracrine sources.

Early light and electron microscopic studies of putative adipocyte precursors had some success in identifying adipocyte progenitors in whole adipose tissue [53]. Recent studies have shown that immature adipocytes cluster near sites of angiogenesis suggesting that *in vivo*, adipogenesis and angiogenesis are causally associated [54, 55]. An increase in *in vivo* neovascularization precedes adipogenesis [56], and this process is in turn regulated by hypertrophic adipocytes [8].

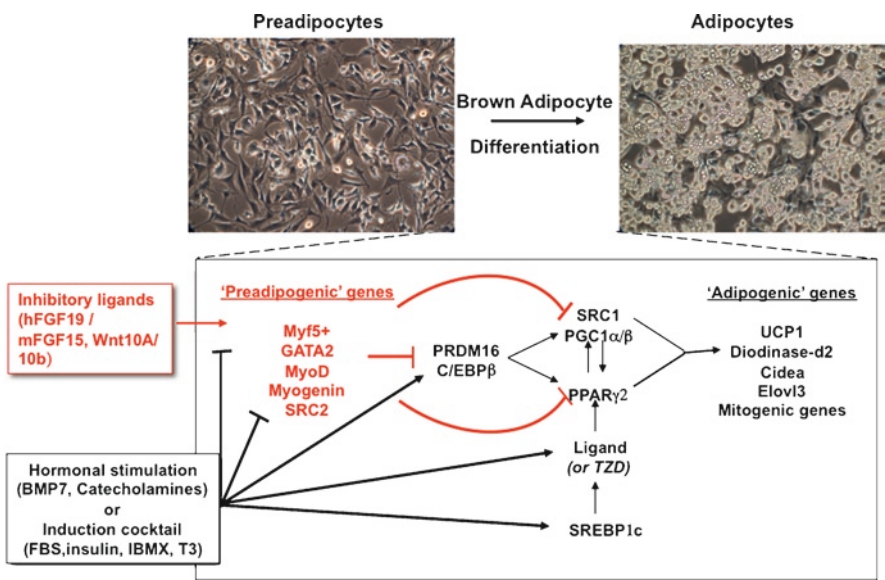
It appears that pericytes found closely associated with blood vessels may in fact represent a subpopulation of adipocyte progenitors. Evidence for this has come from elegant studies using a transgenic approach to generate various PPAR $\gamma$ -reporter mice [55]. PPAR $\gamma$ -positive pericytes retained the ability to proliferate. These cells can be detected prenatally and proliferate during the first month of life, a time when white adipose depots expand. PPAR $\gamma$ -positive SVF cells from 30-day-old mice can also form adipose depots when injected subcutaneously into nude mice. As with the precursors reported by Rodeheffer et al. [52], this SVF subpopulation also expresses stem cell markers Sca1+ and CD34+ but are negative for CD105 (Endoglin), CD45 (protein tyrosine phosphatase, receptor type, C), TER119 (lymphocyte antigen 76), and Mac-1 (CD11b or integrin  $\alpha$  M). Gene expression profiling of the PPAR $\gamma$ -positive SV cells confirmed that they have a preadipocyte-like signature and are distinct from mature adipocytes. These progenitors expressed developmental transcription factors (e.g. goosecoid and twist2), ECM genes (e.g. MMP3) and

anti-angiogenic factors (e.g. Stab1) and signalling receptors (e.g. EGFR and FGF10). Gene expression data are also emerging to suggest that precursors from different WAT depots exhibit distinct transcriptional profiles [57].

These data indicate that the regulation of adipogenesis in vivo is dependent on an intricate interplay between hypertrophic adipocytes, differentiating adipocytes and developing vasculature. This allows for adipogenic potential, storage capacity and nutritional supply to be efficiently but tightly regulated. Potentially, adipose expansion could be manipulated in various depots for the prevention and treatment of diseases associated with obesity [58].

**Brown Adipose Tissue**

BAT primarily functions as a thermogenic tissue in response to sympathetic nerve activity [59]. BAT is composed of lipid-laden adipocytes, blood vessels and nerves. However, the cellular heterogeneity of BAT is less well characterized than that for WAT (Fig. 4). In contrast to the unilocular white adipocytes, brown adipocytes are smaller (<20 µm) multilocular cells with eccentrically located nuclei, and are often so densely packed that it can be difficult to distinguish individual cell boundaries (Fig. 4). Brown adipocytes also have large numbers of mitochondria – the site of thermogenesis. Brown adipocytes are also characterized by the unique expression of the mitochondrial uncoupling protein (UCP)-1. BAT is metabolically very active,



**Fig. 4** Differentiation programme for brown adipocytes

consuming more glucose per gram of tissue than muscle. Indeed, this level of activity only occurs with the brain and tumorigenic tissues.

## In Vivo Regulation of BAT

BAT depots also have a remarkable ability to expand and contract in response to thermogenic demands. The expansion and activation of brown fat is stimulated *in vivo* by chronic cold exposure. Certain high-fat diets that cause overfeeding in rodents (cafeteria diets) can also stimulate the expansion and activation of brown fat. This so-called diet-induced thermogenesis may represent a physiological attempt to restrain weight gain and obesity [60]. BAT is increased by thyroid hormones, which promote non-shivering thermogenesis, and chronic adrenergic stimulation. Factors that stimulate vascularization (e.g. angiopoietin-2) in adipose tissue have also been shown to be important in BAT expansion. Furthermore, Wnt10a [61], Wnt10b [62], FGF19 (FGF15 in mouse) [63, 64] and BMP7 [65], have all been shown to increase BAT. The expansion and activation of BAT is accompanied by stimulated mitochondrial biogenesis and oxidative metabolism.

The programme of brown adipocyte differentiation includes activation of thermogenic genes (UCP-1, PGC-1 $\alpha$  and Deiodinase-D2), mitochondrial genes and other BAT-selective genes (e.g. *cidea* and *elov13*) [66–68]. This occurs in addition to the expression of many adipogenic genes reported for white adipocytes. The transcriptional control of BAT development and differentiation has recently been reviewed in detail [69]. In summary, while BAT has some similarities with the gene expression profiles exhibited by differentiating white adipocytes, it is clear that the programme of brown adipocyte differentiation is distinct. For example, ectopic expression of PPAR $\alpha$  or C/EBP $\alpha$  in mesenchymal cells induces white, and not brown adipocyte differentiation.

Recently, a nuclear scaffold protein, PRDM16, has been shown to drive BAT cell differentiation and function [68]. PRDM16 regulates the co-activators PGC-1 $\alpha$  and PGC-1 $\beta$ , as well as the transcription factors, PPAR $\alpha$  and PPAR $\gamma$ , which collectively induce brown fat cell-selective genes. PRDM16 expression is also associated with the suppression of several white adipocyte genes (i.e. *resistin* and *angiotensinogen*), as well as muscle cell-selective genes (i.e. *myoD*, *myogenin* and *myosin heavy chain*). The former appears to require interaction between PRDM16 and the corepressors, CtBP1 and CtBP2 [66]. These studies have therefore identified a new molecular determinant of brown adipose differentiation and also provide significant support for the existence of a distinct cellular lineage of progenitors that can form new brown adipocytes.

A longstanding debate in BAT biology is whether brown adipocytes are derived from a distinct lineage, share a common lineage with white fat cells or transdifferentiate from existing mature white adipocytes. This debate is fuelled in part by the fact that in addition to defined BAT depots, traditional WAT depots also have the capacity to adopt some key phenotypic characteristics of BAT albeit under

specific circumstances. Indeed,  $\beta$ -adrenergic signalling in WAT promotes the appearance of brown fat cells and the increased and sustained expression of C/EBP $\beta$  in white fat cells has been shown to promote the expression of brown fat cell selective genes [70]. There is also evidence that this capacity may be genetically determined. Whether this represents trans-differentiation, induction of a common functional phenotype or recruitment of pluripotent MSCs remains unclear.

Nonetheless, a recent study provides compelling evidence to suggest that BAT is likely to be derived from a distinct lineage and may share a common ontogeny with muscle progenitor cells. Seale et al. found that primary brown fat progenitors lacking PRDM16 exhibited greater potential for skeletal muscle differentiation. Conversely, overexpression of PRDM16 in myoblasts promoted brown adipocyte differentiation [67]. Additional compelling evidence comes from Myf5-lineage tracing studies that showed that brown fat and skeletal muscle, but not white fat, can be generated from Myf5-expressing progenitors [67]. However, much remains to be done before the debate surrounding the identity and ontogeny of the brown adipocyte progenitor is settled [71]. The association between BAT and muscle may be physiologically significant, since the presence of ectopic BAT in muscle explains the species-specific differences in risk of metabolic syndrome in mice [72].

## Recent Advances in Human Adult BAT

Until recently, it was believed that the presence of BAT was limited to rodents and newborn human infants, where BAT regulates thermogenesis. However, it is now clear that BAT can persist in human adults in variable amounts [73]. The breakthrough has come with the aid of a technique isotopic tracer, 18F-fluorodeoxyglucose (18F-FDG), combined with computed tomography (CT). This technique is used routinely to identify malignant metastatic tissues in the clinic. However, in some subjects, an intense uptake is seen in the supraclavicular regions, and appear to be colocalized with fat tissue rather than muscle [74–76]. Recently, a large study examined PET/CT scans from 1,972 patients, and found a high signal consistent with BAT activity in the anterior region of the neck and chest in 7.5% of women and 3.1% of men [77]. Biopsies were also taken from 33 patients and showed multilocular and UCP-1 positive cells [77]. Similar histological findings were reported from a smaller study of five healthy subjects, in which the tissue biopsies revealed BAT biomarkers, i.e. DIO2, PGC1 $\alpha$ , PRDM16, ADRB3 and mitochondrial protein cytochrome *c* protein [78]. Increased 18F-FDG uptake is highest in the supraclavicular region, but is also present in the areas where BAT have been previously localized [73, 76, 77, 79].

Whether BAT plays a significant role in obesity and the metabolic syndrome is still unresolved. Initial reports suggest that cold-induced glucose uptake is increased in paracervical and supraclavicular adipose tissue in healthy subjects [73, 78], and that BAT activity is positively correlated with resting metabolic rate [73]. BAT activity is also inversely associated with adiposity, at least in healthy men <32 years



old [73, 77]. However, BAT was found most frequently in young women and least frequently in older, overweight men and in patients receiving beta-blockers [77]. The following questions need to be addressed with respect to the importance of BAT in energy balance in humans. Does BAT play a role in adaptive non-shivering thermogenesis in humans? Does BAT expand in response to chronic cold-exposure? A connection between BAT and thermogenesis has been proposed in adult humans who live in cold regions, such as Inuit Eskimo, Athapaskan and Alacaluf Indians, and Norwegian Lapps. In some populations, the basal metabolic rates may increase by 30–40%, allowing people to sleep in ambient temperatures as low as 2–5°C [80]. Another important issue is whether BAT is reduced in obesity, and if so, whether the change in BAT is a cause or consequence of obesity. It seems logical that obesity will result in a shift of energy storage from BAT to WAT. Moreover, obese individuals are better insulated from the cold by WAT, hence BAT is less actively challenged and should become smaller. The answers to these issues could have a significant impact on future therapeutic strategies for obesity, diabetes and other metabolic disorders.

## Conclusion

Fuelled by the desire to better understand the association between obesity and the metabolic syndrome, significant strides have been made in our knowledge of the development, structure and function of both WAT and BAT. With this knowledge has come a renewed appreciation of the degree of communication that exists between adipose tissue and various physiological systems. Adipose tissue exhibits remarkable plasticity in its structure and function in obesity, including activation of innate immunity, and alterations in the levels of circulating fatty acids, adipokines, cytokines and other factors. While insulin resistance is a sine qua non of obesity, most patients do not develop diabetes, likely because of adequate compensation by the pancreatic B cell. The advances in our understanding of the molecular regulation of adipose tissue structure and function could provide novel insights into the how adipose tissue signals to itself and other organs, and how dysregulation of these interactions culminates into obesity-related diseases.

## References

1. Cannon, B., & Nedergaard, J. (2008). Developmental biology: Neither fat nor flesh. *Nature*, 454(7207), 947–948.
2. Beale, L. (1871). The nucleus of adipose tissue. *Nature*, 4, 367–367.
3. Cao, H., Gerhold, K., Mayers, J. R., Wiest, M. M., Watkins, S. M., & Hotamisligil, G. S. (2008). Identification of a lipokine, a lipid hormone linking adipose tissue to systemic metabolism. *Cell* 134(6), 933–944.
4. Kissebah, A. H., & Krakower, G. R (1994). Regional adiposity and morbidity. *Physiological Review* 74(4), 761–811.

5. Weisberg, S. P., McCann, D., Desai, M., Rosenbaum, M., Leibel, R. L., & Ferrante, A. W., Jr. (2003). Obesity is associated with macrophage accumulation in adipose tissue. *Journal of Clinical Investigation*, 112(12), 1796–1808.
6. Xu, H., Barnes, G. T., Yang, Q., et al. (2003). Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *Journal of Clinical Investigation*, 112(12), 1821–1830.
7. Virtue, S., & Vidal-Puig, A. (2008). It's not how fat you are, it's what you do with it that counts. *PLoS Biology*, 6(9), e237.
8. Castellot, J. J., Jr., Karnovsky, M. J., & Spiegelman, B. M. (1982). Differentiation-dependent stimulation of neovascularization and endothelial cell chemotaxis by 3T3 adipocytes. *Proceedings of the National Academy of Sciences of the United States of America* 79(18), 5597–5601.
9. Rajashekhar, G., Traktuev, D. O., Roell, W. C., et al. (2008). IFATS collection: Adipose stromal cell differentiation is reduced by endothelial cell contact and paracrine communication: role of canonical Wnt signaling. *Stem Cells* 26(10), 2674–2681.
10. Cinti, S., Mitchell G., Barbatelli, Get al., (2005). Adipocyte death defines macrophage localization and function in adipose tissue of obese mice and humans. *Journal of Lipid Research* 46(11), 2347–2355.
11. Lumeng, C. N., Bodzin, J. L., & Saltiel, A. R. (2007). Obesity induces a phenotypic switch in adipose tissue macrophage polarization. *Journal of Clinical Investigation* 117(1), 175–184.
12. Heilbronn, L. K., & Campbell, L. V. (2008). Adipose tissue macrophages, low grade inflammation and insulin resistance in human obesity. *Current Pharmaceutical Design* 14(12), 1225–1230.
13. Boschmann, M., Engeli, S., Adams, F., et al. (2005). Adipose tissue metabolism and CD11b expression on monocytes in obese hypertensives. *Hypertension* 46(1), 130–136.
14. Curat, C. A., Miranville, A., Sengenès, C., et al. (2004). From blood monocytes to adipose tissue-resident macrophages: Induction of diapedesis by human mature adipocytes. *Diabetes* 53(5), 1285–1292.
15. Bouloumie, A., Casteilla, L., & Lafontan, M. (2008). Adipose tissue lymphocytes and macrophages in obesity and insulin resistance: Makers or markers, and which comes first? *Arteriosclerosis, Thrombosis, and Vascular Biology* 28(7), 1211–1213.
16. Xu, Y., Malladi, P., Wagner, D. R., & Longaker, M. T. (2005). Adipose-derived mesenchymal cells as a potential cell source for skeletal regeneration. *Current Opinion in Molecular Therapy* 7(4), 300–305.
17. Kang, S. K., Putnam, L. A., Ylostalo, J., et al. (2004). Neurogenesis of Rhesus adipose stromal cells. *Journal of Cell Science* 117(Pt 18), 4289–4299.
18. Moon, M. H., Kim, S. Y., Kim, Y. J., et al. (2006). Human adipose tissue-derived mesenchymal stem cells improve postnatal neovascularization in a mouse model of hindlimb ischemia. *Cellular Physiology and Biochemistry*, 17(5–6), 279–290.
19. Charriere, G. M., Cousin, B., Arnaud, E et al., (2006). Macrophage characteristics of stem cells revealed by transcriptome profiling. *Experimental Cell Research* 312(17):3205–3214.
20. Matsubara, Y., Saito, E., Suzuki, H., Watanabe, N., Murata, M., & Ikeda Y (2009). Generation of megakaryocytes and platelets from human subcutaneous adipose tissues. *Biochemical and Biophysical Research Communications* 378(4), 716–720.
21. Charriere, G., Cousin, B., Arnaud E, et al. (2003). Preadipocyte conversion to macrophage. Evidence of plasticity. *Journal of Biological Chemistry* 278(11), 9850–9855.
22. Kim, C. S., Kawada, T., Yoo, H., Kwon, B. S., & Yu, R. (2003). Macrophage inflammatory protein-related protein-2, a novel CC chemokine, can regulate preadipocyte migration and adipocyte differentiation. *FEBS Letters*, 548(1–3), 125–130.
23. Lanotte, M., Metcalf, D., & Dexter, T. M. (1982). Production of monocyte/macrophage colony-stimulating factor by preadipocyte cell lines derived from murine marrow stroma. *Journal of Cell Physiology*, 112(1), 123–127.
24. Molgat, A. S., Gagnon, A., & Sorisky, A. (2009). Preadipocyte apoptosis is prevented by macrophage-conditioned medium in a PDGF-dependent manner. *American Journal of Physiology. Cell Physiology* 296(4), C757–C765.



25. Hemmrich, K., von Heimburg, D., Rendchen, R., Di Bartolo, C., Milella, E., & Pallua, N. (2005). Implantation of preadipocyte-loaded hyaluronic acid-based scaffolds into nude mice to evaluate potential for soft tissue engineering. *Biomaterials* 26(34), 7025–7037.
26. Hong, L., Peptan, A.I., Colpan, A., & Daw, J. L. (2006). Adipose tissue engineering by human adipose-derived stromal cells. *Cells, Tissues, Organs* 183(3), 133–140.
27. Farooqi, S.I., & O'Rahilly, S. (2007). Genetic factors in human obesity. *Obesity Reviews* 8-(Suppl 1), 37–40.
28. Lee, Y. S. (2009). The role of genes in the current obesity epidemic. *Annals of the Academy of Medicine, Singapore* 38(1), 45–43.
29. Stoger R. (2008). Epigenetics and obesity. *Pharmacogenomics* 9(12), 1851–1860.
30. Cottrell, E. C., & Ozanne, S. E. (2008). Early life programming of obesity and metabolic disease. *Physiology and Behavior* 94(1), 17–28.
31. Vickers, M. H., Krechowec, S. O., & Breier, B. H. (2007). Is later obesity programmed in utero? *Current Drug Targets* 8(8), 923–934.
32. Avram, M. M., Avram, A. S., & James, W. D. (2007). Subcutaneous fat in normal and diseased states 3. Adipogenesis: From stem cell to fat cell. *Journal of the American Academy of Dermatology*, 56(3), 472–492.
33. Farmer, S. R. (2006). Transcriptional control of adipocyte formation. *Cell Metabolism*, 4(4), 263–273.
34. Gesta, S., Tseng, Y. H., & Kahn, C. R. (2007). Developmental origin of fat: Tracking obesity to its source. *Cell* 131(2), 242–256.
35. Lefterova, M. I., & Lazar, M. A. (2009). New developments in adipogenesis. *Trends in Endocrinology and Metabolism*, 20(3), 107–114.
36. Rosen, E. D., & MacDougald, O. A. (2006). Adipocyte differentiation from the inside out. *Nature Reviews. Molecular Cell Biology* 7(12), 885–896.
37. Tontonoz, P., & Spiegelman, B. M. (2008). Fat and beyond: The diverse biology of PPARgamma. *Annual Review of Biochemistry*, 77, 289–312.
38. Darlington, G. J., Ross, S. E., & MacDougald, O. A. (1998). The role of C/EBP genes in adipocyte differentiation. *Journal of Biological Chemistry*, 273(46), 30057–30060.
39. Spalding, K. L., Arner, E., Westermark, P. O., et al. (2008). Dynamics of fat cell turnover in humans. *Nature*, 453(7196), 783–787.
40. Christodoulides, C., Lagathu, C., Sethi, J. K., & Vidal-Puig, A. (2009). Adipogenesis and WNT signalling. *Trends in Endocrinology and Metabolism*, 20(1), 16–24.
41. Prestwich, T. C., & Macdougald, O. A. (2007). Wnt/beta-catenin signaling in adipogenesis and metabolism. *Current Opinion in Cell Biology*, 19(6), 612–617.
42. Lagathu, C., Christodoulides, C., Virtue, S., et al. (2009). Dact1, a nutritionally regulated preadipocyte gene, controls adipogenesis by coordinating the Wnt/beta-catenin signaling network. *Diabetes*, 58(3), 609–619.
43. Anagnostou, S. H., & Shepherd, P. R. (2008). Glucose induces an autocrine activation of the Wnt/beta-catenin pathway in macrophage cell lines. *Biochemistry Journal* 416(2), 211–218.
44. Kim, J. Y., van de Wall, E., Laplante, M., et al. (2007). Obesity-associated improvements in metabolic profile through expansion of adipose tissue. *Journal of Clinical Investigation*, 117(9), 2621–2637.
45. Medina-Gomez, G., Gray, S. L., Yetukuri, L. et al., (2007). PPAR gamma 2 prevents lipotoxicity by controlling adipose tissue expandability and peripheral lipid metabolism. *PLoS Genetics*, 3(4), e64.
46. Permana, P. A., Nair, S., Lee, Y. H., Luczy-Bachman, G., Vozarova De Courten, B., & Tataranni, P. A. (2004). Subcutaneous abdominal preadipocyte differentiation in vitro inversely correlates with central obesity. *American Journal of Physiology. Endocrinology and Metabolism* 286(6), E958–E962.
47. Tchoukalova, Y., Koutsari, C., & Jensen, M. (2007). Committed subcutaneous preadipocytes are reduced in human obesity. *Diabetologia*, 50(1), 151–157.
48. Yang, X., Jansson, P. A., Nagaev, I., et al. (2004). Evidence of impaired adipogenesis in insulin resistance. *Biochemical and Biophysical Research Communication*, 317(4), 1045–1051.

49. Cawthorn, W. P., Heyd, F., Hegyi, K., & Sethi, J. K. (2007). Tumour necrosis factor- $\alpha$  inhibits adipogenesis via a beta-catenin/TCF4(TCF7L2)-dependent pathway. *Cell Death and Differentiation*, 14(7), 1361–1373.
50. Isakson, P., Hammarstedt, A., Gustafson, B., & Smith, U. (2009). Impaired preadipocyte differentiation in human abdominal obesity: role of Wnt, tumor necrosis factor- $\alpha$ , and inflammation. *Diabetes*, 58(7), 1550–1557.
51. Cawthorn, W. P., & Sethi, J. K. (2008). TNF- $\alpha$  and adipocyte biology. *FEBS Lett*582(1), 117–131.
52. Rodeheffer, M. S., Birsoy, K., & Friedman, J. M. (2008). Identification of white adipocyte progenitor cells in vivo. *Cell*135(2), 240–249.
53. Cinti, S., Cigolini, M., Bosello, O., & Bjorntorp, P. (1984). A morphological study of the adipocyte precursor. *Journal of Submicroscopic Cytology and Pathology*, 16(2), 243–251.
54. Rupnick, M. A., Panigrahy, D., Zhang, C. Y., et al. (2002). Adipose tissue mass can be regulated through the vasculature. *Proceedings of the National Academy of Sciences of the United States of America*, 99(16), 10730–10735.
55. Tang, W., Zeve, D., Suh, J. M., et al. (2008). White fat progenitor cells reside in the adipose vasculature. *Science*322(5901), 583–586.
56. Kawaguchi, N., Toriyama, K., Nicodemou-Lena, E., Inou, K., Torii, S., & Kitagawa, Y. (1998). De novo adipogenesis in mice at the site of injection of basement membrane and basic fibroblast growth factor. *Proceedings of the National Academy of Sciences of the United States of America*, 95(3), 1062–1066.
57. Tchkonja, T., Lenburg, M., Thomou, T., et al. (2007). Identification of depot-specific human fat cell progenitors through distinct expression profiles and developmental gene patterns. *American Journal of Physiology. Endocrinology and Metabolism*292(1), E298–E307.
58. Kolonin, M. G., Saha, P. K., Chan, L., Pasqualini, R., & Arap, W. (2004). Reversal of obesity by targeted ablation of adipose tissue. *Nature Medicine*, 10(6), 625–632.
59. Cannon, B., & Nedergaard, J. (2004). Brown adipose tissue: function and physiological significance. *Physiological Review*, 84(1), 277–359.
60. Rothwell, N. J., & Stock, M. J. (1979). A role for brown adipose tissue in diet-induced thermogenesis. *Nature*, 281(5726), 31–35.
61. Tseng, Y. H., Kriacuciunas, K. M., Kokkotou, E., & Kahn, C. R. (2004). Differential roles of insulin receptor substrates in brown adipocyte differentiation. *Molecular and Cell Biology*, 24(5), 1918–1929.
62. Longo, K. A., Wright, W. S., Kang, S., et al. (2004). Wnt10b inhibits development of white and brown adipose tissues. *Journal of Biological Chemistry*, 279(34), 35503–35509.
63. Fu, L., John, L. M., Adams, S. H., et al. (2004). Fibroblast growth factor 19 increases metabolic rate and reverses dietary and leptin-deficient diabetes. *Endocrinology*145(6), 2594–2603.
64. Tomlinson, E., Fu, L., John, L., et al. (2002). Transgenic mice expressing human fibroblast growth factor-19 display increased metabolic rate and decreased adiposity. *Endocrinology*143(5), 1741–1747.
65. Tseng, Y. H., Kokkotou, E., Schulz, T. J., et al., (2008). New role of bone morphogenetic protein 7 in brown adipogenesis and energy expenditure. *Nature*454(7207), 1000–1004.
66. Kajimura, S., Seale, P., Tomaru, T., et al. (2008). Regulation of the brown and white fat gene programs through a PRDM16/CtBP transcriptional complex. *Genes Development*, 22(10), 1397–1409.
67. Seale, P., Bjork, B., Yang, W., et al. (2008). PRDM16 controls a brown fat/skeletal muscle switch. *Nature*454(7207), 961–967.
68. Seale, P., Kajimura, S., Yang, W., et al. (2007). Transcriptional control of brown fat determination by PRDM16. *Cell Metabolism*, 6(1), 38–54.
69. Seale, P., Kajimura, S., Spiegelman, B. M. (2009). Transcriptional control of brown adipocyte development and physiological function – of mice and men. *Genes Development*, 23(7), 788–797.
70. Karamanlidis, G., Karamitri, A., Docherty, K., Hazlerigg, D. G., & Lomax, M. A. (2007). C/EBP $\beta$  reprograms white 3T3-L1 preadipocytes to a Brown adipocyte pattern of gene expression. *Journal of Biological Chemistry*, 282(34), 24660–24669.

71. Fruhbeck, G., Sesma, P., & Burrell, M. A. (2009). PRDM16: the interconvertible adipo-myocyte switch. *Trends in Cellular Biology*, 19(4), 141–146.
72. Almind, K., Manieri, M., Sivitz, W. I., Cinti, S., & Kahn, C. R. (2007). Ectopic brown adipose tissue in muscle provides a mechanism for differences in risk of metabolic syndrome in mice. *Proceedings of the National Academy of Sciences of the United States of America*, 104(7), 2366–2371.
73. van Marken Lichtenbelt, W. D., Vanhommerig, J. W., Smulders, N. M., et al. (2009). Cold-activated brown adipose tissue in healthy men. *New England Journal of Medicine* 360(15), 1500–1508.
74. Cohade, C., Osman, M., Pannu, H. K., & Wahl, R. L. (2003). Uptake in supraclavicular area fat (“USA-Fat”): description on 18F-FDG PET/CT. *Journal of Nuclear Medicine*, 44(2), 170–176.
75. Hany, T. F., Gharehpapagh, E., Kamel, E. M., Buck, A., Himms-Hagen, J., & von Schulthess, G. K. (2002). Brown adipose tissue: a factor to consider in symmetrical tracer uptake in the neck and upper chest region. *European Journal of Nuclear Medicine and Molecular Imaging*, 29(10), 1393–1398.
76. Nedergaard, J., Bengtsson, T., & Cannon, B. (2007). Unexpected evidence for active brown adipose tissue in adult humans. *American Journal of Physiology. Endocrinology and Metabolism*, 293(2), E444–E452.
77. Cypess, A. M., Lehman, S., Williams, G., et al. (2009). Identification and importance of brown adipose tissue in adult humans. *New England Journal of Medicine*, 360(15), 1509–1517.
78. Virtanen, K. A., Lidell, M. E., Orava, J., et al. (2009). Functional brown adipose tissue in healthy adults. *New England Journal of Medicine*, 360(15), 1518–1525.
79. Heaton, J. M. (1972). The distribution of brown adipose tissue in the human. *Journal of Anatomy*, 112(Pt 1), 35–39.
80. Marchand, P. J., & Walker, L. (1996). *Life in the cold: An introduction to winter ecology* (3rd ed.). Hanover, NH: University Press of New England.

# Chapter 4

## Adipokines in Health and Disease

Rexford S. Ahima and Marcus D. Goncalves

### Introduction

Obesity has become a major public health problem [1]. It is currently estimated that more than 1.6 billion adults worldwide are overweight [body mass index (BMI) >25] and 400 million are obese (BMI >30). The incidence of obesity in children is also very high [1]. The obesity epidemic is attributed mainly to excessive intake of foods rich in fat and sugar, and lack of exercise [1]. Obesity increases the risk of diabetes, hypertension, coronary artery disease, sleep apnea, cancer, and various diseases; therefore, there is enormous interest in understanding the pathogenesis of obesity [1, 2].

As discussed in chapter 3, White adipose tissue (WAT) is specialized for storage of fat, mainly in the form of triglycerides. WAT consists of adipocytes filled with triglycerides, precursor cells (preadipocytes), and a variety of immune cells, and has a rich vascular supply and innervation [1, 2]. A sexual dimorphism of fat distribution exists, such that subcutaneous adipose tissue is more abundant in premenopausal women, whereas visceral adipose tissue is prominent in males and postmenopausal women [3]. These differences in fat distribution are determined by sex steroids [3]. Obesity is associated with profound changes in the structure and function of adipose tissue to accommodate the increased demand for triglyceride storage. Adipocytes undergo hyperplasia and hypertrophy, the extracellular matrix expands, and angiogenesis and macrophage infiltration are all increased in obesity [2–4]. In addition, obesity is characterized by ectopic accumulation of triglycerides and other lipid species in the liver, muscle, and pancreatic islets [2]. This condition, called steatosis, predisposes toward insulin resistance, glucose intolerance, and diabetes [2].

The past two decades have witnessed major advances in our understanding of adipose tissue [2, 4]. In addition to releasing fatty acids as a result of lipolysis, adipose tissue secretes many peptides including leptin, adiponectin, resistin,

---

R.S. Ahima (✉)

Division of Endocrinology, Diabetes and Metabolism, and the Institute for Diabetes, Obesity and Metabolism, University of Pennsylvania School of Medicine, 415 Curie Boulevard, 712A Clinical Research Building, Philadelphia, PA 19104, USA  
e-mail: ahima@mail.med.upenn.edu

proinflammatory cytokines, and complement, vasoactive and pro-coagulant factors, and retinol binding protein-4 (RBP4) [2, 4]. These so-called adipokines act as hormones to control feeding, energy balance, and neuroendocrine, immune and cardiovascular systems [2]. Some adipokines also act through autocrine and paracrine mechanisms to control the growth and metabolic functions of adipose tissue [2, 4]. This chapter will focus on the biology of leptin, adiponectin, and resistin. A comprehensive list of adipokines and other factors secreted by adipose tissue is shown in Table 1.

**Table 1** Proteins secreted by adipocytes

Adipokines	Extracellular matrix
<i>Hormones</i>	Cathepsins B, D, L, S
Leptin	Collagen $\alpha 1$ I, III, IV, VI, XV, XIV, XVII
Adiponectin	Collagen $\alpha 2$ I, IV, VI
Resistin	Collagen $\alpha 3$ VI
Retinol binding protein 4	Fibronectin
Insulin-like growth factor (IGF)-1	Galectin 3 binding protein
IGF binding protein 7	Gelsolin
Fasting-induced adipose factor (adiponutrin)	Laminin $\alpha 4$
Apelin	Laminin $\beta 1$
Visfatin	Laminin $\gamma$
Vaspin	Matrilin-2
	Matrix metalloproteinase 1, 2, 3, 7, 9, 10, 11, 12, 13, 14, 15, 16, 17, 19, 23, 24
	Tissue inhibitor of matrix metalloproteinase 1, 2, 3, 4
<i>Immune factors</i>	Osteonectin
$\alpha 1$ acid glycoprotein	Tenacin
Colony-stimulating factor	Thrombospondin 1, 2
Complement C1, 2, 3, 4, 7	
Complement factor B, C, D (adipsin)	
C-reactive protein	
Interleukin-1, 4, 6, 7, 8, 10, 12, 18	
Lipocalin	
Macrophage inhibitory factor 1	
TNF- $\alpha$	
Serum amyloid A3	
Haptoglobulin	
<i>Others</i>	-
Plasminogen activator inhibitor-1	
Tissue factor	
Angiotensinogen	
Angiopoietin 1 and 2	
Transforming growth factor $\beta$	
Galectin 1	
Fibroblast growth factor	
Vascular endothelial growth factor	

## Leptin

### *Regulation of Leptin*

Leptin is a 16-kDa protein synthesized and secreted mainly by white adipocytes (reviewed in Refs. [2, 5]). The levels of leptin in adipose tissue and plasma are proportional to fat stores; therefore, leptin is elevated in obesity and reduced in lean individuals. The production of leptin is also affected by acute changes in energy status and various factors. Leptin falls rapidly during fasting and increases after re-feeding [2, 6, 7]. These changes are partly due to insulin, which stimulates leptin synthesis in rodents and humans [2, 8]. Females have higher leptin levels than males due to increased synthesis in subcutaneous adipose tissue, stimulation by estrogen, and suppression by androgens [2, 5]. Chronically elevated glucocorticoids, as in Cushing's syndrome, and elevated levels of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interleukin-6 (IL-6), increase leptin [2, 5]. In contrast,  $\beta$ 3 adrenergic stimulation and cold exposure both decrease leptin [2, 5]. Leptin has circadian and ultradian rhythms [6, 9]. Leptin peaks at night in humans and at the beginning of the light period in rodents [6, 9]. The circadian rhythm of leptin is controlled by the timing of feeding and alterations in insulin levels [2, 5]. Pulsatile leptin secretion is positively associated with estradiol and growth hormone [9].

Plasma leptin is mainly bound to a soluble receptor (LRe) and albumin. Free (unbound) leptin crosses the blood–brain barrier via a saturable mechanism [10], and acts in the hypothalamus and other brain targets to control feeding, energy expenditure, neuroendocrine axis, and glucose and lipid metabolism [5]. The rapid decline in leptin during fasting inhibits reproduction, thyroid and growth hormones, energy expenditure and immunity, and increases feeding [6, 7, 11]. These responses are blocked by leptin treatment, confirming leptin's role as a major “starvation hormone” [6, 7, 11]. Similarly, congenital leptin deficiency in rodents and humans is characterized by hyperphagia, reduced thermogenesis, hypothyroidism, hypogonadism, and immunosuppression [5, 7]. Together, these findings demonstrate a critical role of leptin as a signal for energy deficiency.

### *Leptin Signaling*

Various leptin receptors (LR) are derived from alternative splicing of the *Lepr* gene product [12]. Short leptin receptors, LRA, LRC, LRD, and LRF (in mice) and the long leptin receptor, LRB, share identical extracellular and transmembrane domains and the first 29 intracellular amino acids; however, LRB is the only leptin receptor with an intracellular domain critical for leptin signal transduction. LRA is widely expressed and conserved among species. Studies have suggested that LRA may serve as a leptin transporter in the brain capillary endothelium [13]. LRe, consisting of the extracellular domain, binds leptin in the plasma and may determine the

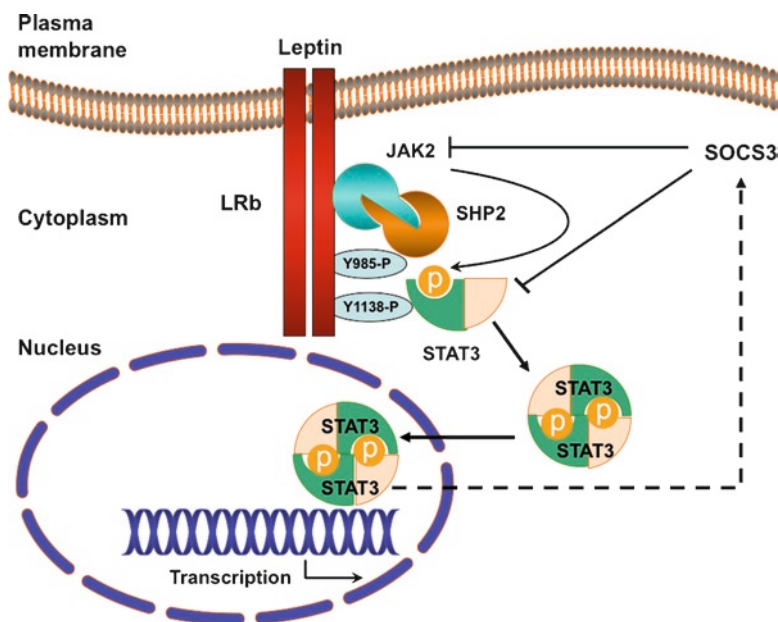
bioavailability of leptin. Consistent with its role as the predominant leptin signaling receptor, *db/db* mice lacking LRb due to a mutation that causes missplicing of the LRb mRNA, and *db3j/db3j* mice lacking all LR isoforms, develop profound hyperphagia and morbid obesity, similar to leptin-deficient *ob/ob* mice [5, 12]. Likewise, human *LEP* and *LEPR* mutations result in hyperphagia and obesity [14, 15].

Two sets of neurons in the hypothalamic arcuate nucleus express high levels of LRb [5]. Neurons that co-express LRb and neuropeptide Y (NPY) and agouti-related peptide (AGRP) synapse on neurons in the paraventricular nucleus (PVN) that express NPY-Y1 and NPY-Y5 receptors and MC4 receptors (MC4R). Arcuate neurons co-expressing LRb and proopiomelanocortin (POMC), the precursor of  $\alpha$ -melanocyte stimulating hormone (MSH), and cocaine and amphetamine-regulated transcript (CART), also project to PVN. The binding of leptin to LRb inhibits NPY/AGRP and stimulates POMC/CART, leading to appetite suppression, increased energy expenditure and weight loss. AGRP normally blocks  $\alpha$ -MSH signaling via MC4R in PVN. Low leptin levels during fasting increase NPY/AGRP and reduce POMC/CART, resulting in hyperphagia, reduced energy expenditure, and weight increase [5]. Similar changes in neuropeptide levels are detected in the hypothalami of *ob/ob* and *db/db* mice [5]. Leptin signaling in PVN is transduced through second order neurons that express TRH, CRH, and oxytocin [5]. Other neuronal targets of leptin are located in the brainstem and limbic areas, and will be discussed in greater detail in Chapter 5.

The binding of leptin to LRb results in transphosphorylation and activation of janus kinase-2 (Jak2), which then phosphorylates other tyrosine residues within the LRb/Jak2 complex (Fig. 1) [5]. Three conserved tyrosine residues, Tyr985, Tyr1077, and Tyr1138, on the intracellular domain of LRb are phosphorylated and mediate leptin signaling. Phosphorylated Tyr985 binds the phosphotyrosine binding (SH2) domain of the tyrosine phosphatase SHP-2, resulting in activation of p21ras and ERK signaling. Phosphorylation of Tyr1138 recruits STAT3 to LRb/Jak2 complex, resulting in the tyrosine phosphorylation and translocation of STAT3 to the nucleus to regulate transcription of neuropeptide and SOCS-3 (suppressor of cytokine signaling-3) (Fig. 1). The latter binds to Tyr985 of LRb and mediates the feedback inhibition of LRb-STAT3 signaling.

As expected, deletion of LRb or STAT3 leads to obesity [16]. A mutant LRbS1138 containing a substitution mutation of Tyr1138 (the STAT3 binding site), failed to activate STAT3 activation in response to leptin, resulting in hyperphagia, reduced thermogenesis, tertiary hypothyroidism, and early-onset obesity [17]. These features recapitulated the phenotype of *db/db* mice. However, LRbS1138 mice showed glucose tolerance, fertility, linear growth and immunity were improved, in contrast to *db/db* mice [17]. Moreover, POMC expression was reduced in both LRbS1138 and *db/db* mice, while NPY and AGRP were reduced in LRbS1138 but increased in *db/db* mice. These results show distinct roles of LRb–Tyr1138–STAT3 signaling in the regulation of feeding, energy expenditure, neuroendocrine axis, and glucose homeostasis [17]. A mutation of Tyr985 prevented phosphorylation of this site and activation of SOCS-3. As expected, leptin signaling was enhanced, resulting in reduction of food intake and weight loss [18].





**Fig. 1** Leptin signal transduction. Leptin binding to its receptor LRb activates Janus kinase (JAK) which autophosphorylates and then phosphorylates LRb on tyrosine multiple residues. Tyr<sub>1138</sub> mediates the phosphorylation and activation of STAT3, which is then translocated into the nucleus to regulate the transcription of neuropeptides. Suppressor of cytokine signaling-3 (SOCS-3) is also induced by pSTAT3 and terminates leptin signaling

## *Leptin Resistance*

The inability of high endogenous or exogenous leptin to inhibit feeding and decrease weight in obesity is suggestive of “leptin resistance” [12]. A number of mechanisms have been proposed to explain leptin resistance in diet-induced obesity. The transport of leptin across the blood–brain barrier is impaired in diet-induced obesity [12]. The specific leptin transport defect is unknown, but lipids could play an important role [19]. Hyperleptinemia in obesity is associated with decreased LRb Tyr985-mediated phosphorylation of STAT3, and induction of SOCS-3 expression in the hypothalamus [12]. Tyr985 and SOCS-3 contribute to leptin resistance, as evidenced by increased leptin sensitivity and leanness when SOCS-3 was deleted in arcuate hypothalamic POMC neurons [20]. Another possible mediator of leptin resistance is the tyrosine phosphatase PTP1B, which dephosphorylates Jak2 and blunts LRb signaling [21]. Neuron-specific deletion of PTP1B increased leptin sensitivity and protected against obesity, while PTP1B deletion in adipocytes and liver did not decrease weight [22].



The robust signaling of low leptin levels is likely to have evolved to maximize feeding and energy storage as a defense against starvation [5]. On the other hand, leptin resistance appears to have emerged as a problem in modern human history, where food is abundant and physical activity is sparse [12]. However, there appears to be a physiological need for leptin resistance in some circumstances. For example, seasonal animals, e.g., hibernators exhibit leptin resistance as a means of increasing food intake and promoting energy storage [23]. Leptin resistance has also been proposed as a means of maintaining high food consumption and energy storage to meet the high demands of pregnancy and lactation [24, 25].

### *Other Mechanisms of Leptin Signaling in Neurons*

LRb is expressed in the nucleus of the solitary tract (NTS) and lateral parabrachial nucleus in the brain stem, and ventral tegmental area in the midbrain [5, 26, 27]. Leptin modulates the feeding-reward circuitry by inducing STAT3 phosphorylation in dopamine and GABA ( $\gamma$ -amino butyric acid) neurons of the ventral tegmental area and mesoaccumbens [26, 27]. AMP-activated protein kinase (AMPK) is another important leptin target [28]. AMPK is phosphorylated and activated in response during cellular stress or fasting, which results in fatty acid oxidation and inhibition of anabolic pathways [28]. AMPK is co-localized with LRb, STAT3, NPY, and other hypothalamic neuropeptides. Leptin inhibits AMPK in the hypothalamus, in parallel with appetite suppression and weight loss [28]. Leptin also engages insulin signaling pathways in the hypothalamus, via Jak2, PI3K (phosphoinositide 3-kinase) and IRS1 and IRS2 (insulin receptor substrate 1 and 2), leading to suppression of feeding [29].

Not all of leptin's effects in the brain can be explained on the basis of Jak2–STAT3 signaling. For example, leptin depolarizes arcuate POMC neurons, and decreases the inhibitory tone of GABA on POMC neurons [30]. Leptin hyperpolarizes and inactivates NPY neurons in the arcuate nucleus [30]. In contrast, low leptin during fasting depolarizes NPY/AGRP neurons and stimulates feeding [30]. Leptin hyperpolarizes glucose-responsive neurons in the hypothalamus by opening KATP channels, resulting in inhibition of feeding and weight loss [5].

Leptin modulates synaptic density in NPY and POMC neurons in the hypothalamus within a few hours [31]. Leptin also affects brain structure, as evidenced by reduction of brain size in humans and rodents with congenital leptin deficiency [32, 33]. In *ob/ob* mice, leptin deficiency is associated with neuronal loss and impaired myelination [34, 35]. These deficits are partially reversed by leptin treatment [32, 33]. Leptin also plays an important role in the development of hypothalamic arcuate to PVN neuronal projections during the early postnatal period [36]. This action is also attenuated in diet-induced obesity, providing a structural explanation to abnormal neurotransmission in obesity [37].

Functional magnetic resonance imaging (fMRI) has revealed profound effects of leptin in brain activity [23, 38, 39]. Obese patients were food restricted, maintained at 10% below their starting weight, and given leptin replacement therapy or placebo [39]. Declining leptin levels during weight loss increased brain activity in areas involved in emotional, cognitive, and sensory control of food intake. Leptin replacement reversed the changes in brain activity and maintained weight loss, confirming leptin is a critical factor linking reduced energy stores to feeding [39]. Leptin treatment blunted the desire to eat in patients with congenital leptin deficiency [23]. Interestingly, leptin inhibited of striatal brain activity, consistent with its role in the regulation of pleasure and reward responses to food [23]. In another study in patients with congenital leptin deficiency, leptin treatment decreased activity of brain areas that sense hunger, and increased activity in areas linked to satiety [38]. Leptin also stimulates hippocampal activity and cognitive function [38]. Recently, high leptin levels have been associated with reduced incidence of dementia and Alzheimer's disease in the Framingham cohort [40].

### ***Other Actions of Leptin***

Leptin resistance in obesity has been linked to steatosis, lipotoxicity, and organ dysfunction [41]. Although the brain is the major site of leptin action, low levels of LRb are expressed in peripheral tissues and involved in metabolism. Leptin normalizes plasma glucose and insulin in *ob/ob* mice without significantly reducing body weight [42]. Analysis of liver mRNA using microarrays identified IGF binding protein 2 (IGFBP2) as being regulated by leptin. Overexpression of IGFBP2 via adenovirus reversed diabetes in *ob/ob*, as well as agouti (Ay/a) and diet-induced obese mice [42]. These results show that leptin-inducible IGFBP2 can regulate glucose metabolism, a finding with potential implications for the treatment of diabetes. Leptin suppresses insulin gene expression and secretion in human pancreatic islet [43]. Deletion of LRb from pancreatic  $\beta$  cells increased islet mass, and impaired glucose-stimulated insulin release and glucose tolerance [44].

Leptin has major effects on immunity [45]. LRb is expressed by CD34+ hematopoietic bone-marrow precursors, monocytes and macrophages and T and B cells. Leptin promotes innate immunity through activation of monocytes/macrophages, neutrophils, and natural killer cells [45]. The effect of leptin on bone biology has attracted a lot of attention [46]. Although *ob/ob* mice have hypothalamic hypogonadism and markedly elevated glucocorticoids, bone density is surprisingly normal [46]. Leptin reduces bone mass via the sympathetic nervous system and CART [46]. As predicted, deletion of neuronal LRb increased bone formation and resorption, resulting in a high bone mass [47]. Bone mass was also enhanced when leptin signaling was increased through a Y985L substitution in LRb [47]. Furthermore, leptin decreased the levels of osteocalcin, revealing an important connection between leptin and bone biology [47].

## Adiponectin

### *Regulation of Adiponectin*

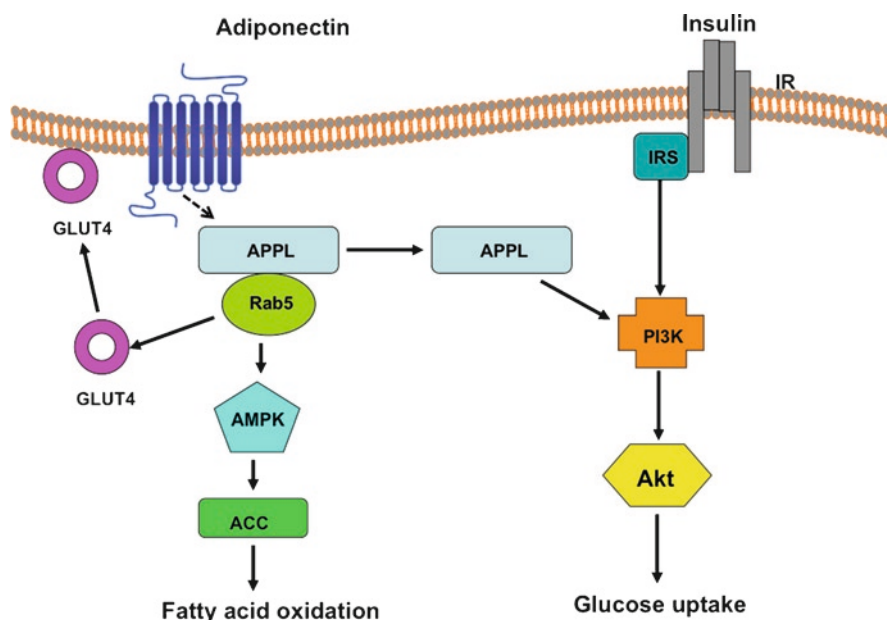
Adiponectin is synthesized and secreted by white adipocytes. The primary structure of adiponectin is composed of an N-terminal sequence, hypervariable domain, 15 collagenous repeats, and a C-terminal domain [48]. A trimeric form of adiponectin is secreted by adipocytes, and forms hexamers [low molecular weight (LMW)] and six trimers [18 mers, high molecular weight (HMW)], via non-covalent bonding [48]. HMW adiponectin is the bioactive form of adiponectin in plasma, while trimeric and hexameric forms are predominant in cerebrospinal fluid (CSF) [34, 49]. Adiponectin also undergoes other post-translational modifications, including glycosylation [48]. Women have higher concentrations of both total and HMW adiponectin than males, partly because adiponectin is decreased by androgens. Unlike the fall of leptin, adiponectin is reduced in obesity and increased during prolonged fasting and severe weight reduction. HMW adiponectin is increased by thiazolidinediones, and is thought to mediate the insulin sensitizing effect of this class of antidiabetic drug [48, 49].

### *Adiponectin Signaling*

Adiponectin receptors, AdipoR1 and AdipoR2, containing seven transmembrane domains with an internal N-terminus and an external C-terminus, mediate the signaling of adiponectin [35]. AdipoR1 is highly expressed in skeletal muscle, while AdipoR2 is mainly expressed in liver [35]. AdipoR1 has a higher affinity for the globular form of adiponectin than for full-length adiponectin. AdipoR2 has an intermediate affinity for both globular and full-length adiponectin [35].

Adiponectin increases glucose uptake and fatty acid oxidation in skeletal muscle via AdipoR1 and adaptor protein containing pleckstrin homology domain, phosphotyrosine domain, and leucine zipper domain (APPL) (Fig. 2) [50]. Adiponectin stimulates APPL binding to the intracellular region of AdipoR1, which activates Rab5, a small GTPase that increases the membrane translocation of glucose transporter-4 and glucose uptake in muscle [50]. APPL also interacts with PI3 kinase and Akt, suggesting a means by which adiponectin enhances insulin signaling [50]. Association of APPL and AdipoR1 stimulates phosphorylation and activation of AMPK, and inhibits ACC. Since ACC stimulates production of malonyl-CoA production and inhibits fatty acid oxidation, AdipoR-mediated activation of AMPK has a net effect to enhance fatty acid oxidation and decrease adiposity (Fig. 2). AMPK activation increases glucose uptake and lactate production in muscle and suppresses gluconeogenesis (Fig. 2).

Expression of AdipoR1 and R2 activated AMPK and PPAR $\alpha$  signaling in the liver of *db/db* mice, decreased gluconeogenesis, and enhanced fatty acid oxidation [51].



**Fig. 2** Adiponectin and insulin signaling in muscle. Adiponectin binds to AdipoR1, which binds to the adapter protein, APPL, which then binds to Rab5, a small GTPase required for the membrane translocation of the glucose transporter (GLUT4). Activation of AdipoR1 leads to the activation of AMP-activated protein kinase (AMPK) which inhibits acetyl-CoA carboxylase (ACC) to increase fatty acid oxidation. APPL has also been associated with the activation of PI3 kinase and Akt in the insulin signaling pathway, resulting in an increase in glucose uptake

In contrast, AdipoR1 deficiency decreased adiponectin-induced AMPK activation, while AdipoR2 deficiency decreased PPAR $\alpha$  signaling [51]. Disruption of both AdipoR1 and AdipoR2 abolished adiponectin binding, induced lipid accumulation in liver and muscle, and induced inflammation, oxidative stress and insulin resistance [51]. Together, these genetic manipulations highlight the importance of adiponectin in glucose and lipid metabolism.

### *Adiponectin and Metabolic Syndrome*

Total adiponectin deficiency in rodents increases hepatic insulin resistance, inflammation, and vascular injury [48, 52, 53]. Similarly, hypoadiponectinemia in humans is associated with insulin resistance, inflammation, dyslipidemia, and risk of atherogenic vascular disease [48]. Individuals with a family history of type 2 diabetes display skeletal muscle insulin resistance and impaired mitochondrial function strongly associated with adiponectin deficiency [54]. Adiponectin treatment of human myotubes increased mitochondrial biogenesis, fatty acid oxidation, and citrate synthase activity, suppressed reactive oxygen species production, and increased glucose uptake [54].

In rodents, adiponectin treatment attenuates the progression of vascular injury and atherosclerosis in rodents [52]. Adiponectin stimulates production of nitric oxide in endothelial cells, reduces reactive oxygen species, and protects against inflammation, by activating AMPK [55]. Adiponectin is also protective against ischemic–reperfusion injury in the heart via cyclo-oxygenase-2-mediated suppression of TNF signaling, and inhibition of oxidative stress and apoptosis [56]. Adiponectin inhibits monocyte adhesion, macrophage transformation, proliferation and migration of vascular smooth muscle cells, by activating AMPK and inhibiting NF- $\kappa$ B (nuclear factor  $\kappa$ B) [55].

Obesity is associated with accumulation of lipids not only in adipose tissue, but also in the liver, skeletal muscle, and pancreatic islet. Ectopic lipid accumulation has been linked to insulin resistance and pancreatic  $\beta$ -cell failure [41]. It has been suggested that ectopic fat has more adverse consequences than excess adipose tissue. In fact, removal of subcutaneous adipose tissue through liposuction did not improve diabetes and other obesity-associated metabolic abnormalities [57]. Moreover, thiazolidinediones stimulate adipogenesis while improving insulin sensitivity. The connection between adiponectin and metabolic changes associated with obesity was examined in a mouse model overexpressing a modest amount of adiponectin [58]. An increase in adiponectin in *ob/ob* mice resulted in dramatic expansion of subcutaneous adipose tissue. However, hepatic steatosis, insulin resistance and islet function, were all improved in these massively obese mice [58]. Whether adiponectin promotes adipogenesis and lipid storage directly in adipose tissue is unknown [58]. Nonetheless, this is a novel example of metabolically benign obesity [59].

### ***CNS Action of Adiponectin***

Adiponectin affects energy balance via neuronal circuits in the brain [60]. Adiponectin is present in the CSF in rodents and humans, and CSF adiponectin is increased following peripheral adiponectin administration, suggesting that adiponectin can cross the blood–brain barrier [34, 60, 61]. Central administration of adiponectin stimulated energy expenditure and decreased weight and fat content in mice [60]. Adiponectin also enhanced AMPK activity in the arcuate nucleus through AdipoR1, increased food intake, and decreased energy expenditure [61]. In contrast, adiponectin knockout mice showed decreased AMPK phosphorylation in the arcuate nucleus, inhibition of food intake, increased energy expenditure, and resistance to obesity [61]. Serum and CSF levels of adiponectin and AdipoR1 expression in the arcuate nucleus are increased in response to fasting, indicating that adiponectin is a major signal for the physiological adaptation to fasting [61].

An opposite effect of adiponectin was observed in another study [62]. Intracerebroventricular injection of adiponectin inhibited food intake, and increased the activities of IRS1/2, ERK, Akt, FOXO1, Jak2 and STAT3, via AdipoR1 in the hypothalamus [62]. In contrast, others have shown that deletion of AdipoR

increased adiposity, and decreased glucose tolerance, locomotor activity and energy expenditure. AdipoR2 knockout mice were lean, and had improved glucose tolerance, higher locomotor activity and energy expenditure, and reduced plasma cholesterol levels [63].

Adiponectin has rapid electrophysiological actions in the brain [64–67]. The area postrema (AP) in the brainstem lacks a blood–brain barrier and is a critical homeostatic integrator for humoral and neural signals. AP neurons expressing both AdipoR1 and AdipoR2 were depolarized by adiponectin, and direct injection of adiponectin into AP increased blood pressure [64]. However, adiponectin decreased blood pressure by modulating the excitability of NPY neurons in the NTS [67]. Adiponectin also depolarized CRH neurons in PVN, and increased plasma ACTH levels [65]. In contrast, adiponectin did not affect TRH neurons in the PVN [65]. Instead, adiponectin depolarized both pre-autonomic TRH and oxytocin neurons, revealing distinct populations of PVN neurons involved in autonomic and neuroendocrine functions of adiponectin [66].

## Resistin

### *Regulation of Resistin*

Resistin belongs to a family of cystine-rich peptides called resistin-like molecules [68]. Resistin is expressed and secreted by adipocytes in rodents and induces insulin resistance [68]. In rodents, serum resistin levels are increased in obesity, while resistin mRNA levels in adipose tissue are reduced [68–70]. Multimeric complexes of resistin and resistin-like molecule- $\beta$  have been identified in mouse serum [71]. Each promoter consists of a COOH-terminal disulfide-rich  $\beta$ -sandwich head and an NH<sub>2</sub>-terminal  $\alpha$ -helical tail, which associates to form three-stranded coils, linked by interchain disulfide linkages to form tail-to-tail hexamers. Resistin levels are higher in females, fall during fasting, and increase after re-feeding [69]. The nutritional regulation of resistin is under the control of insulin and glucose [69].

Resistin is also regulated by incretin hormones and lipoprotein lipase (LPL) activity [72, 73]. Resistin did not increase when mice lacking receptors for glucagon-like peptide 1 and gastric inhibitory polypeptide (GIP) were fed a high-fat diet [72]. In contrast, chronic elevation of GIP levels increased plasma resistin levels in Zucker rats [73]. Furthermore, treatment of 3T3-L1 adipocytes with resistin or GIP inhibited activities of AMPK and LPL [73]. RNA interference-mediated suppression of resistin attenuated the effect of GIP on AMPK and LPL pathways in 3T3-L1 adipocytes, indicating that resistin acts distally to GIP [73].

Peripheral resistin injection or adenovirus-mediated overexpression of resistin induces insulin resistance in mice [74, 75]. In contrast, ablation of the *retn* gene or inhibition of resistin via antisense oligonucleotides improved insulin sensitivity [76, 77].

Resistin inhibits adipogenesis, whereas resistin deficiency increases body weight and fat, and improves insulin sensitivity [78, 79]. Thus, resistin has profound effects on energy and glucose metabolism. In agreement, we found that loss of resistin in *ob/ob* mice increased body weight and fat by decreasing energy expenditure [80]. Insulin sensitivity was improved in *ob/ob* mice lacking resistin, and reversed by resistin treatment [80]. While the resistin receptor is not known, resistin attenuated AMPK phosphorylation and increased SOCS-3 expression, suggesting an overlap in signaling pathways with leptin and adiponectin [80].

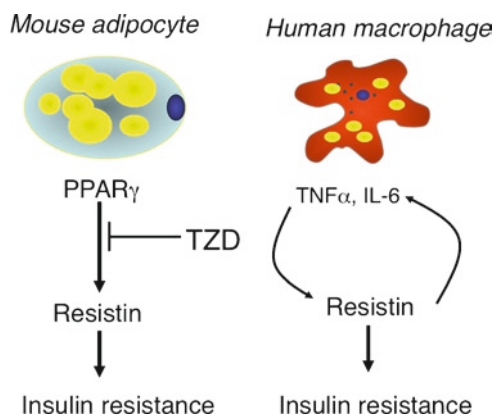
### ***CNS Effects of Resistin***

Resistin has been detected in CSF, and inhibits the release of dopamine and norepinephrine from hypothalamic synaptosomes [81]. In rats, microinfusion of wild type resistin or an active cysteine mutant of resistin into the mediobasal hypothalamus stimulated induced hepatic insulin resistance, and increased TNF- $\alpha$ , IL-6, and SOCS-3 [82]. In contrast, antagonism of resistin action in the hypothalamus improved hepatic insulin sensitivity [82]. These findings have been confirmed in mice, in which intracerebroventricular resistin treatment induced hepatic insulin resistance and inflammation [83]. The effects were associated with induction of TNF- $\alpha$ , IL-6, and SOCS-3 in the liver. Interestingly, resistin increased NPY and AGRP in the hypothalamus, and the central effect on glucose homeostasis was abrogated in NPY-deficient mice as well as by pharmacological blockade of NPY-Y1 receptor [83].

### ***Biology of Human Resistin***

In contrast to rodents, human resistin is synthesized and secreted by macrophages (Fig. 3) [84, 85]. Plasma resistin levels and single-nucleotide polymorphisms have been linked to obesity and lipid and glucose abnormalities in some studies [86–89], although others have failed to establish such a relationship [90, 91]. Resistin has been associated with inflammation, atherosclerosis, and heart failure [92–94]. Resistin is strongly related to the levels of soluble TNF receptor-2, IL-6- and lipoprotein-associated phospholipase A2, and severity of coronary artery calcification [94]. The connection between resistin and inflammation was examined in an experimental endotoxemia paradigm in humans [95]. Infusion of a low dose of lipopolysaccharide (LPS) induced fever and increased adipose TNF- $\alpha$  and IL-6 levels, and insulin resistance. LPS increased resistin and leptin, suggesting a link between inflammation, adipokines, and glucose metabolism [96]. Neutralization of TNF- $\alpha$  in individuals with the metabolic syndrome increased total adiponectin but not HMW adiponectin, and decreased resistin. However, these changes did not affect insulin sensitivity [96, 97].





**Fig. 3** Sources of resistin in mice and humans. Mouse resistin is expressed by adipocytes and induces insulin resistance. Mouse resistin is inhibited by thiazolidinediones (TZD), which could partly explain the insulin sensitizing effect of this anti-diabetic drug. Human resistin is expressed by macrophages, and increased by inflammatory cytokines. Human resistin induces insulin resistance when expressed in resistin knock out mice

We examined the biology of human resistin by creating mice that lack adipocyte-derived mouse resistin but produce human resistin in a pattern similar to that found in humans, i.e., in macrophages. When fed a high-fat diet, the “humanized resistin” mice developed accelerated WAT inflammation, leading to increased lipolysis and increased serum free fatty acids. Over time, these mice accumulated lipids, including diacylglycerol, in skeletal muscle, resulting in insulin resistance. Thus, human resistin contributes to insulin resistance, despite the difference in the tissue origins of resistin in humans and mice [98].

## Other Adipokines Related to Obesity

### *Proinflammatory Cytokines*

TNF- $\alpha$  is expressed by adipocytes, stromovascular cells, and macrophages in adipose tissue [99]. TNF- $\alpha$  induces the expression of genes involved in cholesterol and fatty acid synthesis, and inhibits the expression of genes involved in fatty acid oxidation and glucose uptake in liver [99]. Obesity is associated with increased TNF- $\alpha$  expression, insulin resistance, and hyperlipidemia [100, 101]. Conversely, deletion of TNF- $\alpha$  or its receptors improved insulin sensitivity and reduced the levels of circulating free fatty acids in obese mice [102]. TNF- $\alpha$  attenuates insulin signaling partly by activating the NF- $\kappa$ B pathway [103]. IKK $\beta$  overexpression attenuates insulin signaling. TNF- $\alpha$  also induces insulin resistance by activating the Jun N-terminal kinase family of serine/threonine protein kinases, which phosphorylates



IRS-1/IRS-2 on serine residues, thus disrupting the insulin signaling cascade in muscle, adipose tissue, and liver [104].

Interleukin-6 is another proinflammatory cytokine that is increased in obesity [105]. Adipocytes and stromal cells express IL-6 and its receptor (IL-6R), which belongs to the same cytokine receptor family as Lr $\beta$ . IL-6 binding to IL-6R and gp130 results in activation of Jak/STAT3 signaling pathway [106]. Elevation of serum IL-6 parallels the development of insulin resistance in humans. IL-6 inhibits insulin signaling in hepatocytes by decreasing tyrosine phosphorylation of the insulin receptor, association with PI3K to IRS-1, and activation of Akt [106]. IL-6 also induces the expression of SOCS-3, which inhibits insulin signaling [107]. Intracerebroventricular injection of IL-6 in rodents increases energy expenditure, resulting in weight loss. Conversely, mice lacking IL-6 develop obesity that is reversed by IL-6 treatment [107].

### ***Retinol Binding Protein-4***

This adipokine produced by adipose tissue and liver, is increased in obese rodents, but the levels are highly variable in humans [108–111]. Studies revealed that RBP4 treatment induced insulin resistance in mice, while a reduction of RBP4 in obese mice reduced glucose levels [108]. Further analysis on the mechanism of action of RBP4 demonstrated that increased serum levels of RBP4 inhibited PI3K activity in muscle, thus decreasing insulin signaling [108]. Furthermore, administration of RBP4 in wild type mice reduced tyrosine phosphorylation of IRS-1 [108]. Administration of RBP4 stimulated the expression of PEPCK, which led to an increase of hepatic glucose production [108].

### **Concluding Remarks**

Adipose tissue has gained recognition not only as the main energy storage organ, but also as a source of secreted peptides. This review highlights the roles of leptin, adiponectin and proinflammatory cytokines in obesity, diabetes, and related disorders. Current research areas include the origin of adipose tissue, and specific functions of subcutaneous and visceral adipose tissue, and how they relate to normal physiology and disease. Our knowledge of adipokine signaling has benefited immensely from animal models, but there are potential pitfalls, e.g., differences in the sources of adipokines and target tissues. Moreover, important differences exist between rodent and human circadian rhythms, thermoregulation, immune function and glucose and lipid metabolism. Thus, it is necessary to confirm discoveries about adipokine signaling in humans under normal physiological conditions and disease states.

**Acknowledgements** This work was supported by grant RO1-DK62348 and PO1-DK49210 from the National Institutes of Health.

## References

1. James, W. P. (2008). The epidemiology of obesity: the size of the problem. *Journal of Internal Medicine* 263(4), 336–352.
2. Badman, M. K., & Flier, J. S. (2007). The adipocyte as an active participant in energy balance and metabolism. *Gastroenterology* 132(6), 2103–2115.
3. Belanger, C., Luu-The, V., Dupont, P., & Tchernof A. (2002). Adipose tissue intracrinology: potential importance of local androgen/estrogen metabolism in the regulation of adiposity. *Hormone and Metabolic Research*, 34(11–12), 737–745.
4. Wellen, K. E., & Hotamisligil, G. S. (2003). Obesity-induced inflammatory changes in adipose tissue. *Journal of Clinical Investigation*, 112(12), 1785–1788.
5. Ahima, R. S., Saper, C. B., Flier, J. S., & Elmquist, J. K. (2000). Leptin regulation of neuroendocrine systems. *Frontiers in Neuroendocrinology*, 21(3), 263–307.
6. Ahima, R. S., Prabakaran, D., Mantzoros, C., et al. (1996). Role of leptin in the neuroendocrine response to fasting. *Nature*, 382(6588), 250–252.
7. Farooqi, I. S., Matarese, G., Lord, G. M., et al. (2002). Beneficial effects of leptin on obesity, T cell hyporesponsiveness, and neuroendocrine/metabolic dysfunction of human congenital leptin deficiency. *Journal of Clinical Investigation*, 110(8), 1093–1103.
8. Ahima, R. S., Qi, Y., Singhal, N. S., Jackson, M. B., & Scherer, P. E. (2006). Brain adipocytokine action and metabolic regulation. *Diabetes*, 55(Suppl 2), S145–S154.
9. Licinio, J., Negrao, A. B., Mantzoros, C., et al. (1998). Sex differences in circulating human leptin pulse amplitude: clinical implications. *Journal of Clinical Endocrinology and Metabolism*, 83(11), 4140–4147.
10. Banks, W. A., Kastin, A. J., Huang, W., Jaspan, J. B., & Maness, L. M. (1996). Leptin enters the brain by a saturable system independent of insulin. *Peptides*, 17(2), 305–311.
11. Lord, G. M., Matarese, G., Howard, J. K., Baker, R. J., Bloom, S. R., & Lechler, R. I. (1998). Leptin modulates the T-cell immune response and reverses starvation-induced immunosuppression. *Nature*, 394(6696), 897–901.
12. Munzberg, H., & Myers, M. G., Jr. (2005). Molecular and anatomical determinants of central leptin resistance. *Nature Neuroscience*, 8(5), 566–570.
13. Bjorbaek, C., Uotani, S., da Silva, B., & Flier, J. S. (1997). Divergent signaling capacities of the long and short isoforms of the leptin receptor. *Journal of Biological Chemistry*, 272(51), 32686–32695.
14. Clement, K., Vaisse, C., Lahlou, N., et al. (1998). A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. *Nature*, 392(6674), 398–401.
15. Montague, C. T., Farooqi, I. S., Whitehead, J. P., et al. (1997). Congenital leptin deficiency is associated with severe early-onset obesity in humans. *Nature*, 387(6636), 903–908.
16. Cohen, P., Zhao, C., Cai, X., et al. (2001). Selective deletion of leptin receptor in neurons leads to obesity. *Journal of Clinical Investigation*, 108(8), 1113–1121.
17. Bates, S. H., Stearns, W. H., Dundon, T. A., et al. (2003). STAT3 signalling is required for leptin regulation of energy balance but not reproduction. *Nature*, 421(6925), 856–859.
18. Bjornholm, M., Munzberg, H., Leshan, R. L., et al. (2007). Mice lacking inhibitory leptin receptor signals are lean with normal endocrine function. *Journal of Clinical Investigation*, 117(5), 1354–1360.
19. Banks, W. A., & Farrell, C. L. (2003). Impaired transport of leptin across the blood-brain barrier in obesity is acquired and reversible. *American Journal of Physiology. Endocrinology and Metabolism*, 285(1), E10–E15.

20. Kievit, P., Howard, J. K., Badman, M. K., et al. (2006). Enhanced leptin sensitivity and improved glucose homeostasis in mice lacking suppressor of cytokine signaling-3 in POMC-expressing cells. *Cellular Metabolism*, 4(2), 123–132.
21. Zabolotny, J. M., Bence-Hanulec, K. K., Stricker-Krongrad, A., et al. (2002). PTP1B regulates leptin signal transduction in vivo. *Developmental Cell*, 2(4), 489–495.
22. Bence, K. K., Delibegovic, M., Xue, B., et al. (2006). Neuronal PTP1B regulates body weight, adiposity and leptin action. *Nature Medicine*, 12(8), 917–924.
23. Farooqi, I. S., Bullmore, E., Keogh, J., Gillard, J., O'Rahilly, S., & Fletcher, P. C. (2007). Leptin regulates striatal regions and human eating behavior. *Science*, 317(5843), 1355.
24. Dark, J. (2005). Annual lipid cycles in hibernators: integration of physiology and behavior. *Annual Review of Nutrition*, 25, 469–497.
25. Grattan, D. R., Ladyman, S. R., & Augustine, R. A. (2007). Hormonal induction of leptin resistance during pregnancy. *Physiology & Behavior*, 91(4), 366–374.
26. Fulton, S., Pissios, P., Manchon, R. P., et al. (2006). Leptin regulation of the mesoaccumbens dopamine pathway. *Neuron*, 51(6), 811–822.
27. Hommel, J. D., Trinko, R., Sears, R. M., et al. (2006). Leptin receptor signaling in midbrain dopamine neurons regulates feeding. *Neuron*, 51(6), 801–810.
28. Kahn, B. B., Alquier, T., Carling, D., & Hardie, D. G. (2005). AMP-activated protein kinase: ancient energy gauge provides clues to modern understanding of metabolism. *Cellular Metabolism*, 1(1), 15–25.
29. Niswender, K. D., Baskin, D. G., & Schwartz, M. W. (2004). Insulin and its evolving partnership with leptin in the hypothalamic control of energy homeostasis. *Trends in Endocrinology and Metabolism*, 15(8), 362–369.
30. Cowley, M. A., Smart, J. L., Rubinstein, M., et al. (2001). Leptin activates anorexigenic POMC neurons through a neural network in the arcuate nucleus. *Nature*, 411(6836), 480–484.
31. Pinto, S., Roseberry, A. G., Liu, H., et al. (2004). Rapid rewiring of arcuate nucleus feeding circuits by leptin. *Science*, 304(5667), 110–115.
32. Ahima, R. S., Bjorbaek, C., Osei, S., & Flier, J. S. (1999). Regulation of neuronal and glial proteins by leptin: implications for brain development. *Endocrinology*, 140(6), 2755–2762.
33. Matochik, J. A., London, E. D., Yildiz, B. O., et al. (2005). Effect of leptin replacement on brain structure in genetically leptin-deficient adults. *Journal of Clinical Endocrinology and Metabolism*, 90(5), 2851–2854.
34. Kusminski, C. M., McTernan, P. G., Schraw, T., et al. (2007). Adiponectin complexes in human cerebrospinal fluid: distinct complex distribution from serum. *Diabetologia*, 50(3), 634–642.
35. Yamauchi, T., Kamon, J., Ito, Y., et al. (2003). Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. *Nature*, 423(6941), 762–769.
36. Bouret, S. G., Draper, S. J., & Simerly, R. B. (2004). Trophic action of leptin on hypothalamic neurons that regulate feeding. *Science*, 304(5667), 108–110.
37. Bouret, S. G., Gorski, J. N., Patterson, C. M., Chen, S., Levin, B. E., & Simerly, R. B. (2008). Hypothalamic neural projections are permanently disrupted in diet-induced obese rats. *Cellular Metabolism*, 7(2), 179–185.
38. Baicy, K., London, E. D., Monterosso, J., et al. (2007). Leptin replacement alters brain response to food cues in genetically leptin-deficient adults. *Proceedings of the National Academy of Sciences of the United States of America*, 104(46), 18276–18279.
39. Rosenbaum, M., Sy, M., Pavlovich, K., Leibel, R. L., & Hirsch, J. (2008). Leptin reverses weight loss-induced changes in regional neural activity responses to visual food stimuli. *Journal of Clinical Investigation*, 118(7), 2583–2591.
40. Lieb, W., Beiser, A. S., Vasan, R. S., et al. (2009). Association of plasma leptin levels with incident Alzheimer disease and MRI measures of brain aging. *JAMA*, 302(23), 2565–2572.
41. Unger, R. H. (2003). The physiology of cellular liporegulation. *Annual Reviews in Physiology*, 65, 333–347.

42. Hedbacker, K., Birsoy, K., Wysocki, R. W., et al. (2010). Antidiabetic effects of IGFBP2, a leptin-regulated gene. *Cellular Metabolism*, 11(1), 11–22.
43. Seufert, J., Kieffer, T. J., Leech, C. A., et al. (1999). Leptin suppression of insulin secretion and gene expression in human pancreatic islets: implications for the development of adipogenic diabetes mellitus. *Journal of Clinical Endocrinology and Metabolism*, 84(2), 670–676.
44. Morioka, T., Asilmaz, E., Hu, J., et al. (2007). Disruption of leptin receptor expression in the pancreas directly affects beta cell growth and function in mice. *Journal of Clinical Investigation*, 117(10), 2860–2868.
45. De Rosa, V., Procaccini, C., Cali, G., et al. (2007). A key role of leptin in the control of regulatory T cell proliferation. *Immunity*, 26(2), 241–255.
46. Karsenty, G. (2006). Convergence between bone and energy homeostases: leptin regulation of bone mass. *Cellular Metabolism*, 4(5), 341–348.
47. Shi, Y., Yadav, V. K., Suda, N., et al. (2008). Dissociation of the neuronal regulation of bone mass and energy metabolism by leptin in vivo. *Proceedings of the National Academy of Sciences of the United States of America*, 105(51), 20529–20533.
48. Kadowaki, T., & Yamauchi, T. (2005). Adiponectin and adiponectin receptors. *Endocrine Reviews*, 26(3), 439–451.
49. Pajvani, U. B., Hawkins, M., Combs, T. P., et al. (2004). Complex distribution, not absolute amount of adiponectin, correlates with thiazolidinedione-mediated improvement in insulin sensitivity. *Journal of Biological Chemistry*, 279(13), 12152–12162.
50. Mao, X., Kikani, C. K., Riojas, R. A., et al. (2006). APPL1 binds to adiponectin receptors and mediates adiponectin signalling and function. *Nature Cell Biology*, 8(5), 516–523.
51. Yamauchi, T., Nio, Y., Maki, T., et al. (2007). Targeted disruption of AdipoR1 and AdipoR2 causes abrogation of adiponectin binding and metabolic actions. *Nature Medicine*, 13(3), 332–339.
52. Kubota, N., Terauchi, Y., Yamauchi, T., et al. (2002). Disruption of adiponectin causes insulin resistance and neointimal formation. *Journal of Biological Chemistry*, 277(29), 25863–25866.
53. Maeda, N., Shimomura, I., Kishida, K., et al. (2002). Diet-induced insulin resistance in mice lacking adiponectin/ACRP30. *Nature Medicine*, 8(7), 731–737.
54. Civitarese, A. E., Ukropcova, B., Carling, S., et al. (2006). Role of adiponectin in human skeletal muscle bioenergetics. *Cellular Metabolism*, 4(1), 75–87.
55. Goldstein, B. J., Scalia, R. (2004). Adiponectin: A novel adipokine linking adipocytes and vascular function. *Journal of Clinical Endocrinology and Metabolism*, 89(6), 2563–2568.
56. Shibata, R., Sato, K., Pimentel, D. R., et al. (2005). Adiponectin protects against myocardial ischemia-reperfusion injury through AMPK- and COX-2-dependent mechanisms. *Nature Medicine*, 11(10), 1096–1103.
57. Klein, S., Fontana, L., Young, V. L., et al. (2004). Absence of an effect of liposuction on insulin action and risk factors for coronary heart disease. *New England Journal of Medicine*, 350(25), 2549–2557.
58. Kim, J. Y., van de Wall, E., Laplante, M., et al. (2007). Obesity-associated improvements in metabolic profile through expansion of adipose tissue. *Journal of Clinical Investigation*, 117(9), 2621–2637.
59. Stefan, N., Kantartzis, K., Machann, J., et al. (2008). Identification and characterization of metabolically benign obesity in humans. *Archives in Internal Medicine*, 168(15), 1609–1616.
60. Qi, Y., Takahashi, N., Hileman, S. M., et al. (2004). Adiponectin acts in the brain to decrease body weight. *Nature Medicine*, 10(5), 524–529.
61. Kubota, N., Yano, W., Kubota, T., et al. (2007). Adiponectin stimulates AMP-activated protein kinase in the hypothalamus and increases food intake. *Cellular Metabolism*, 6(1), 55–68.
62. Coope, A., Milanski, M., Araujo, E. P., et al. (2008). AdipoR1 mediates the anorexigenic and insulin/leptin-like actions of adiponectin in the hypothalamus. *FEBS Letters*, 582(10), 1471–1476.

63. Bjursell, M., Ahnmark, A., Bohlooly, Y. M., et al. (2007). Opposing effects of adiponectin receptors 1 and 2 on energy metabolism. *Diabetes*, 56(3), 583–593.
64. Fry, M., Smith, P. M., Hoyda, T. D., et al. (2006). Area postrema neurons are modulated by the adipocyte hormone adiponectin. *Journal of Neuroscience*, 26(38), 9695–9702.
65. Hoyda, T. D., Fry, M., Ahima, R. S., & Ferguson, A. V. (2007). Adiponectin selectively inhibits oxytocin neurons of the paraventricular nucleus of the hypothalamus. *Journal of Physiology*, 585(Pt 3), 805–816.
66. Hoyda, T. D., Samson, W. K., & Ferguson, A. V. (2009). Adiponectin depolarizes parvocellular paraventricular nucleus neurons controlling neuroendocrine and autonomic function. *Endocrinology*, 150(2), 832–840.
67. Hoyda, T. D., Smith, P. M., & Ferguson, A. V. (2009). Adiponectin acts in the nucleus of the solitary tract to decrease blood pressure by modulating the excitability of neuropeptide Y neurons. *Brain Research*, 1256, 76–84.
68. Steppan, C. M., Bailey, S. T., Bhat, S., et al. (2001). The hormone resistin links obesity to diabetes. *Nature*, 409(6818), 307–312.
69. Rajala, M. W., Qi, Y., Patel, H. R., et al. (2004). Regulation of resistin expression and circulating levels in obesity, diabetes, and fasting. *Diabetes*, 53(7), 1671–1679.
70. Way, J. M., Gorgun, C. Z., Tong, Q., et al. (2001). Adipose tissue resistin expression is severely suppressed in obesity and stimulated by peroxisome proliferator-activated receptor gamma agonists. *Journal of Biological Chemistry*, 276(28), 25651–25653.
71. Patel, S. D., Rajala, M. W., Rossetti, L., Scherer, P. E., Shapiro, L. (2004). Disulfide-dependent multimeric assembly of resistin family hormones. *Science*, 304(5674), 1154–1158.
72. Hansotia, T., Maida, A., Flock, G., et al. (2007). Extrapankreatic incretin receptors modulate glucose homeostasis, body weight, and energy expenditure. *Journal of Clinical Investigation*, 117(1), 143–152.
73. Kim, S. J., Nian, C., & McIntosh, C. H. (2007). Resistin is a key mediator of glucose-dependent insulinotropic polypeptide (GIP) stimulation of lipoprotein lipase (LPL) activity in adipocytes. *Journal of Biological Chemistry*, 282(47), 34139–34147.
74. Rajala, M. W., Obici, S., Scherer, P. E., & Rossetti, L. (2003). Adipose-derived resistin and gut-derived resistin-like molecule-beta selectively impair insulin action on glucose production. *Journal of Clinical Investigation*, 111(2), 225–230.
75. Satoh, H., Nguyen, M. T., Miles, P. D., Imamura, T., Usui, I., Olefsky, J. M. (2004). Adenovirus-mediated chronic “hyper-resistinemia” leads to in vivo insulin resistance in normal rats. *Journal of Clinical Investigation*, 114(2), 224–231.
76. Banerjee, R. R., Rangwala, S. M., Shapiro, J. S., et al. (2004). Regulation of fasted blood glucose by resistin. *Science*, 303(5661), 1195–1198.
77. Muse, E. D., Obici, S., Bhanot, S., et al. (2004). Role of resistin in diet-induced hepatic insulin resistance. *Journal of Clinical Investigation*, 114(2), 232–239.
78. Kim, K. H., Lee, K., Moon, Y. S., & Sul, H. S. (2001). A cysteine-rich adipose tissue-specific secretory factor inhibits adipocyte differentiation. *Journal of Biological Chemistry*, 276(14), 11252–11256.
79. Kim, K. H., Zhao, L., Moon, Y., Kang, C., & Sul, H. S. (2004). Dominant inhibitory adipocyte-specific secretory factor (ADSF)/resistin enhances adipogenesis and improves insulin sensitivity. *Proceedings of the National Academy of Sciences of the United States of America*, 101(17), 6780–6785.
80. Qi, Y., Nie, Z., Lee, Y. S., et al. (2006). Loss of resistin improves glucose homeostasis in leptin deficiency. *Diabetes*, 55(11), 3083–3090.
81. Kos, K., Harte, A. L., da Silva, N. F., et al. (2007). Adiponectin and resistin in human cerebrospinal fluid and expression of adiponectin receptors in the human hypothalamus. *Journal of Clinical Endocrinology and Metabolism*, 92(3), 1129–1136.
82. Muse, E. D., Lam, T. K., Scherer, P. E., & Rossetti, L. (2007). Hypothalamic resistin induces hepatic insulin resistance. *Journal of Clinical Investigation*, 117(6), 1670–1678.
83. Singhal, N. S., Lazar, M. A., & Ahima, R. S. (2007). Central resistin induces hepatic insulin resistance via neuropeptide Y. *Journal of Neuroscience*, 27(47), 12924–12932.

84. Lazar, M. A. (2007). Resistin- and obesity-associated metabolic diseases. *Hormone and Metabolic Research*, 39(10), 710–716.
85. Savage, D. B., Sewter, C. P., Klenk, E. S., et al. (2001). Resistin/Fizz3 expression in relation to obesity and peroxisome proliferator-activated receptor-gamma action in humans. *Diabetes*, 50(10), 2199–2202.
86. Conneely, K. N., Silander, K., Scott, L. J., et al. (2004). Variation in the resistin gene is associated with obesity and insulin-related phenotypes in Finnish subjects. *Diabetologia*, 47(10), 1782–1788.
87. Menzaghi, C., Coco, A., Salvemini, L., et al. (2006). Heritability of serum resistin and its genetic correlation with insulin resistance-related features in nondiabetic Caucasians. *Journal of Clinical Endocrinology and Metabolism*, 91(7), 2792–2795.
88. Ochi, M., Osawa, H., Hirota, Y., et al. (2007). Frequency of the G/G genotype of resistin single nucleotide polymorphism at -420 appears to be increased in younger-onset type 2 diabetes. *Diabetes*, 56(11), 2834–2838.
89. Xu, J. Y., Sham, P. C., Xu, A., et al. (2007). Resistin gene polymorphisms and progression of glycaemia in southern Chinese: a 5-year prospective study. *Clinical Endocrinology (Oxford)*, 66(2), 211–217.
90. Gerber, M., Boettner, A., Seidel, B., et al. (2005). Serum resistin levels of obese and lean children and adolescents: biochemical analysis and clinical relevance. *Journal of Clinical Endocrinology and Metabolism*, 90(8), 4503–4509.
91. Lee, J. H., Chan, J. L., Yiannakouris, N., et al. (2003). Circulating resistin levels are not associated with obesity or insulin resistance in humans and are not regulated by fasting or leptin administration: cross-sectional and interventional studies in normal, insulin-resistant, and diabetic subjects. *Journal of Clinical Endocrinology and Metabolism*, 88(10), 4848–4856.
92. Frankel, D. S., Vasan, R. S., D'Agostino, R. B., Sr., et al. (2009). Resistin, adiponectin, and risk of heart failure the Framingham offspring study. *Journal of the American College of Cardiology*, 53(9), 754–762.
93. Osawa, H., Tabara, Y., Kawamoto, R., et al. (2007). Plasma resistin, associated with single nucleotide polymorphism -420, is correlated with insulin resistance, lower HDL cholesterol, and high-sensitivity C-reactive protein in the Japanese general population. *Diabetes Care*, 30(6), 1501–1506.
94. Reilly, M. P., Lehrke, M., Wolfe, M. L., Rohatgi, A., Lazar, M. A., & Rader, D. J. (2005). Resistin is an inflammatory marker of atherosclerosis in humans. *Circulation*, 111(7), 932–939.
95. Anderson, P. D., Mehta, N. N., Wolfe, M. L., et al. (2007). Innate immunity modulates adipokines in humans. *Journal of Clinical Endocrinology and Metabolism*, 92(6), 2272–2279.
96. Lo, J., Bernstein, L. E., Canavan, B., et al. (2007). Effects of TNF-alpha neutralization on adipocytokines and skeletal muscle adiposity in the metabolic syndrome. *American Journal of Physiology. Endocrinology and Metabolism*, 293(1), E102–E109.
97. Bernstein, L. E., Berry, J., Kim, S., Canavan, B., & Grinspoon, S. K. (2006). Effects of etanercept in patients with the metabolic syndrome. *Archives in Internal Medicine*, 166(8), 902–908.
98. Qatanani, M., Szwegold, N. R., Greaves, D. R., Ahima, R. S., & Lazar, M. A. (2009). Macrophage-derived human resistin exacerbates adipose tissue inflammation and insulin resistance in mice. *Journal of Clinical Investigation*, 119(3)531–539.
99. Tilg, H., & Moschen, A. R. (2006). Adipocytokines: mediators linking adipose tissue, inflammation and immunity. *Nature Reviews. Immunology*, 6(10), 772–783.
100. Hotamisligil, G. S., Shargill, N. S., & Spiegelman, B. M. (1993). Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. *Science*, 259(5091), 87–91.
101. Ruan, H., Miles, P. D., Ladd, C. M., et al. (2002). Profiling gene transcription in vivo reveals adipose tissue as an immediate target of tumor necrosis factor-alpha: implications for insulin resistance. *Diabetes*, 51(11), 3176–3188.



102. Uysal, K. T., Wiesbrock, S. M., Marino, M. W., & Hotamisligil, G. S. (1997). Protection from obesity-induced insulin resistance in mice lacking TNF- $\alpha$  function. *Nature*, 389(6651), 610–614.
103. Yuan, M., Konstantopoulos, N., Lee, J., et al. (2001). Reversal of obesity- and diet-induced insulin resistance with salicylates or targeted disruption of I $\kappa$ kbeta. *Science*, 293(5535), 1673–1677.
104. Hirosumi, J., Tuncman, G., Chang, L., et al. (2002). A central role for JNK in obesity and insulin resistance. *Nature*, 420(6913), 333–336.
105. Senn, J. J., Klover, P. J., Nowak, I. A., & Mooney, R. A. (2002). Interleukin-6 induces cellular insulin resistance in hepatocytes. *Diabetes*, 51(12), 3391–3399.
106. Senn, J. J., Klover, P. J., Nowak, I. A., et al. (2003). Suppressor of cytokine signaling-3 (SOCS-3), a potential mediator of interleukin-6-dependent insulin resistance in hepatocytes. *Journal of Biological Chemistry*, 278(16), 13740–13746.
107. Wallenius, V., Wallenius, K., Ahren, B., et al. (2002). Interleukin-6-deficient mice develop mature-onset obesity. *Nature Medicine*, 8(1), 75–79.
108. Yang, Q., Graham, T. E., Mody, N., et al. (2005). Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. *Nature*, 436(7049), 356–362.
109. Graham, T. E., Yang, Q., Bluher, M., et al. (2006). Retinol-binding protein 4 and insulin resistance in lean, obese, and diabetic subjects. *New England Journal of Medicine*, 354(24), 2552–2563.
110. Janke, J., Engeli, S., Boschmann, M., et al. (2006). Retinol-binding protein 4 in human obesity. *Diabetes*, 55(10), 2805–2810.
111. Graham, T. E., Wason, C. J., Bluher, M., & Kahn, B. B. (2007). Shortcomings in methodology complicate measurements of serum retinol binding protein (RBP4) in insulin-resistant human subjects. *Diabetologia*, 50(4), 814–823.

# Chapter 5

## Neural Control of Feeding and Energy Homeostasis

Emilie Caron and Rexford S. Ahima

### Introduction

Hunger is defined as a strong desire for food, while satiety is defined as a feeling of being full. These subjective characteristics of eating behavior arise from afferent neuronal and humoral signals from the gastrointestinal tract, adipose tissue, and other peripheral organs to the brain. Food intake provides energy to meet the requirements of basal metabolism, thermogenesis, and physical activity. It is logical that evolution has favored the development of mechanisms that promote eating and energy storage in order to protect us from the threat of starvation. Ironically, this genetic advantage toward positive energy balance has contributed to the obesity epidemic in modern societies where food is plentiful and exercise is sparse. Over time, the mismatch between energy intake and expenditure leads to excessive energy storage in the form of triglycerides in adipose tissue.

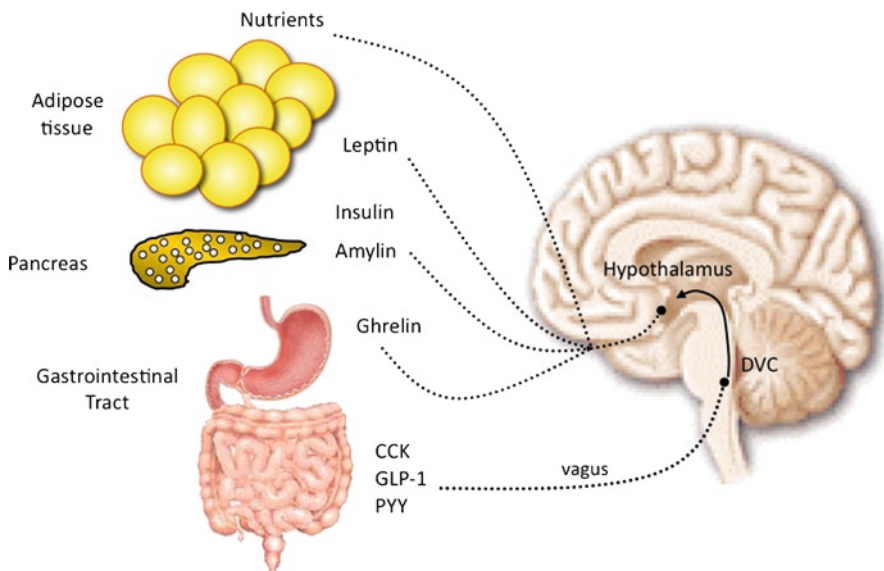
A role for the hypothalamus in the control of feeding was proposed in the 1940s based on lesion experiments [1]. Lesions of the ventromedial hypothalamus resulted in overeating and rapid weight increase, while lesions of the lateral hypothalamus resulted in failure of spontaneous feeding and starvation. These classic studies provided a conceptual framework for feeding regulation, in which the lateral hypothalamus was considered the “feeding center,” and the ventromedial hypothalamus was considered the “satiety center.” However, the hypothalamic lesions were not precise and often disrupted major fiber systems connecting the hypothalamus to other areas of the brain [1]. Nonetheless, the importance of the hypothalamus was rekindled in later studies involving parabiosis (i.e., cross-circulation) of obese ventromedial hypothalamic-lesioned rats (VMH) and normal (lean) rats [2].

---

R.S. Ahima (✉)

Division of Endocrinology, Diabetes and Metabolism, and the Institute for Diabetes, Obesity and Metabolism, University of Pennsylvania School of Medicine,  
415 Curie Boulevard, 712A Clinical Research Building, Philadelphia, PA 19104, USA  
e-mail: ahima@mail.med.upenn.edu





**Fig. 1** Brain–gut–adipose interactions. Nutrients, gastrointestinal hormones, adipokines, and vagal afferents signal to the hypothalamus and brainstem to coordinate feeding and metabolic adaptations

The lean rats ate less food and lost weight, while the VMH rats gained weight, suggesting the existence of a circulating satiety factor probably acting in the brain to control feeding and body weight [2]. Subsequently, the *ob* and *db* mutations, both of which caused hyperphagia and early onset obesity, were discovered in mice [3, 4]. In the 1990s the *ob* gene was shown to encode leptin while the *db* gene was shown to encode the leptin receptor [1, 5]. This chapter will focus on how hypothalamic and brainstem nuclei receive and integrate signals from the gastrointestinal tract and adipose tissue, and integrate the information for short- and long-term regulation of feeding and energy homeostasis (Fig. 1).

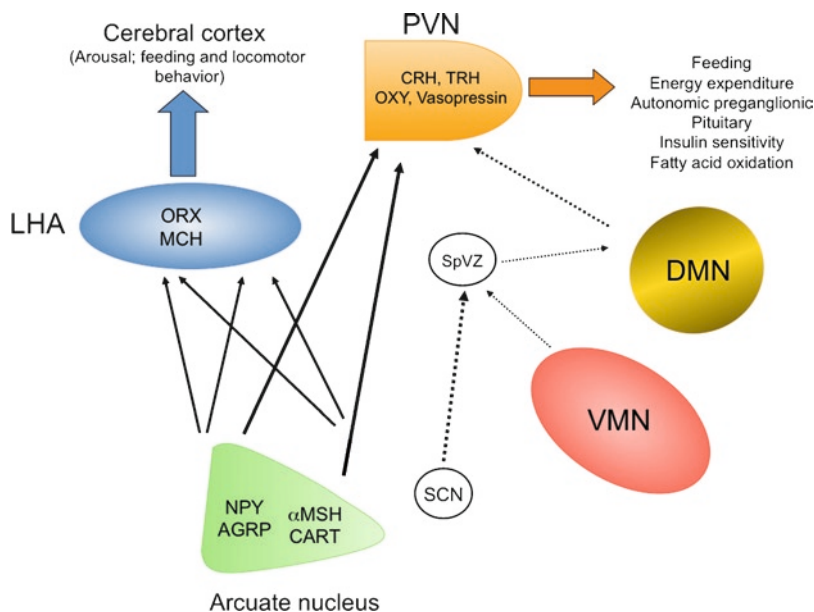
## Hypothalamic Regulation of Feeding and Energy Homeostasis

### *Arcuate Nucleus*

The arcuate nucleus is located in the basal hypothalamus, above the median eminence and pituitary stalk. Two distinct populations of neurons within the arcuate nucleus have been shown to be important in controlling energy homeostasis [1, 5]. One population of neurons co-expresses neuropeptide Y (NPY) and agouti-related peptide (AGRP). Intracerebroventricular (ICV) injection of either NPY or AGRP potently

stimulates food intake and weight gain [6, 7]. The second population of neurons in the arcuate nucleus co-expresses cocaine and amphetamine-related transcript (CART) and  $\alpha$ -melanocyte-stimulating hormone (MSH), derived from a precursor protein pro-opiomelanocortin (POMC) [1, 5]. CART and  $\alpha$ -MSH inhibit food intake when administered ICV [8, 9]. NPY exerts its orexigenic action by activating NPY Y1 and Y5 receptor, while  $\alpha$ -MSH inhibits feeding via melanocortin (MC)-4 receptors [1, 5]. AGRP acts mainly as an antagonist to  $\alpha$ -MSH [10]. NPY/AGRP and POMC/CART neurons project from the arcuate nucleus to the paraventricular nucleus (PVN), ventromedial nucleus (VMN), dorsomedial nucleus (DMN), and perifornical and lateral hypothalamic areas (LHA) (Fig. 2) [1, 5]. These nuclei project to the dorsal vagal complex (DVC), which includes the nucleus tractus solitarius (NTS) and the dorsal motor nucleus of the vagus (DMV) [1].

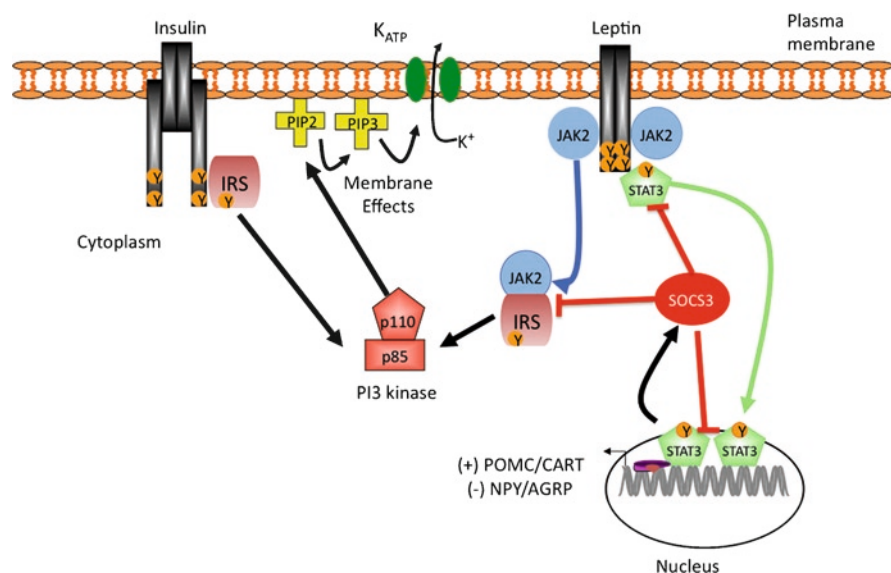
The pharmacological effects of neuropeptides expressed in the arcuate nucleus have been confirmed in some genetic models (Tables 1 and 2). Transgenic over-expression of NPY or AGRP causes hyperphagia and obesity in mice [11, 12]. POMC deficiency causes hyperphagia, obesity, and hypopigmentation in mice and humans [13, 14]. Ablation of the *mc4r* gene in mice or mutations that disrupt MC4 receptor signaling in humans causes hyperphagia, obesity, and increased linear growth



**Fig. 2** Hypothalamic neuronal circuitry. Neurons in the arcuate nucleus expressing NPY/AGRP or POMC/CART project to the paraventricular (PVN) and lateral hypothalamic area (LHA) to regulate feeding, energy expenditure, and neuroendocrine axis, through the expression of corticotropin-releasing hormone (CRH), thyrotropin-releasing hormone (TRH), oxytocin (OXY), orexins (ORX), and melanin-concentrating hormone (MCH). Neuronal projections from the suprachiasmatic nucleus (SCN), ventromedial nucleus (VMN), dorsomedial nuclei (DMN), and subparaventricular zone (SpVz), are involved in the control of feeding and circadian rhythms

[15, 16]. MC4 receptor-deficient patients are also protected from hypertension, reflecting a permissive effect of central melanocortin signaling on sympathetic nervous control of blood pressure [17]. Surprisingly, deletion of *npy* did not affect feeding and body weight [18]. However, later studies showed that NPY-deficient *ob/ob* mice were less hyperphagic and leaner than *ob/ob* mice [19]. NPY deficiency also attenuated post-fast hyperphagia and weight regain in diabetic or starved mice, suggesting that a major action of NPY is to promote feeding and replenish energy stores [20, 21]. The orexigenic action of NPY is thought to occur through the Y5 receptor in the hypothalamus; however, ablation of this gene produced an opposite phenotype, resulting instead in obesity [22]. Furthermore, AGRP deficiency did not affect feeding and in neonatal mice, whereas adult mice lacking AGRP died of starvation [23, 24]. Together, these findings highlight the complex and redundant roles of hypothalamic neuropeptides.

Leptin and insulin inhibit feeding by suppressing NPY/AGRP neurons and stimulating POMC neurons [5] (Fig. 3). Since the brain is protected by a blood–brain barrier (BBB), how do hormones reach neurons in the arcuate nucleus? A potential mechanism is that insulin and leptin cross the BBB endothelium via carrier-mediated transport [25, 26]. Systemic administration of horseradish peroxidase has been shown to penetrate the arcuate nucleus, suggesting that large molecules in the circulation may reach neurons in the arcuate nucleus outside the BBB [27]. The circumventricular organs (CVOs) are structures lining the cavity of the third



**Fig. 3** Signaling of leptin and insulin in the hypothalamus. Leptin binds to the long leptin receptor, which activates Janus kinase (JAK2) leading to nuclear translocation of STAT3 to mediate the transcriptional regulation of neuropeptides and suppressor of cytokine signaling-3 (SOCS3). SOCS3 inhibits leptin signaling. Leptin and insulin interact via activation of phosphatidylinositol-3 kinase (PI3K) to suppress feeding

**Table 1** Orexigenic neuropeptides, neurotransmitters, and hormones

	Targets	Effects
Neuropeptides		
Neuropeptide Y (NPY)	PVN, LHA, PFA	<p>mRNAs increased by food restriction</p> <p>Stimulates food intake when injected icv or in PVN, DMN or LHA</p> <p>Y1 and Y5 receptors mediate central effects of NPY on feeding</p> <p>Stimulates carbohydrate intake</p> <p>Knockout blunts post-fast hyperphagia and attenuates obesity in <i>ob/ob</i> mice</p>
Agouti-related peptide (AGRP)	PVN, LHA, PFA	<p>Expression restricted to the arcuate nucleus and co-localized with NPY</p> <p>Potent antagonist of <math>\alpha</math>-MSH at MC4 receptor</p> <p>Expression increased during fasting</p> <p>Prolonged stimulation of food intake when injected ICV or into the PVN</p> <p>Sustained action in the brain may be mediated via syndecans</p> <p>Specific deletion of <i>agrp</i> in NPY/AGRP in adult mice results in starvation</p>
Orexins	Cortex, limbic	<p>Close proximity with MCH-secreting neurons</p> <p>Orexin-expressing neurons receive inputs from NPY, AGRP and <math>\alpha</math>-MSH</p> <p>ICV injection potently increases food intake and induces feeding-related activities</p>
Melanin-concentrating hormone (MCH)	Cortex, limbic	<p>Expression restricted to the LHA and zona incerta</p> <p>Inputs from NPY/AGRP and POMC/CART neurons</p> <p>Expression increased during fasting</p> <p>ICV injection stimulates food intake</p> <p>Overexpression causes obesity and insulin-resistance</p> <p>Knockout results in hypophagia, hyperactivity and leanness</p>
Galanin	PVN	<p>mRNAs increased with obesity</p> <p>Injection ICV or directly into the PVN stimulates food intake</p> <p>Chronic infusion does not lead to obesity</p> <p>Knockout has no phenotype</p>
Galanin-like peptide (GALP)	PVN	<p>Mostly expressed in arcuate nucleus</p> <p>Injection ICV or directly into the PVN increases food intake</p> <p>Stimulates food intake by decreasing CART and increasing NPY</p>

(continued)

**Table 1** (continued)

	Targets	Effects
$\beta$ -endorphin	PVN, VMN	Increases consumption of highly palatable food ICV injection stimulates food intake Chronic treatment with opioid antagonists (naloxone and naltrexone) suppresses feeding and decreases body weight Knockout results in mild late-onset obesity
Norepinephrine	PVN, VMN	Stimulates feeding when injected into the PVN Chronic infusion into the VMN stimulates feeding and induces hyperinsulinemia and obesity Stimulates carbohydrate intake Effects on feeding mediated via $\alpha_2$ receptor
Gamma amino butyric acid (GABA)	PVN	Injection ICV or in the PVN of GABA <sub>A</sub> receptor agonist stimulates food intake Systemic or ICV injection of GABA <sub>B</sub> receptor agonist increases food intake Preferential expression of GABA <sub>A</sub> R in POMC/CART neurons Preferential expression of GABA <sub>B</sub> R in NPY/AGRP neurons Knockout of $\beta 3$ subunit of the GABA <sub>A</sub> receptor or glutamic acid decarboxylase (GAD)-65 did not affect body weight
Glucocorticoids	PVN, LHA, VMN, DMN	Enhances feeding probably through interaction with NPY, norepinephrine, and galanin. Effect on feeding mediated by type 2 corticosteroid receptor
Ghrelin	Arc, PVN, NTS	Increases feeding and weight Ghrelin receptor knockout develop mild resistance to diet-induced obesity

ventricle (neurohypophysis, vascular organ of the lamina terminalis, subfornical organ, pineal gland, and subcommissural organ) and of the fourth ventricle (area postrema). In contrast to capillaries in the rest of the brain which have tight junctions, the CVO capillaries have fenestrated endothelium and are very rich in peptidergic receptors [28]. The CVOs are well situated to detect hormones and relay the information to hypothalamic nuclei to control energy homeostasis. The arcuate nucleus is not considered a CVO, but it is situated in close proximity to the median eminence, raising the possibility that leptin and other peptide hormones can reach neurons in the arcuate via passive transport, and regulate NPY/AGRP and POMC/CART neurons, leading to coordinated changes in food intake, energy expenditure, and neuroendocrine function. Gut-derived hormones have been shown to signal in the area postrema and subfornical organ, and the information is then transmitted to the hypothalamus and other areas of the forebrain [29].

**Table 2** Anorexigenic neuropeptides, neurotransmitters, and hormones

	Targets	Effects
<b>Neuropeptides</b>		
Alpha melanocyte-stimulating hormone ( $\alpha$ -MSH)	PVN	ICV injection of $\alpha$ -MSH inhibits food intake Effects mediated mainly through MC4R and partly by MC3R Knockout of MC3R, MC4R and POMC leads to obesity
Cocaine and amphetamine-regulated transcript (CART)	PVN	ICV injection decreases food intake Expression decreased during fasting Co-expressed with POMC in the arcuate nucleus Implicated in taste aversion
Thyrotropin-releasing hormone (TRH)	DMN, and pituitary	ICV injection decreases both feeding and drinking Expression decreased in the PVN during fasting Depending on the feeding status of the mice, TRH neurons are either activated by CART or $\alpha$ -MSH, or inhibited by NPY Knockout did not affect feeding or weight, but caused glucose intolerance
Corticotropin-releasing hormone (CRH)	VMN, and pituitary	ICV injection decreases food intake Effects on feeding mediated by CRH-2 receptors CRH knockout: no change in feeding or weight CRH-2 knockout: decreased post-fast feeding
Glucagon-like peptide-1 (GLP-1)	PVN, DMN, Arc	ICV injection reduces food intake ICV injection reduces fasting and NPY-induced food intake Effects mediated through PVN and arcuate nucleus GLP-1 knockout has no change in feeding or weight
<b>Neurotransmitters</b>		
Serotonin	PVN, VMN, Arc	ICV injection inhibits food intake Serotonin reuptake inhibition reduces feeding Decreases food intake partly via activation of MC4 receptors in Arc 5HT <sub>2C</sub> receptor knockout: hyperphagia, obesity, insulin resistance
Histamine	PVN, VMN	Activation of H1 or H3 receptor decreases food intake Knockout of H3 receptor results in obesity
<b>Hormones</b>		
Insulin	Multiple	Inhibits food intake when injected ICV or in the hypothalamus Neuronal knockout developed mild obesity Deletion of <i>insr</i> + <i>lepr</i> in POMC neurons resulted in insulin resistance
Leptin	Arc, VMN, DMN	Decreases food intake and body weight when injected peripherally, ICV or directly in the PVN <i>ob/ob</i> : hyperphagia, obesity, impaired thermogenesis, hypogonadism

### ***Paraventricular Nucleus***

The paraventricular nucleus (PVN) is a pennant shaped structure which lies adjacent to the dorsal aspect of the third ventricle, and is critically involved in integrating feeding, autonomic, and neuroendocrine functions [1, 5]. Microinjection of NPY into the PVN stimulates feeding, while microinjection of  $\alpha$ -MSH or MC3/4 receptor agonists inhibits feeding [30, 31]. Electrophysiological studies have also demonstrated profound changes in PVN activity in response to leptin, adiponectin, cholecystokinin, and GLP-1 [32–35]. The PVN receives input from NPY/AGRP and POMC/CART neuron in the arcuate nucleus. The PVN contains neurons expressing corticotrophin-releasing hormone (CRH), thyrotropin-releasing hormone (TRH), oxytocin and vasopressin [1, 5]. These second order neurons transmit the effects of NPY and  $\alpha$ -MSH and AGRP, leading to profound changes in feeding and energy expenditure [1, 5] (Fig. 2). CRH, TRH, vasopressin, and oxytocin play critical roles in the regulation of glucocorticoids, thyroid hormone, water balance, uterine contraction, and milk secretion respectively. The PVN shares reciprocal connections with areas in the brainstem such as the NTS, which receives vagal innervation from the gastrointestinal tract and has extensive reciprocal connections with the area postrema [36]. In turn, the area postrema monitors circulating signals, and transmits the information to the NTS to be relayed to the PVN and other forebrain nuclei [37].

### ***Ventromedial Nucleus***

As discussed earlier, the important role of the ventromedial nucleus (VMN) in the control of feeding behavior was first demonstrated in hypothalamic lesion studies [1]. Lesions of VMN caused overeating, impaired thermogenesis, hypogonadism, and morbid obesity [1]. VMN neurons are activated by gastric distention, an effect mediated by the vagus nerve [38]. Glucose-sensing neurons have also been characterized in the VMN, and mediate the counter-regulatory response to hypoglycemia [39].

### ***Dorsomedial Nucleus***

The dorsomedial nucleus (DMN) has extensive connections with other hypothalamic pathways important for regulation of feeding [40]. The DMN also receives input from the suprachiasmatic nucleus and subparaventricular zone, suggesting an involvement in the control of circadian rhythms [41] (Fig. 2). It has been proposed that the DMN is involved in the feeding entrainment of circadian rhythms [42]. NPY is not normally expressed in the DMN but is induced in response to suckling and chronic food restriction [43, 44]. These changes are independent of leptin and thought to drive hyperphagia and energy storage [43, 44].

### ***Lateral Hypothalamic Area***

Bilateral lesions of the lateral hypothalamic area (LHA) produce aphagia, while electrical stimulation induces feeding, even in satiated animals [45, 46]. The LHA contains glucose-sensitive neurons which are activated by low glucose levels and inhibited by leptin [47, 48]. Two distinct populations of LHA neurons express orexins and melanin concentrating hormone (MCH) [1, 5]. Orexin A and B are released at terminals widely distributed in brain areas involved in the control of arousal, feeding, and autonomic function. Loss-of-function mutations of orexin receptors cause narcolepsy [49, 50]. Neurons expressing orexin A, are stimulated by starvation and by hypoglycemia [51, 52], and inhibited by visceral feeding signals, likely through vagal sensory pathways relayed by the NTS [53].

MCH is increased during fasting and stimulates food intake when injected ICV or directly into the hypothalamus [54]. As predicted, ablation of *mch* or *mchr1* genes resulted in hypophagia and lean phenotype [55, 56]. However, a major feature of these mutant mice is hyperactivity, possibly reflecting the diffuse projections of MCH neurons from the LHA to cortical areas involved with arousal and locomotor activity [55, 56]. Leptin inhibits MCH expression indirectly via projections from the arcuate nucleus to the LHA [1, 5]. MCH is increased in *ob/ob* mice, and ablation of *mch* attenuates hyperphagia and obesity [57].

### **Brainstem Regulation of Feeding and Energy Homeostasis**

The caudal brainstem receives afferent innervation from the gastrointestinal tract, and has extensive reciprocal connections with the hypothalamus [58]. The NTS receives information from chemoreceptors and mechanoreceptors conveying information about the taste, texture and chemical composition of food, and gastric distension [59]. This information is then relayed to the PVN and limbic areas in the forebrain. In addition to expressing glucagon-like peptide (GLP)-1, there is also evidence to suggest that the NTS contains POMC which is activated by cholecystokinin (CCK), resulting in inhibition of feeding and decrease in body weight [60, 61]. The NTS lies adjacent to the area postrema which lacks the normal BBB, and contains a variety of peptidergic receptors [29]. The area postrema is responsive to adiponectin, CCK, and orexin A [28, 29]. Ghrelin increases food intake partly by targeting the area postrema [29]. GLP-1, amylin and CCK also suppress feeding via the area postrema [29]. Leptin activates neurons in the NTS and lateral parabrachial nucleus, both of which receive vagal input from the gastrointestinal tract, and have extensive projections to the PVN and other forebrain areas [62, 63]. The NTS has been implicated in taste aversion, which may involve GLP-1, CCK, PYY, and other neuropeptides [64].



## Short-Term Regulation of Energy Homeostasis by Nutrients and Gut Hormones

In addition to providing a conduit for food and secreting enzymes involved in the digestion of carbohydrates, lipids, and proteins, endocrine cells in the gastrointestinal tract produce peptides which control motility, appetite, satiety, and insulin secretion [65]. The presence of food in the gut stimulates stretch receptors and chemoreceptors which inhibit appetite and induce satiety. Gut-derived signals may be integrated in the brainstem via vagal afferents in the NTS, or the hypothalamus via the circulation. Neurons in the brainstem and hypothalamus respond acutely to changes in glucose, fatty acids, and amino acids, leading to initiation or termination of meals, as well as alterations in fuel metabolism in the liver and other organs [66, 67].

Several gut hormones have been shown to influence feeding [65] (Tables 1 and 2). CCK decreases both meal size and duration thereby inhibiting food intake [68, 69]. CCK is released from the small intestine and activates gastric and duodenal vagal afferents sensitive to food volume. CCK acts synergistically with leptin to inhibit feeding [70]. Otsuka Long-Evans Tokushima Fatty (OLETF) rats lacking functional CCK1 receptors develop hyperphagia, diet-induced obesity and diabetes [71]. In contrast, disruption of CCK1 receptor in mice prevented the satiety effect of CCK but did not affect body weight in the long-term [72].

Ghrelin is produced mainly by the gastric mucosa, though ghrelin expression has also been detected in the hypothalamus and pancreatic islets [73]. A unique feature of ghrelin is the bioactive hormone is octanoylated on the third serine residue by Ghrelin-O-Acyltransferase (GOAT), which is expressed in the gastric mucosa [73, 74]. Ghrelin acts via growth hormone secretagogue (GHS) receptor-1 to increase food intake and body weight [75]. Secretion of ghrelin is increased in response to starvation and suppressed by meals. The pre-prandial rise in plasma ghrelin levels may signal meal initiation. An earlier study suggested that the pre-prandial increase in ghrelin levels was blunted after bariatric surgery and may contribute to weight loss following this surgical procedure [76]. However, such an association between ghrelin and bariatric surgery was not confirmed by other investigators [77, 78]. High ghrelin levels precede the development of Prader–Willi syndrome, which may explain overeating and obesity typical of this condition [79]. Although ghrelin tends to be reduced in primary (common) obesity, it is unclear if this contributes to the pathogenesis of obesity [80, 81].

In rodents, peripheral and especially ICV ghrelin treatment increases food intake and body weight [75]. The orexigenic effect of ghrelin is disrupted by vagotomy indicating that gastric vagal afferents play a major role in conveying ghrelin's signal to the brain [82]. Ghrelin activates NPY/AGRP neurons and inhibits POMC neurons in the arcuate nucleus, and these effects are abolished in the presence of Y1 and GABA<sub>A</sub> receptor antagonists [83]. Mice that are incapable of ghrelin signaling were resistant to diet-induced obesity, although the phenotype was very mild [84]. Similarly, ghrelin-deficient *ob/ob* mice did not show any obvious change in body weight, but insulin sensitivity was significantly improved [85]. Mice with deletion of *goat* had normal weight but became severely hypoglycemic in response to food restriction [86]. Infusion of ghrelin or growth hormone restored glucose to normal levels [86].

Amylin is co-secreted with insulin from pancreatic  $\beta$  cells in response to food intake [65, 87]. Amylin lowers plasma glucose by delaying gastric emptying and intestinal glucose absorption, and suppressing glucagon. This hypoglycemic property was the basis for developing amylin analogs, e.g., pramlintide, for treatment of diabetes. Injection of amylin ICV or intrahypothalamically slows gastric emptying and induces satiety. Chronic amylin treatment reduces body weight by inhibiting food intake and increasing energy expenditure [88]. Amylin exerts these actions on energy homeostasis via neuronal circuits in the brainstem and hypothalamus [88].

PYY is produced by the intestinal L-cells [65]. The bioactive peptide, PYY<sub>3-36</sub>, is stimulated in proportion to the energy content of food and peaks 1–2 h postprandially. PYY<sub>3-36</sub> has a high affinity for Y2 receptors and lower affinity for Y1 and Y5 receptors. Peripheral administration of PYY inhibits food intake in rodents and humans [89]. Electrophysiological studies demonstrated that PYY<sub>3-36</sub> depolarized and activated POMC neurons in the arcuate nucleus [89]. In addition, PYY<sub>3-36</sub> acted presynaptically at Y2 receptors to decrease glutamatergic transmission between the NTS and DMV [90]. Although there is agreement that PYY reduces gastric emptying and pancreatic secretions, and increases intestinal absorption of fluids and electrolytes, the anorexiogenic effect of PYY<sub>3-36</sub> could not be reproduced in other studies [90, 91].

Glucagon-like peptides (GLP-1 and GLP-2) and oxyntomodulin are produced by the post-translational processing of preproglucagon gene in the intestinal L-cells [92]. GLP-2 acts mainly to protect the intestinal mucosa. Like PYY, GLP-1, and oxyntomodulin are released into the circulation following a meal. GLP-1 acts as an incretin to stimulate glucose-dependent insulin secretion and delay gastric emptying [92]. Chronic administration of GLP-1 in the brain decreases food intake and body weight, mainly by targeting the PVN [92]. High-affinity binding sites for GLP-1 have been identified in the arcuate nucleus, area postrema, and NTS [92]. Although the important role of GLP-1 in insulin secretion has been confirmed by GLP-1 mimetics and dipeptidyl peptidase IV inhibitors which prevent the degradation of GLP-1, ablation of *glp1r* did not affect feeding and body weight, suggesting GLP-1 is not critically involved in energy homeostasis [93–95]. In contrast, oxyntomodulin acts in the brainstem to inhibit food intake and increase energy expenditure [96].

## Long-Term Regulation of Energy Stores by Adiposity Hormones

As discussed in chapter 4, adipokines act in the brain to control feeding and energy stores in adipose tissue. Congenital leptin deficiency causes hyperphagia, impaired thermoregulation (in rodents), steatosis, insulin resistance, hypogonadism, and immunosuppression [1, 5]. Leptin reverses these abnormalities mostly through CNS mechanisms. Leptin activates Jak2-STAT3 in NPY/AGRP and POMC/CART neurons in the arcuate nucleus, which project to the PVN and LHA [1, 5] (Figs. 2 and 3). Second order neurons transmit the leptin signal to the brainstem, limbic areas, and cortex, leading to coordinated changes in eating behavior, energy expenditure, and neuroendocrine function [1, 5]. Most obese people have leptin resistance, but

whether this is the result of impaired leptin transport in the brain and/or attenuation of leptin signaling via SOCS3 is unknown [97].

Leptin receptors are expressed in multiple sites besides the hypothalamus and brain-stem [1, 5]. Within the ventral tegmental area (VTA), which is critical for the reward circuitry, leptin targets dopamine and GABA neurons, inducing phosphorylation of signal-transducer-and-activator-of-transcription-3 (STAT3) [98]. Leptin-sensitive VTA neurons project to the nucleus accumbens to regulate hedonic aspects of feeding behavior. Electrically stimulated dopamine release from nucleus accumbens shell terminals was attenuated in *ob/ob* brain slices and restored by leptin [98]. Direct injection of leptin into the VTA decreased food intake in mice, while RNAi-mediated knockdown of leptin receptors in the VTA increased food intake and preference for highly palatable food. These findings support a role for leptin in integrating motivated behavior [99].

Adiponectin is reduced in obesity and has been shown to regulate energy balance in rodents [100, 101]. Adiponectin increases energy expenditure in *ob/ob* mice, partly by activating MC4 receptors in the PVN [101]. Adiponectin is increased during fasting and targets the arcuate nucleus to stimulate feeding [100].

Insulin levels increase with meals and decrease in response to fasting. Insulin is also positively correlated with adiposity. Long before the discovery of leptin, it was reported that administration of insulin into the brain inhibited feeding and decreased body weight [102]. Insulin receptor and signaling molecules are widely distributed in the brain [103]. Genetic deletion of neuronal insulin receptors caused hyperphagia and disrupted estrus cycles in obesity in female mice [104]. Within the arcuate nucleus, insulin receptors are co-localized with  $\alpha$ -MSH and NPY, and interact with leptin signaling via PI-3 kinase [103]. ICV insulin administration during fasting inhibits NPY mRNA levels in the arcuate nucleus [103]. However, genetic ablation of insulin receptor in NPY/AGRP or POMC neurons did not alter feeding or energy homeostasis [105, 106]. Instead, central insulin signaling is important for glucose homeostasis [105–107]. Reduction of insulin signaling molecules in the arcuate nucleus induced hepatic insulin resistance and increased glucose production [107].

Glucocorticoids exert a permissive effect on feeding, by promoting the expression of NPY and other orexigenic peptides [108]. Anorexia is a prominent feature of adrenal insufficiency, while excess glucocorticoids in Cushing's syndrome stimulate feeding. Estrogen inhibits food intake, partly through interactions with leptin and insulin [109]. Proinflammatory cytokines also inhibit feeding and increase energy expenditure via hypothalamic mechanisms [110].

## Role of Biogenic Amines in Feeding Regulation and Energy Homeostasis

Central biogenic amine pathways have been studied for their effects on feeding behavior and body weight [111, 112]. Monoaminergic neuronal circuits that use dopamine, norepinephrine, and serotonin (5-hydroxytryptamine, 5-HT) as

neurotransmitters have been major targets of drug development. For example, amphetamines, phentermine, and ephedrine, release monoamines from neuronal stores, and their anti-obesity effect appears to be mediated through norepinephrine. Sibutramine is a serotonin and norepinephrine reuptake inhibitor which causes sustained weight reduction by inducing satiety and increasing energy expenditure. Selective serotonin reuptake inhibitors have been used in the treatment of depression and eating disorders but have been less effective in the treatment of obesity. Nonetheless, genetic models support a major role of serotonin in energy homeostasis. Deletion of 5HT<sub>2c</sub> receptors, especially in POMC neurons of the arcuate nucleus, resulted in obesity [113, 114]. Furthermore, 5HT<sub>2c</sub> agonists improved glucose homeostasis through interactions with the central melanocortin system [115]. Histamine receptors, H<sub>1</sub> and H<sub>2</sub>, have been implicated in energy homeostasis. Deletion of H<sub>3</sub> receptor resulted in obesity [116]. These findings suggest that 5-HT<sub>2c</sub> and histamine (H<sub>3</sub>) receptors may be suitable for developing drugs for obesity and diabetes.

Obesity is common among patients with schizophrenia and often associated with detrimental health consequences. As in the general population, overconsumption of energy-dense food and sedentary lifestyle are major factors in the development of obesity in schizophrenia. However, the introduction of atypical antipsychotic agents, e.g., clozapine and olanzapine, had led to higher incidences of obesity, diabetes, and dyslipidemia [117, 118]. The underlying mechanisms are unclear, but studies have implicated a dysregulation of serotonin, opioid, and neuropeptide pathways [119, 120].

## Conclusions

The past two decades has seen tremendous advances in our knowledge of brain circuits which control feeding and energy homeostasis. Contrary to the notion that feeding is regulated by discrete populations of neurons, emerging evidence points to a distributed circuitry across the hypothalamus, brainstem, limbic, and higher cortical areas. Neuropeptides and classical neurotransmitters that respond to metabolic signals from the gut, adipose tissue, and other organs, contribute to the integrated control of hunger, satiety, and energy storage. The central neuronal circuits involved in feeding and energy expenditure are hard-wired, but also capable of adapting to short- and long-term alterations in energy requirements [121]. Leptin, estrogen, glucocorticoids, and ghrelin influence synaptic plasticity, and are capable of modulating feeding behavior under varying physiological and pathological conditions [122–124]. Dysregulation of these complex systems contributes to obesity, diabetes, and other metabolic diseases. Advances in molecular genetics and neuroimaging techniques will increase our understanding of the pathogenesis of obesity and enable the development of novel therapies.

## References

1. Elmquist, J. K., Elias, C. F., & Saper, C. B. (1999). From lesions to leptin: Hypothalamic control of food intake and body weight. *Neuron*, 22(2), 221–232.
2. Hervey, G. R. (1959). The effects of lesions in the hypothalamus in parabiotic rats. *The Journal of Physiology*, 145(2), 336–352.
3. Coleman, D. L. (1973). Effects of parabiosis of obese with diabetes and normal mice. *Diabetologia*, 9(4), 294–298.
4. Hummel, K. P., Dickie, M. M., & Coleman, D. L. (1966). Diabetes, a new mutation in the mouse. *Science*, 153(740), 1127–1128.
5. Ahima, R. S., Saper, C. B., Flier, J. S., & Elmquist, J. K. (2000). Leptin regulation of neuroendocrine systems. *Frontiers in Neuroendocrinology*, 21(3), 263–307.
6. Clark, J. T., Sahu, A., Kalra, P. S., Balasubramaniam, A., & Kalra, S. P. (1987). Neuropeptide Y (NPY)-induced feeding behavior in female rats: Comparison with human NPY ([Met17] NPY), NPY analog ([norLeu4]NPY) and peptide YY. *Regulatory Peptides*, 17(1), 31–39.
7. Rossi, M., Kim, M. S., Morgan, D. G., et al. (1998). A C-terminal fragment of Agouti-related protein increases feeding and antagonizes the effect of alpha-melanocyte stimulating hormone in vivo. *Endocrinology*, 139(10), 4428–4431.
8. Edwards, C. M., Abbott, C. R., Sunter, D., et al. (2000). Cocaine- and amphetamine-regulated transcript, glucagon-like peptide-1 and corticotrophin releasing factor inhibit feeding via agouti-related protein independent pathways in the rat. *Brain Research*, 866(1–2), 128–134.
9. Wirth, M. M., & Giraudo, S. Q. (2000). Agouti-related protein in the hypothalamic paraventricular nucleus: Effect on feeding. *Peptides*, 21(9), 1369–1375.
10. Ollmann, M. M., Wilson, B. D., Yang, Y. K., et al. (1997). Antagonism of central melanocortin receptors in vitro and in vivo by agouti-related protein. *Science*, 278(5335), 135–138.
11. Kaga, T., Inui, A., Okita, M., et al. (2001). Modest overexpression of neuropeptide Y in the brain leads to obesity after high-sucrose feeding. *Diabetes*, 50(5), 1206–1210.
12. Wilson, B. D., Ollmann, M. M., & Barsh, G. S. (1999). The role of agouti-related protein in regulating body weight. *Molecular Medicine Today*, 5(6), 250–256.
13. Coll, A. P., Challis, B. G., Yeo, G. S., et al. (2004). The effects of proopiomelanocortin deficiency on murine adrenal development and responsiveness to adrenocorticotropin. *Endocrinology*, 145(10), 4721–4727.
14. Krude, H., Biebermann, H., Luck, W., Horn, R., Brabant, G., & Gruters, A. (1998). Severe early-onset obesity, adrenal insufficiency and red hair pigmentation caused by POMC mutations in humans. *Nature Genetics*, 19(2), 155–157.
15. Huszar, D., Lynch, C. A., Fairchild-Huntress, V., et al. (1997). Targeted disruption of the melanocortin-4 receptor results in obesity in mice. *Cell*, 88(1), 131–141.
16. Yeo, G. S., Lank, E. J., Farooqi, I. S., Keogh, J., Challis, B. G., & O’Rahilly, S. (2003). Mutations in the human melanocortin-4 receptor gene associated with severe familial obesity disrupts receptor function through multiple molecular mechanisms. *Human Molecular Genetics*, 12(5), 561–574.
17. Greenfield, J. R., Miller, J. W., Keogh, J. M., et al. (2009). Modulation of blood pressure by central melanocortinergic pathways. *The New England Journal of Medicine*, 360(1), 44–52.
18. Erickson, J. C., Clegg, K. E., & Palmiter, R. D. (1996). Sensitivity to leptin and susceptibility to seizures of mice lacking neuropeptide Y. *Nature*, 381(6581), 415–421.
19. Erickson, J. C., Hollopeter, G., & Palmiter, R. D. (1996). Attenuation of the obesity syndrome of ob/ob mice by the loss of neuropeptide Y. *Science*, 274(5293), 1704–1707.
20. Patel, H. R., Qi, Y., Hawkins, E. J., et al. (2006). Neuropeptide Y deficiency attenuates responses to fasting and high-fat diet in obesity-prone mice. *Diabetes*, 55(11), 3091–3098.
21. Sindelar, D. K., Mystkowski, P., Marsh, D. J., Palmiter, R. D., & Schwartz, M. W. (2002). Attenuation of diabetic hyperphagia in neuropeptide Y – deficient mice. *Diabetes*, 51(3), 778–783.

22. Marsh, D. J., Hollopeter, G., Kafer, K. E., & Palmiter, R. D. (1998). Role of the Y5 neuropeptide Y receptor in feeding and obesity. *Nature Medicine*, 4(6), 718–721.
23. Bewick, G. A., Gardiner, J. V., Dhillon, W. S., et al. (2005). Post-embryonic ablation of AgRP neurons in mice leads to a lean, hypophagic phenotype. *The FASEB Journal*, 19(12), 1680–1682.
24. Luquet, S., Perez, F. A., Hnasko, T. S., & Palmiter, R. D. (2005). NPY/AgRP neurons are essential for feeding in adult mice but can be ablated in neonates. *Science*, 310(5748), 683–685.
25. Banks, W. A., Kastin, A. J., Huang, W., Jaspan, J. B., & Maness, L. M. (1996). Leptin enters the brain by a saturable system independent of insulin. *Peptides*, 17(2), 305–311.
26. Baura, G. D., Foster, D. M., Porte, D., Jr., et al. (1993). Saturable transport of insulin from plasma into the central nervous system of dogs in vivo. A mechanism for regulated insulin delivery to the brain. *The Journal of Clinical Investigation*, 92(4), 1824–1830.
27. Broadwell, R. D., & Brightman, M. W. (1976). Entry of peroxidase into neurons of the central and peripheral nervous systems from extracerebral and cerebral blood. *The Journal of Comparative Neurology*, 166(3), 257–283.
28. Cottrell, G. T., & Ferguson, A. V. (2004). Sensory circumventricular organs: Central roles in integrated autonomic regulation. *Regulatory Peptides*, 117(1), 11–23.
29. Hoyda, T. D., Smith, P. M., & Ferguson, A. V. (2009). Gastrointestinal hormone actions in the central regulation of energy metabolism: Potential sensory roles for the circumventricular organs. *International Journal of Obesity (London)*, 33(Suppl 1), S16–S21.
30. Rossi, M., Beak, S. A., Choi, S. J., et al. (1999). Investigation of the feeding effects of melanin concentrating hormone on food intake – action independent of galanin and the melanocortin receptors. *Brain Research*, 846(2), 164–170.
31. Taylor, K., Lester, E., Hudson, B., & Ritter, S. (2007). Hypothalamic and hindbrain NPY, AGRP and NE increase consummatory feeding responses. *Physiology & Behavior*, 90(5), 744–750.
32. Hamamura, M., Leng, G., Emson, P. C., & Kiyama, H. (1991). Electrical activation and c-fos mRNA expression in rat neurosecretory neurones after systemic administration of cholecystokinin. *The Journal of Physiology*, 444, 51–63.
33. Hoyda, T. D., Fry, M., Ahima, R. S., & Ferguson, A. V. (2007). Adiponectin selectively inhibits oxytocin neurons of the paraventricular nucleus of the hypothalamus. *The Journal of Physiology*, 585(Pt 3), 805–816.
34. Larsen, P. J., Tang-Christensen, M., & Jessop, D. S. (1997). Central administration of glucagon-like peptide-1 activates hypothalamic neuroendocrine neurons in the rat. *Endocrinology*, 138(10), 4445–4455.
35. Powis, J. E., Bains, J. S., & Ferguson, A. V. (1998). Leptin depolarizes rat hypothalamic paraventricular nucleus neurons. *The American Journal of Physiology*, 274(5 Pt 2), R1468–R1472.
36. Ruggiero, D. A., Giuliano, R., Anwar, M., Stornetta, R., & Reis, D. J. (1990). Anatomical substrates of cholinergic-autonomic regulation in the rat. *The Journal of Comparative Neurology*, 292(1), 1–53.
37. Smith, P. M., & Ferguson, A. V. (1996). Paraventricular nucleus efferents influence area postrema neurons. *The American Journal of Physiology*, 270(2 Pt 2), R342–R347.
38. Sun, X., Tang, M., Zhang, J., & Chen, J. D. (2006). Excitatory effects of gastric electrical stimulation on gastric distension responsive neurons in ventromedial hypothalamus (VMH) in rats. *Neuroscience Research*, 55(4), 451–457.
39. McCrimmon, R. (2009). Glucose sensing during hypoglycemia: Lessons from the lab. *Diabetes Care*, 32(8), 1357–1363.
40. Bellinger, L. L., & Bernardis, L. L. (2002). The dorsomedial hypothalamic nucleus and its role in ingestive behavior and body weight regulation: Lessons learned from lesioning studies. *Physiology & Behavior*, 76(3), 431–442.
41. Saper, C. B., Lu, J., Chou, T. C., & Gooley, J. (2005). The hypothalamic integrator for circadian rhythms. *Trends in Neurosciences*, 28(3), 152–157.
42. Gooley, J. J., Schomer, A., & Saper, C. B. (2006). The dorsomedial hypothalamic nucleus is critical for the expression of food-entrainable circadian rhythms. *Nature Neuroscience*, 9(3), 398–407.



43. Grove, K. L., Brogan, R. S., & Smith, M. S. (2001). Novel expression of neuropeptide Y (NPY) mRNA in hypothalamic regions during development: Region-specific effects of maternal deprivation on NPY and Agouti-related protein mRNA. *Endocrinology*, 142(11), 4771–4776.
44. Li, C., Chen, P., & Smith, M. S. (1998). The acute suckling stimulus induces expression of neuropeptide Y (NPY) in cells in the dorsomedial hypothalamus and increases NPY expression in the arcuate nucleus. *Endocrinology*, 139(4), 1645–1652.
45. Anand, B. K., & Brobeck, J. R. (1951). Localization of a “feeding center” in the hypothalamus of the rat. *Proceedings of the Society for Experimental Biology and Medicine*, 77(2), 323–324.
46. Stein, E. A., Carr, K. D., & Simon, E. J. (1990). Brain stimulation-induced feeding alters regional opioid receptor binding in the rat: An in vivo autoradiographic study. *Brain Research*, 533(2), 213–222.
47. Burdakov, D., Luckman, S. M., & Verkhatsky, A. (2005). Glucose-sensing neurons of the hypothalamus. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, 360(1464), 2227–2235.
48. Williams, G., Bing, C., Cai, X. J., Harrold, J. A., King, P. J., & Liu, X. H. (2001). The hypothalamus and the control of energy homeostasis: Different circuits, different purposes. *Physiology & Behavior*, 74(4–5), 683–701.
49. Brisbare-Roch, C., Dingemans, J., Koberstein, R., et al. (2007). Promotion of sleep by targeting the orexin system in rats, dogs and humans. *Nature Medicine*, 13(2), 150–155.
50. Chemelli, R. M., Willie, J. T., Sinton, C. M., et al. (1999). Narcolepsy in orexin knockout mice: Molecular genetics of sleep regulation. *Cell*, 98(4), 437–451.
51. Karteris, E., Machado, R. J., Chen, J., Zervou, S., Hillhouse, E. W., & Randeva, H. S. (2005). Food deprivation differentially modulates orexin receptor expression and signaling in rat hypothalamus and adrenal cortex. *American Journal of Physiology. Endocrinology and Metabolism*, 288(6), E1089–1100.
52. Tkacs, N. C., Pan, Y., Sawhney, G., Mann, G. L., & Morrison, A. R. (2007). Hypoglycemia activates arousal-related neurons and increases wake time in adult rats. *Physiology & Behavior*, 91(2–3), 240–249.
53. Zheng, H., Patterson, L. M., & Berthoud, H. R. (2005). Orexin-A projections to the caudal medulla and orexin-induced c-Fos expression, food intake, and autonomic function. *The Journal of Comparative Neurology*, 485(2), 127–142.
54. Qu, D., Ludwig, D. S., Gammeltoft, S., et al. (1996). A role for melanin-concentrating hormone in the central regulation of feeding behaviour. *Nature*, 380(6571), 243–247.
55. Marsh, D. J., Weingarh, D. T., Novi, D. E., et al. (2002). Melanin-concentrating hormone 1 receptor-deficient mice are lean, hyperactive, and hyperphagic and have altered metabolism. *Proceedings of the National Academy of Sciences of the United States of America*, 99(5), 3240–3245.
56. Shimada, M., Tritos, N. A., Lowell, B. B., Flier, J. S., & Maratos-Flier, E. (1998). Mice lacking melanin-concentrating hormone are hypophagic and lean. *Nature*, 396(6712), 670–674.
57. Segal-Lieberman, G., Bradley, R. L., Kokkotou, E., et al. (2003). Melanin-concentrating hormone is a critical mediator of the leptin-deficient phenotype. *Proceedings of the National Academy of Sciences of the United States of America*, 100(17), 10085–10090.
58. Berthoud, H. R., Sutton, G. M., Townsend, R. L., Patterson, L. M., & Zheng, H. (2006). Brainstem mechanisms integrating gut-derived satiety signals and descending forebrain information in the control of meal size. *Physiology & Behavior*, 89(4), 517–524.
59. Rolls, E. T. (1997). Taste and olfactory processing in the brain and its relation to the control of eating. *Critical Reviews in Neurobiology*, 11(4), 263–287.
60. Hayes, M. R., Bradley, L., & Grill, H. J. (2009). Endogenous hindbrain glucagon-like peptide-1 receptor activation contributes to the control of food intake by mediating gastric satiation signaling. *Endocrinology*, 150(6), 2654–2659.
61. Rogers, R. C., & Hermann, G. E. (2008). Mechanisms of action of CCK to activate central vagal afferent terminals. *Peptides*, 29(10), 1716–1725.
62. Elias, C. F., Kelly, J. F., Lee, C. E., et al. (2000). Chemical characterization of leptin-activated neurons in the rat brain. *The Journal of Comparative Neurology*, 423(2), 261–281.

63. Hayes, M. R., Skibicka, K. P., & Lechner, T. M., et al. Endogenous leptin signaling in the caudal nucleus tractus solitarius and area postrema is required for energy balance regulation. *Cell Metabolism*, 11(1), 77–83.
64. Tang-Christensen, M., Vrang, N., & Larsen, P. J. (2001). Glucagon-like peptide containing pathways in the regulation of feeding behaviour. *International Journal of Obesity and Related Metabolic Disorders*, 25(Suppl 5), S42–S47.
65. Wren, A. M., & Bloom, S. R. (2007). Gut hormones and appetite control. *Gastroenterology*, 132(6), 2116–2130.
66. Cota, D., Proulx, K., & Seeley, R. J. (2007). The role of CNS fuel sensing in energy and glucose regulation. *Gastroenterology*, 132(6), 2158–2168.
67. Lam, T. K. Neuronal regulation of homeostasis by nutrient sensing. *Nature Medicine*, 16(4), 392–395.
68. Moran, T. H., & Kinzig, K. P. (2004). Gastrointestinal satiety signals II. Cholecystokinin. *American Journal of Physiology. Gastrointestinal and Liver Physiology*, 286(2), G183–188.
69. Moran, T. H., Ladenheim, E. E., & Schwartz, G. J. (2001). Within-meal gut feedback signaling. *International Journal of Obesity and Related Metabolic Disorders*, 25(Suppl 5), S39–S41.
70. Matson, C. A., Reid, D. F., & Ritter, R. C. (2002). Daily CCK injection enhances reduction of body weight by chronic intracerebroventricular leptin infusion. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology*, 282(5), R1368–R1373.
71. Laye, M. J., Rector, R. S., Warner, S. O., et al. (2009). Changes in visceral adipose tissue mitochondrial content with type 2 diabetes and daily voluntary wheel running in OLETF rats. *The Journal of Physiology*, 587(Pt 14), 3729–3739.
72. Kopin, A. S., Mathes, W. F., McBride, E. W., et al. (1999). The cholecystokinin-A receptor mediates inhibition of food intake yet is not essential for the maintenance of body weight. *The Journal of Clinical Investigation*, 103(3), 383–391.
73. Castaneda, T. R., Tong, J., Datta, R., Culler, M., & Tschop, M. H. (2010). Ghrelin in the regulation of body weight and metabolism. *Frontiers in Neuroendocrinology*, 31(1), 44–60.
74. Sakata, I., Yang, J., Lee, C. E., et al. (2009). Colocalization of ghrelin O-acyltransferase and ghrelin in gastric mucosal cells. *American Journal of Physiology. Endocrinology and Metabolism*, 297(1), E134–E141.
75. Kirchner, H., Gutierrez, J. A., Solenberg, P. J., et al. (2009). GOAT links dietary lipids with the endocrine control of energy balance. *Nature Medicine*, 15(7), 741–745.
76. Cummings, D. E., Weigle, D. S., Frayo, R. S., et al. (2002). Plasma ghrelin levels after diet-induced weight loss or gastric bypass surgery. *The New England Journal of Medicine*, 346(21), 1623–1630.
77. Couce, M. E., Cottam, D., Esplen, J., Schauer, P., & Burguera, B. (2006). Is ghrelin the culprit for weight loss after gastric bypass surgery? A negative answer. *Obesity Surgery*, 16(7), 870–878.
78. Foschi, D., Corsi, F., Colombo, F., et al. (2008). Different effects of vertical banded gastroplasty and Roux-en-Y gastric bypass on meal inhibition of ghrelin secretion in morbidly obese patients. *Journal of Investigative Surgery*, 21(2), 77–81.
79. Feigerlova, E., Diene, G., Conte-Auriol, F., et al. (2008). Hyperghrelinemia precedes obesity in Prader-Willi syndrome. *The Journal of Clinical Endocrinology and Metabolism*, 93(7), 2800–2805.
80. Marzullo, P., Verti, B., Savia, G., et al. (2004). The relationship between active ghrelin levels and human obesity involves alterations in resting energy expenditure. *The Journal of Clinical Endocrinology and Metabolism*, 89(2), 936–939.
81. Pacifico, L., Poggiogalle, E., Costantino, F., et al. (2009). Acylated and nonacylated ghrelin levels and their associations with insulin resistance in obese and normal weight children with metabolic syndrome. *European Journal of Endocrinology*, 161(6), 861–870.
82. Hosoda, H., & Kangawa, K. (2008). The autonomic nervous system regulates gastric ghrelin secretion in rats. *Regulatory Peptides*, 146(1–3), 12–18.
83. Cowley, M. A., Cone, R. D., Enriori, P., Louiselle, I., Williams, S. M., & Evans, A. E. (2003). Electrophysiological actions of peripheral hormones on melanocortin neurons. *Annals of the New York Academy of Sciences*, 994, 175–186.



84. Zigman, J. M., Nakano, Y., Coppari, R., et al. (2005). Mice lacking ghrelin receptors resist the development of diet-induced obesity. *The Journal of Clinical Investigation*, 115(12), 3564–3572.
85. Sun, Y., Asnicar, M., Saha, P. K., Chan, L., & Smith, R. G. (2006). Ablation of ghrelin improves the diabetic but not obese phenotype of ob/ob mice. *Cell Metabolism*, 3(5), 379–386.
86. Zhao, T. J., Liang, G., Li, R. L., et al. Ghrelin O-acyltransferase (GOAT) is essential for growth hormone-mediated survival of calorie-restricted mice. *Proceedings of the National Academy of Sciences of the United States of America*, 107(16), 7467–7472.
87. Riediger, T., Zuend, D., Becskei, C., & Lutz, T. A. (2004). The anorectic hormone amylin contributes to feeding-related changes of neuronal activity in key structures of the gut-brain axis. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology*, 286(1), R114–R122.
88. Turek, V. F., Trevaskis, J. L., Levin, B. E., et al. Mechanisms of amylin/leptin synergy in rodent models. *Endocrinology*, 151(1), 143–152.
89. Batterham, R. L., Cowley, M. A., Small, C. J., et al. (2002). Gut hormone PYY(3–36) physiologically inhibits food intake. *Nature*, 418(6898), 650–654.
90. Browning, K. N., & Travagli, R. A. (2003). Neuropeptide Y and peptide YY inhibit excitatory synaptic transmission in the rat dorsal motor nucleus of the vagus. *The Journal of Physiology*, 549(Pt 3), 775–785.
91. Halatchev, I. G., & Cone, R. D. (2005). Peripheral administration of PYY(3–36) produces conditioned taste aversion in mice. *Cell Metabolism*, 1(3), 159–168.
92. Baggio, L. L., & Drucker, D. J. (2007). Biology of incretins: GLP-1 and GIP. *Gastroenterology*, 132(6), 2131–2157.
93. Drucker, D. J. (2007). Dipeptidyl peptidase-4 inhibition and the treatment of type 2 diabetes: Preclinical biology and mechanisms of action. *Diabetes care*, 30(6), 1335–1343.
94. Lovshin, J. A., & Drucker, D. J. (2009). Incretin-based therapies for type 2 diabetes mellitus. *Nature Reviews. Endocrinology*, 5(5), 262–269.
95. Scrocchi, L. A., Hill, M. E., Saleh, J., Perkins, B., & Drucker, D. J. (2000). Elimination of glucagon-like peptide 1R signaling does not modify weight gain and islet adaptation in mice with combined disruption of leptin and GLP-1 action. *Diabetes*, 49(9), 1552–1560.
96. Baggio, L. L., Huang, Q., Brown, T. J., & Drucker, D. J. (2004). Oxyntomodulin and glucagon-like peptide-1 differentially regulate murine food intake and energy expenditure. *Gastroenterology*, 127(2), 546–558.
97. Myers, M. G., Cowley, M. A., & Munzberg, H. (2008). Mechanisms of leptin action and leptin resistance. *Annual Review of Physiology*, 70, 537–556.
98. Fulton, S., Pissios, P., Manchon, R. P., et al. (2006). Leptin regulation of the mesoaccumbens dopamine pathway. *Neuron*, 51(6), 811–822.
99. Lommel, J. D., Trinko, R., Sears, R. M., et al. (2006). Leptin receptor signaling in midbrain dopamine neurons regulates feeding. *Neuron*, 51(6), 801–810.
100. Kubota, N., Yano, W., Kubota, T., et al. (2007). Adiponectin stimulates AMP-activated protein kinase in the hypothalamus and increases food intake. *Cell Metabolism*, 6(1), 55–68.
101. Qi, Y., Takahashi, N., Hileman, S. M., et al. (2004). Adiponectin acts in the brain to decrease body weight. *Nature Medicine*, May 10(5), 524–529.
102. Woods, S. C., Lotter, E. C., McKay, L. D., & Porte, D., Jr. (1979). Chronic intracerebroventricular infusion of insulin reduces food intake and body weight of baboons. *Nature*, 282(5738), 503–505.
103. Niswender, K. D., Baskin, D. G., & Schwartz, M. W. (2004). Insulin and its evolving partnership with leptin in the hypothalamic control of energy homeostasis. *Trends in Endocrinology and Metabolism*, 15(8), 362–369.
104. Bruning, J. C., Gautam, D., Burks, D. J., et al. (2000). Role of brain insulin receptor in control of body weight and reproduction. *Science*, 289(5487), 2122–2125.
105. Hill, J. W., Elias, C. F., Fukuda, M., et al. Direct insulin and leptin action on pro-opiomelanocortin neurons is required for normal glucose homeostasis and fertility. *Cell Metabolism*, 11(4), 286–297.

106. Konner, A. C., Janoschek, R., Plum, L., et al. (2007). Insulin action in AgRP-expressing neurons is required for suppression of hepatic glucose production. *Cell Metabolism*, 5(6), 438–449.
107. Obici, S., Zhang, B. B., Karkanias, G., & Rossetti, L. (2002). Hypothalamic insulin signaling is required for inhibition of glucose production. *Nature Medicine*, 8(12), 1376–1382.
108. Dallman, M. F., Warne, J. P., Foster, M. T., & Pecoraro, N. C. (2007). Glucocorticoids and insulin both modulate caloric intake through actions on the brain. *The Journal of Physiology*, 583(Pt 2), 431–436.
109. Shi, H., Seeley, R. J., & Clegg, D. J. (2009). Sexual differences in the control of energy homeostasis. *Frontiers in Neuroendocrinology*, 30(3), 396–404.
110. Plata-Salaman, C. R. (1989). Immunomodulators and feeding regulation: A humoral link between the immune and nervous systems. *Brain, Behavior, and Immunity*, 3(3), 193–213.
111. Hainer, V., Kabrnova, K., Aldhoon, B., Kunesova, M., & Wagenknecht, M. (2006). Serotonin and norepinephrine reuptake inhibition and eating behavior. *Annals of the New York Academy of Sciences*, 1083, 252–269.
112. Meister, B. (2007). Neurotransmitters in key neurons of the hypothalamus that regulate feeding behavior and body weight. *Physiology & Behavior*, 92(1–2), 263–271.
113. Nonogaki, K., Strack, A. M., Dallman, M. F., & Tecott, L. H. (1998). Leptin-independent hyperphagia and type 2 diabetes in mice with a mutated serotonin 5-HT<sub>2C</sub> receptor gene. *Nature Medicine*, 4(10), 1152–1156.
114. Xu, Y., Jones, J. E., Kohno, D., et al. (2008). 5-HT<sub>2C</sub>Rs expressed by pro-opiomelanocortin neurons regulate energy homeostasis. *Neuron*, 60(4), 582–589.
115. Zhou, L., Sutton, G. M., Rochford, J. J., et al. (2007). Serotonin 2C receptor agonists improve type 2 diabetes via melanocortin-4 receptor signaling pathways. *Cell Metabolism*, 6(5), 398–405.
116. Takahashi, K., Suwa, H., Ishikawa, T., & Kotani, H. (2002). Targeted disruption of H3 receptors results in changes in brain histamine tone leading to an obese phenotype. *The Journal of Clinical Investigation*, 110(12), 1791–1799.
117. Allison, D. B., Newcomer, J. W., Dunn, A. L., et al. (2009). Obesity among those with mental disorders: A National Institute of Mental Health meeting report. *American Journal of Preventive Medicine*, 36(4), 341–350.
118. Reynolds, G. P., & Kirk, S. L. Metabolic side effects of antipsychotic drug treatment – pharmacological mechanisms. *Pharmacology & Therapeutics*, 125(1), 169–179.
119. Basile, V. S., Masellis, M., McIntyre, R. S., Meltzer, H. Y., Lieberman, J. A., & Kennedy, J. L. (2001). Genetic dissection of atypical antipsychotic-induced weight gain: Novel preliminary data on the pharmacogenetic puzzle. *The Journal of Clinical Psychiatry*, 62(Suppl 23), 45–66.
120. Kuzman, M. R., Medved, V., Bozina, N., Hotujac, L., Sain, I., & Bilusic, H. (2008). The influence of 5-HT<sub>2C</sub> and MDR1 genetic polymorphisms on antipsychotic-induced weight gain in female schizophrenic patients. *Psychiatry Research*, 160(3), 308–315.
121. Horvath, T. L. (2005). The hardship of obesity: A soft-wired hypothalamus. *Nature Neuroscience*, 8(5), 561–565.
122. Diano, S., Farr, S. A., Benoit, S. C., et al. (2006). Ghrelin controls hippocampal spine synapse density and memory performance. *Nature Neuroscience*, 9(3), 381–388.
123. Gao, Q., Mezei, G., Nie, Y., et al. (2007). Anorectic estrogen mimics leptin's effect on the rewiring of melanocortin cells and Stat3 signaling in obese animals. *Nature Medicine*, 13(1), 89–94.
124. Horvath, T. L. (2006). Synaptic plasticity mediating leptin's effect on metabolism. *Progress in Brain Research*, 153, 47–55.



# Chapter 6

## Gastrointestinal Hormones and Obesity

Yan Wang and Efi Kokkotou

### Introduction

Apart from digesting and absorbing nutrients, the gastrointestinal (GI) tract also possesses important sensing and signaling functions. It is estimated that more than 50 hormones and regulatory peptides are synthesized in the GI, primarily in response to food entering the digestive system [1, 2]. The majority of the bioactive peptides are generated from a larger precursor (pro-hormone) by proteolytic cleavage mediated by various proconvertases (PC), and followed by modifications such as amidation [3]. Gut hormones are secreted from specialized enteroendocrine cells, different types of which are located in the stomach (G-cells), duodenum (D-cells), and the large intestine (L-cells).

The released molecules diffuse through the interstitial fluid and act locally, for example by activating nearby extrinsic sensory fibers. Hence, the effects of most gut hormones on food intake are abolished by vagotomy [5, 6]. They also enter the circulation and reach the feeding centers in various brain areas, including the brainstem, hypothalamus and midbrain regions involved in reward processing [7, 8]. The ability of gut hormones to penetrate the blood–brain barrier and their potential interactions with other hormones are major determinants of their central actions. Their gastrointestinal actions affect the secretion of gastric acid and pancreatic enzymes, gastric emptying, and intestinal motility [9, 10]. Among the most studied gastrointestinal peptides that regulate food intake are ghrelin, cholecystokinin (CCK), glucagon-like peptide 1 (GLP-1), glucose-dependent insulintropic polypeptide (GIP), oxyntomodulin, peptide YY (PYY), pancreatic polypeptide (PP), and amylin [1, 11, 12]. With the exception of ghrelin, which is considered a “hunger hormone,” the rest of the GI peptides and hormones mediate “satiation,” a fullness signal that leads to meal size reduction and termination of feeding [7].

---

E. Kokkotou (✉)

Gastroenterology Division, Department of Medicine,  
Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA  
e-mail: ekokkoto@bidmc.harvard.edu

Satiation is distinct from “satiety,” which is associated with lack of a desire to start eating and thus postpones the initiation of the next meal [13]. Hormones secreted from the lower GI tend to have both satiation and satiety effects [11, 14].

Experimental approaches that have been applied in elucidating the physiological roles of gut hormones can be summarized in: (a) pharmacological studies using hormone agonists or antagonists; (b) analysis of the feeding behavior of hormone- or hormone receptor-deficient mice, which do not produce consistent results with pharmacology; (c) studies in humans, which are usually descriptive, though critical information has been gathered in particular from the study of obese patients who were subjected to bariatric surgery [15]. A major limitation in understanding the physiology of the gut hormones has been their extremely short half-life, a few minutes, due to degradation of the enzyme dipeptidyl peptidase-IV (DPP-IV). This also explains the lack of reliable serum detection assays for most of the gut hormones, in addition to their structural similarities leading to cross-reactivity [1]. The regulation of their bioavailability is also complex, and is affected by serum-binding proteins and proteolytic enzymes. Gut hormone receptors belong to the G-protein coupled family that is characterized by seven-transmembrane regions. Often, the same hormone utilizes more than one receptor subtype and the same receptor can have multiple ligands [16]. In addition, there is significant functional interaction between gut hormones and hormones that regulate energy balance at the long-term, for example leptin, in particular at the hypothalamic level [17].

It is well accepted that disruption of the gut hormone homeostasis results in dysregulation of energy balance, most often toward obesity [18]. Several mechanisms have been proposed for these effects, including inappropriate hormone secretion in relation to food intake, lack of rhythmicity, and hormone resistance at the receptor level [19].

## Hormones Secreted by the Upper Gastrointestinal Tract

### *Ghrelin*

Ghrelin is a 28-amino acid acylated hormone, resulting from proteolytic cleavage of a pre-pro-hormone [20]. Ghrelin-producing cells are primarily localized in the stomach, with higher density in the gastric fundus, though ghrelin production from pancreatic islet cells and the distal intestine has also been described [12, 21]. Ghrelin acts as an endogenous ligand for the orphan growth hormone secretagogue receptor (GHS-R), which is also expressed in hypothalamic nuclei, the dorsal vagal complex, and the mesolimbic dopaminergic systems [22]. Hence, ghrelin possesses both appetite-stimulating and growth hormone-releasing activities. From the preproghrelin gene, a second putative peptide is produced, obestatin, which is postulated to have the opposite effects of ghrelin and induces satiety [23].

Plasma ghrelin levels exhibit a diurnal variation and are higher during the night. They rise by fasting and before a meal and are suppressed within 1 h of eating

suggesting a role in meal initiation [24–26]. Ghrelin levels also correlate with rates of gastric emptying [24]. In both lean and obese individuals, administration of ghrelin increased hunger scores and caloric intake from a buffet meal [27, 28]. In rodents, chronic ghrelin injections induce hyperphagia and weight gain beyond the effects of caloric intake, suggesting that perhaps ghrelin has also an effect on metabolic rate [29]. Indeed, ghrelin-deficient mice are hypophagic, have increased fat oxidation and are protected from diet-induced obesity [30]. Moreover, they have improved insulin sensitivity compared with mice with similar levels of adiposity. Additional studies have shown that in adipose tissue ghrelin induces enzymes involved in fat storage, thus leading to reduced fat mobilization [31].

Fasting ghrelin levels tend to be low in obese humans and this is postulated to constitute a feedback mechanism to reduce appetite. However, the major defects in obesity appear to be lack of post-prandial ghrelin suppression and an overall increase in ghrelin sensitivity [12]. Following dieting and weight loss, ghrelin levels rise, along with hunger levels, and this adoptive response might account for the failure to sustain weight loss [32]. However, this is not the case after gastric-bypass, which is associated with low ghrelin levels, a factor that accounts, at least in part, for the success of gastric bypass in permanent weight loss [33]. Interestingly, several polymorphisms in the pre-pro-ghrelin genes have been described, that modulate the risk for obesity and the metabolic syndrome [34]. Of note, serum ghrelin levels are particularly high in patients with Prader–Willi syndrome, that is characterized by severe hyperphagia and obesity [35].

In preclinical studies, the bioavailability of ghrelin has been targeted using a vaccination approach. Rats vaccinated against different fragments of the native peptide had slower rates of weight gain and accumulation of fat, most likely due to a decrease in feed efficiency [36].

### ***Cholecystokinin (CCK)***

CCK, a hormone structurally related to gastrin, is primarily synthesized in the duodenum and jejunum and circulates in several molecular forms, as a 8-, 33-, 39-, and 54-aminoacid polypeptide [37]. Although two receptors have been described for CCK, CCK-1, and CCK-2, the major receptor mediating its food-related effects is CCK-1 (or CCK-A) receptor, which is localized in the GI, for example pancreas and afferent fibers of the vagus nerve, and also in brain (brainstem and hypothalamus). Notably, the peptide itself is widely expressed in the brain, in regions regulating both homeostatic and hedonic aspects of feeding [38].

CCK is short-acting and its levels peak after 15–30 min of meal ingestion, in particular when the meal is rich in fat and protein content, and return to basal levels within 3–5 h [39]. CCK is a potent satiety hormone and inhibits food intake while increasing nutrient absorption. Those effects are mediated by delaying gastric emptying, stimulating pancreatic secretions and increasing gall bladder contractions, a property that gave the peptide its name [40].

In rodent studies, administration of CCK reduced meal size and duration, which was compensated by an increase in meal frequency [41]. Moreover, chronic CCK antagonism accelerated weight gain in rodents independently of food intake, suggesting additional functions of this hormone in energy balance. This notion is further supported by studies demonstrating that CCK potentiates the effects of leptin, when the two hormones are co-administered [42].

The regulation of CCK during obesity awaits further clarification. Overall, it appears that CCK is increased in obesity and decreased in anorexia nervosa, most likely as a result, and not a drive, of food intake [43]. Genetic studies have shown that certain CCK alleles (CCK-H3) predispose to consumption of larger portions of meals and that certain CCK-1 receptor variants are associated with increased risk for obesity [44].

CCK's value as an anti-obesity drug has not yet been fully explored, mainly due to its short-lived effects. Studies using CCK-8 have shown that the peptide should be administered within 15 min prior to initiation of a meal, in order to be able to reduce meal size [39]. Development of receptor de-sensitization and tolerance has also been reported after continuous CCK infusion in rats or humans [1]. However, based on its strong effects on meal regulation, both peripherally and centrally, drugs that mimic the effects of CCK are highly desirable [38].

### ***Incretins and Enteroinsular Axis***

Incretins are gut-derived peptides that act as insulin secretagogues and thus increase glucose-stimulated insulin secretion from the pancreas. It has been observed that an amount of glucose given orally results in greater insulin secretion than the same amount given intravenously, and this difference is attributed to the effects of incretins [45]. Glucagon-like peptide-1 (GLP-1) and Glucose-dependent insulinotropic polypeptide (GIP), previously known as gastric-inhibitory peptide, are the two known incretins in the gut [45]. It has been estimated that GIP acting in concert with GLP-1 account for more than 60% of postprandial insulin secretion [46]. In addition to their effects in insulin secretion, incretins also inhibit glucagon release from the pancreas, the hormone that counteracts the action of insulin, while in parallel inhibit acid secretion and delay gastric emptying, decreasing overall food intake [10, 47].

***Glucagon-Like Peptide (GLP)-1*** The preproglucagon gene has a complex evolution and encodes for oxyntomodulin, glucagon, GLP-1, and GLP-2 [48]. In the glucagon family also belongs GIP [49]. GLP-1 is produced by L-cells in the distal intestine. GLP-1 levels start to increase within 5–10 min after eating, peak at 30 min and remain elevated for 2–3 h after a meal [1]. GLP-1 infusion resulted in 10–15% reduction of caloric intake in both lean and obese individuals, and was associated with reduced rates of gastric emptying [50, 51]. However, mice with

ablation of GLP-1 receptor had normal body weight and food intake, but were found to be glucose intolerant [52].

Recently, our understanding of GLP-1 physiology has been revolutionized following the discovery of sweet taste receptors expressed by the enteroendocrine L-cells [53]. These sensors regulate GLP-1 secretion and their blockade results in impaired GLP-1 responses [54]. Obese individuals tend to have an attenuated GLP-1 response post-prandially, while dieting has been associated with low fasting GLP-1 levels, most likely reflecting a drive to eat [1, 55]. In contrast, following gastric bypass, both fasting and post-prandial levels of GLP-1 are increased [56].

The GLP-1 system appears to be a compelling candidate for drug development, primarily due to its insulinotropic activity and glucose-lowering effects [47, 57]. In fact, two long-lasting GLP-1 mimetics have been approved for the treatment of type II diabetes, Exenatide and Liraglutide [58]. A limitation of these drugs is that they must be administered via subcutaneous injections. Exendins are fragments of bioactive peptides first isolated from the lizard venom. Exendin 4 (exenatide) in particular acts as a potent agonist for the GLP-1 receptor and is not subject to DPP-IV degradation [59]. Most common effects associated with this drug are nausea, vomiting, and diarrhea, which decrease overtime [12]. Rare adverse effects include kidney malfunction and development of acute pancreatitis [60]. When exenatide is prescribed together with other glucose-lowering agents, the risk of hypoglycemia increases and the medication doses should be readjusted. Development of antibodies to the drug has also been described, the clinical significance of which remains unknown [61].

Liraglutide is a modified GLP-1 with the addition of a fatty acid molecule. Compared to GLP-1, liraglutide has greater stability, along with a slow and consistent release from albumin. The major advantage of liraglutide is that it rarely leads to hypoglycemia, as most of the glucose-lowering drugs [62]. Inhibitors of DPP-IV have also been investigated as antidiabetic drugs with the advantage that can be taken orally [58]. One of them, sitagliptin has gained FDA approval to be used alone or in combination with other antidiabetic drugs [63].

**Glucose-Dependent Insulinotropic Polypeptide** Glucose-Dependent Insulinotropic Polypeptide (GIP), a 42-amino acid gastrointestinal hormone also known as gastric inhibitory peptide, is the second known incretin and binds to GIP receptor (GIPR) which is widely distributed in peripheral tissues [46]. Ingested fat is a potent stimulus for GIP secretion. Besides its effects on insulin secretion, GIP influences addition aspects of metabolism and energy balance [46, 64]. For example, it can inhibit gluconeogenesis in the liver, enhance glucose uptake in muscle and promote the proliferation, survival, and differentiation of pancreatic  $\beta$ -cells. Moreover, treatment with GIP stimulated the synthesis and secretion of lipoprotein lipase in rat adipose tissue [65]. GIP receptor-deficient mice exhibit a high-fat diet induced obesity resistant phenotype and remain insulin sensitive, suggesting that GIP, or perhaps additional ligands of this receptor, might play an important role in the pathogenesis of obesity [66].



In obese individuals, bioactive GIP levels in response to meal ingestion were found to be reduced, most likely due to increased activity of DPP-4 in obese subjects [55]. Most importantly, and in contrast to GLP-1, it has been reported that the incretin effects of GIP are abolished in patients with diabetes type 2 and their first degree relatives [67].

There is still controversy on whether GIP-mimetic drugs or GIP antagonists can be used for the treatment of obesity and insulin resistance [46]. Based on the lack of GIP-induced incretin effects in diabetic patients and phenotype of the GIPR mice, it appears that GIP antagonists could be potentially used as anti-obesity drugs.

## Hormones Secreted by the Distal Gut

### *Oxyntomodulin (OXM)*

OXM is a 37-aminoacid peptide that contains the whole sequence of glucagon with eight additional aminoacids [49]. It is synthesized by L-cells in the lower intestine and colon, the same cells that also secrete GLP-1 and PYY(3-36) (see below) [1]. Studies using GLP-1R-deficient mice suggest that OXM mediates some of its appetite regulating effects via GLP-1, though its affinity for this receptor is much lower than GLP-1 [68]. It is speculated that additional receptors for OXM do exist.

OXM levels rise within 30 min of a meal and it takes several hours for them to return to baseline. OXM inhibits gastric acid secretion, delays gastric emptying and promotes both satiation and satiety [69]. Rodents given OXM lose more body weight than vehicle-treated pair-fed controls, suggesting that OXM may reduce body weight by upregulating energy expenditure [70]. Acute administration of OXM, but not GLP-1, has also been shown to enhance voluntary activity in obese individuals, consistent with experiments in rodents [71]. In the long term, preprandial subcutaneous injections of OXM to obese subjects for 4 weeks resulted in a moderate weight loss attributed to reduction in fat mass [72]. However, treatment with OXM appears to lack the glucose-lowering effects associated with GLP-1 treatment.

Very little is known about the changes in OXM levels during obesity, mainly due to the lack of a commercially available test that do not cross-react with the other peptides of the family [1]. Interestingly, and consistent with the pharmacological studies, postprandial levels of OXM have been reported to be elevated after gastric bypass [54].

Treatment with OXM would benefit only obese individuals with normal glucose metabolism. A potent, long-lasting OXM analogue, which is injectable, has been developed and tested in clinical trials [1]. However, treatment with OXM lacks the glucose-lowering effects associated with GLP-1 treatment [73]. It has also been reported that in obese individuals, the appetite suppressing effects of OXM and PYY are additive [74].

## ***Peptide YY (PYY)***

PYY, along with pancreatic polypeptide (PP) and neuropeptide Y (NPY), form a family of neuropeptides based on structural similarities (PP-fold) and sharing common receptors. Members of the PP-fold family exert their effects via the G-protein coupled receptors Y1, Y2, Y4, and Y5 and their particular biological effects depend on their receptor distribution and functional antagonism among the members of the family [1, 16]. PYY is secreted by the same cells that secrete also GLP-1 and OXM, localized primarily in the distal gut. Endogenous PYY appears in two forms, PYY1-36 and PYY3-36. PYY3-36 is the major circulating form and produced by DPP-4-mediated proteolytic cleavage of the N-terminal residues of PYY1-36. PYY3-36 has high affinity for Y2 and some affinity for the Y1 and Y5 receptors [75].

Release of PYY is stimulated within 30 min of meal ingestion, in proportion to energy content and its levels remain high for several hours after eating [1]. PYY acts as an “ileal break” by slowing gastric emptying and intestinal transit of the meal, in order to increase nutrient absorption by the small intestine [76]. As such, it reduces feeding [75]. More recent studies show that PYY mediates in particular protein-induced satiation [77]. In healthy subjects, PYY3-36 administration at high doses resulted in the reduction of total energy intake, duration of the meal, and hunger scores for several hours following its infusion, suggesting that it acts both as a satiation and satiety factor [78]. Moreover, as is the case with additional gut hormones, PYY3-36 infusion resulted in an increase in fat oxidation and overall energy expenditure. However, inconsistent results about the PYY-mediated inhibition of food intake have been reported from different studies, raising some concerns about its effectiveness when administered at physiological concentrations [1, 75, 76]. PYY ablation in mice resulted in the development of obesity and insulin resistance, confirming an essential role of PYY in the regulation of food intake and energy balance [77].

Some studies describe reduced fasting and postprandial levels of PYY in obese individuals [79], while others could not confirm this finding [80]. Paradoxically, patients with anorexia nervosa also appear to have even higher fasting PYY levels [80]. More consistent is the observation in both humans and rodents that in the obesity state, more calories need to be consumed in order to stimulate a PYY release in response to a meal similar to that seen in controls [81]. Of clinical significance, PYY infusion had comparable effects in reducing caloric intake in both obese and lean subjects suggesting that sensitivity to PYY is sustained during obesity [79]. A functional mutation in PYY (Q62P) has also been linked to obesity [82].

Efforts have been made to produce an oral form of PYY without altering its chemical properties using synthetic carriers with so far limited success [1]. Despite the fact that these oral preparations induce a rapid increase in serum PYY levels, they were not effective to reduce caloric intake during a test meal. A synthetic analogue of PYY and PP (obinepitide) that targets both Y2 and Y4 receptors is currently under investigation for its effects in the long-term inhibition of food intake [1]. In a totally different approach, weight loss was achieved by long-term protein augmentation in the diet and the results were attributed to an increase of endogenous PYY levels [77].

## Pancreatic Hormones

### *Pancreatic Polypeptide (PP)*

PP is a 36-aminoacid peptide primarily synthesized by the F-cells of the pancreatic islets (64), and to a lesser extent by the large intestine [1]. PP binds with highest affinity to Y4 followed by Y1 and Y5 receptors [16].

PP plasma levels rise within 30 min after food intake in proportion to caloric content of the meal and remain elevated for several hours, suggesting that the main effect of PP is to induce satiety [11, 81, 83]. PP intravenous infusion in normal-weight humans resulted in 20–25% reduction of daily food intake, an effect lasting up to 24 h post-treatment [84]. Chronic administration of PYY in obese mice results in reductions in food intake and weight gain, and in an increase in locomotor activity [83]. As a proof of concept, transgenic mice with pancreatic islet-specific overexpression of PP exhibit a hypophagic and lean phenotype, that can be abrogated by treatment with anti-PP antibodies [85].

So far, the effect of PP on appetite and body weight regulation in obese subjects still remains unclear and further studies are warranted to explore whether PP has the potential to be a novel treatment for obesity [1, 11]. For instance, PP levels are not significantly altered following bariatric surgery, thus this peptide may not account for the anorexia and weight loss following this procedure [86]. On the other hand, PP secretion was found to increase in obese children who lost weight compared to the ones who did not [11]. Overall, it appears that fasting levels of PP are not regulated the same way as the postprandial ones during obesity or weight loss.

As is the case with most of the gut hormones, the main problem of using PP as an anti-obesity treatment is its rapid degradation. Hence, synthetic analogs with prolonged half-life have been developed. One of them, currently evaluated in clinical trials, is a selective Y4 receptor agonist [12].

### *Amylin (Islet Amyloid Polypeptide)*

Amylin is a 37-amino acid amyloid polypeptide derived from an 89-aminoacid precursor by proteolytic cleavage and post-translational modifications. Amylin shares a motif (cystein in position 2,7) that is found in calcitonin-gene related peptides (CGRP). It was first purified from pancreatic amyloid, and such deposits are increased in patients with diabetes type II [87]. It has been demonstrated that amylin utilizes the calcitonin receptor in the presence of receptor activity-modifying proteins (RAMP) 1 or 3 [88].

In response to food intake, amylin is co-released from pancreatic  $\beta$ -cells with insulin in a molar ratio 1:100, respectively [1]. While amylin levels increase rapidly, they remain elevated for several hours after meal termination [13]. Amylin has an

anorectic effect and decreases meal size, at least in part by inhibiting gastric emptying and promoting satiation [89]. However, its main effects on appetite regulation appear to be mediated centrally by areas of brainstem and the hypothalamus, and thus amylin acts as a satiety factor [1, 13]. Most importantly, amylin suppresses postprandial glucagon secretion, which renders it a compelling anti-diabetic agent [90].

Postprandial levels of amylin are increased in obese individuals independently of their diabetic status, and are normalized following weight loss [11]. However, fasting levels of amylin are differentially affected by obesity and diabetes. Obese subjects with normal glucose metabolism exhibit fasting hyperamylinemia while in the presence of glucose intolerance or diabetes, they have lower amylin levels [91].

Due to amylin's effects on glycemic control, it appears that patients with diabetes (type I or II) could benefit from its use, in combination with insulin or other anti-diabetic regimens [92]. The major drawback of using the native peptide is its tendency to form toxic amyloid deposits in the pancreas that further impair insulin secretion [25]. Thus non-amyloidogenic amylin analogs (pramlintide) have been developed and approved for the treatment of diabetes [90, 93]. The weight-reducing effects of amylin in the absence of diabetes need to be further investigated [94].

## Other Gut Hormones

The list of gut hormones and peptides is constantly evolving, not only by the recognition of new members, the majority of which are derived from post-translational modifications of the same precursor, but also by the discovery of novel functions of the existing members. Below we discuss some additional gut peptides which might play a role in the overall regulation of energy balance.

## Bombesin-Related Peptides

In this family belong bombesin, which has been isolated from the amphibian skin, and its mammalian counterparts Gastrin-releasing polypeptide (GRP) and neuromedin B [5, 95]. These are quite potent neuropeptides and act at nanomolar concentrations to modulate the secretion of GLP-1 and PYY from ileal explants [96]. In humans, bombesin infusion results in weight loss, but only in lean individuals [97]. Three G-protein coupled receptors for the bombesin-like peptide have been identified in mammals: GPR receptor (GPR-R), neuromedin B receptor (NMB-R), and bombesin receptor subtype-3 (BRS-3) [98]. Of them, only BRS-3 has been implicated in feeding behavior and energy balance. Specifically, mice deficient for BRS-3 are hyperphagic, obese, and have impaired glucose tolerance [99]. BRS-3 selective agonists are currently evaluated for the treatment of obesity [100].

### ***Neuromedin U (NMU)***

NMU is a ubiquitous neuropeptide with higher expression levels in brain and the gut [101]. NMU inhibits feeding and mice lacking NMU are prone to diet-induced obesity due to hyperphagia and reduced energy expenditure [102]. There are also certain NMU gene polymorphisms that have been associated with obesity [101].

### ***Glucagon-Like Peptide-2 (GLP-2)***

In contrast to GLP-1, GLP-2 is primarily a trophic factor for the intestine and has cytoprotective effects. It stimulates crypt-cell proliferation, inhibits apoptosis of the enterocytes, and increases barrier function [103]. Thus, the primary pharmacological use of GLP-2 appears to be in cases of intestinal injury, inflammation, and defective mucosal healing [47].

### ***Endogenous Cannabinoids***

The cannabis plant has long been known for its appetite-stimulating effect, attributed to D9-tetrahydrocannabinol (THC). Interestingly, endogenous peptides that mediate “hunger” signal, called endocannabinoids, have been described, including anandamide, 2-arachidonoyl glycerol, and others [104]. Two endocannabinoid receptors, CB1 and CB2 have been identified, with differential distribution in brain and peripheral tissues. A selective CB1 receptor antagonist (Rimonabant) has been used as an anti-obesity drug, with moderate effects on weight loss. However, severe side effects made this drug less popular [105].

### ***Others***

The gut is also a significant source of additional neuropeptides, e.g., substance P (SP), neurotensin, melanin-concentrating hormone, and corticotropin-releasing factor.

## **Hormonal Interactions**

An important concept to better understand the functions and the overall effects of the gut hormones in the regulation of energy balance is their interaction with other hormones, at the level of the gut, brain, or adipose tissue [7]. This is of particular significance when such hormones are used as targets for drug development. For example, amylin administration increases central leptin sensitivity [94] and

infusion of PYY and OXM suppress ghrelin levels [21]. Moreover, a synergistic effect of CCK-1 and leptin has been described at the level of vagal afferents [106], while CCK interacts with various brain neurotransmitters, including serotonin and dopamine, that in addition to feeding behavior, they regulate pain perception, anxiety, reward processing, and memory [107]. The experience so far from clinical trials of anti-obesity drugs suggests that combinations of treatments might be the only viable approach to combat this epidemic [108]. For example, treatment with YY3-36 and GLP-1 have additive effects in inhibiting food intake [109].

## Lessons Learned

Overall, the physiology of gut hormones is more complex than originally thought, and involves a bidirectional communication between the brain and the gut, which is also called the “little brain.” Indeed, the majority of the gut hormones and/or their receptors are also found in brain. Moreover, effects of gut hormones to tissues that affect energy balance, for example the adipose tissue, do occur, either directly or via centrally mediated pathways [17]. Disruption or inappropriate regulation of the gut hormonal networks has been described in obesity. However, whether this represents one of the causes of the disease or its consequence remains to be seen in long-term prospective studies. Moreover, the overlap of obesity and diabetes in many cases, may account for some inconsistent results, in particular those involving the incretins. Important insights on the significance of gut hormones in the regulation of food intake, energy balance, and glycemic control have been derived from the study of obese patients subjected to gastric bypass [86]. For example, a drop in ghrelin levels and upregulation of PYY, GLP-1, and OXM appear to represent major contributors to reduction of food intake and can also explain the improvement of glycemic control in these patients that precedes the weight loss [110]. Pharmacological approaches to treat obesity by targeting gut hormones have not achieved great success, for several reasons, including the need of parenteral administration and the presence of side effects that result in poor compliance or alter the human behavior [12].

## References

1. Neary, M. T. & Batterham, R. L. (2009). Gut hormones: implications for the treatment of obesity. *Pharmacology & Therapeutics*, 124, 44–56.
2. Strader, A. D. & Woods, S. C. (2005). Gastrointestinal hormones and food intake. *Gastroenterology*, 128, 175–191.
3. Bataille, D. (2007). Pro-protein convertases in intermediary metabolism: islet hormones, brain/gut hormones and integrated physiology. *Journal of Molecular Medicine (Berlin, Germany)*, 85, 673–684.
4. Williams, G. T. (2007). Endocrine tumours of the gastrointestinal tract—selected topics. *Histopathology*, 50, 30–41.

5. Bray, G. A. (2000). Afferent signals regulating food intake. *The Proceedings of the Nutrition Society*, 59, 373–384.
6. Berthoud, H. R. (2008). Vagal and hormonal gut-brain communication: from satiation to satisfaction. *Neurogastroenterology and Motility: The Official Journal of the European Gastrointestinal Motility Society*, 20, Suppl 1, 64–72.
7. Cummings, D. E. & Overduin, J. (2007). Gastrointestinal regulation of food intake. *The Journal of Clinical Investigation*, 117, 13–23.
8. Chaudhri, O., Small, C. & Bloom, S. (2006). Gastrointestinal hormones regulating appetite. *Philosophical Transactions of the Royal Society of London. Series B, Biological sciences*, 361, 1187–1209.
9. Wren, A. M. & Bloom, S. R. (2007). Gut hormones and appetite control. *Gastroenterology*, 132, 2116–2130.
10. Camilleri, M. (2009). Peripheral mechanisms in the control of appetite and related experimental therapies in obesity. *Regulatory Peptides*, 156, 24–27.
11. Roth, C. L. & Reinehr, T. Roles of gastrointestinal and adipose tissue peptides in childhood obesity and changes after weight loss due to lifestyle intervention. *Archives of Pediatrics & Adolescent Medicine*, 164, 131–138.
12. Field, B. C., Chaudhri, O. B. & Bloom, S. R. (2009). Obesity treatment: novel peripheral targets. *British Journal of Clinical Pharmacology*, 68, 830–843.
13. Moran, T. H. (2009). Gut peptides in the control of food intake. *International Journal of Obesities (London)*, 33, Suppl 1, S7–10.
14. Vincent, R. P. & le Roux, C. W. (2008). The satiety hormone peptide YY as a regulator of appetite. *Journal of Clinical Pathology*, 61, 548–552.
15. le Roux, C. W., Aylwin, S. J., Batterham, R. L., Borg, C. M., Coyle, F., Prasad, V., et al. (2006). Gut hormone profiles following bariatric surgery favor an anorectic state, facilitate weight loss, and improve metabolic parameters. *Annals of Surgery*, 243, 108–114.
16. Lindner, D., Stichel, J. & Beck-Sickinger, A. G. (2008). Molecular recognition of the NPY hormone family by their receptors. *Nutrition (Burbank, Los Angeles County, California)*, 24, 907–917.
17. Badman, M. K. & Flier, J. S. (2005). The gut and energy balance: visceral allies in the obesity wars. *Science*, 307, 1909–1914.
18. Scharf, M. T. & Ahima, R. S. (2004). Gut peptides and other regulators in obesity. *Seminars in Liver Disease*, 24, 335–347.
19. Vincent, R. P., Ashrafian, H. & le Roux, C. W. (2008). Mechanisms of disease: the role of gastrointestinal hormones in appetite and obesity. *Nature Clinical Practice Gastroenterology & Hepatology*, 5, 268–277.
20. Kojima, M., Hosoda, H., Date, Y., Nakazato, M., Matsuo, H. & Kangawa, K. (1999). Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature*, 402, 656–660.
21. Tritos, N. A. & Kokkotou, E. G. (2006). The physiology and potential clinical applications of ghrelin, a novel peptide hormone. *Mayo Clinic Proceedings, Mayo Clinic*, 81, 653–660.
22. Sun, Y., Wang, P., Zheng, H. & Smith, R. G. (2004). Ghrelin stimulation of growth hormone release and appetite is mediated through the growth hormone secretagogue receptor. *Proceedings of the National Academy of Sciences of the United States of America*, 101, 4679–4684.
23. Zhang, J. V., Ren, P. G., Avsian-Kretchmer, O., Luo, C. W., Rauch, R., Klein, C., et al. (2005). Obestatin, a peptide encoded by the ghrelin gene, opposes ghrelin's effects on food intake. *Science*, 310, 996–999.
24. Tschop, M., Wawarta, R., Riepl, R. L., Friedrich, S., Bidlingmaier, M., Landgraf, R., et al. (2001). Post-prandial decrease of circulating human ghrelin levels. *Journal of Endocrinological Investigation*, 24, RC19–21.
25. Karra, E. & Batterham, R. L. The role of gut hormones in the regulation of body weight and energy homeostasis. *Molecular and Cellular Endocrinology*, 316, 120–128.
26. Cummings, D. E., Purnell, J. Q., Frayo, R. S., Schmidova, K., Wisse, B. E. & Weigle, D. S. (2001). A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. *Diabetes*, 50, 1714–1719.



27. Wren, A. M., Seal, L. J., Cohen, M. A., Brynes, A. E., Frost, G. S., Murphy, K. G., et al. (2001). Ghrelin enhances appetite and increases food intake in humans. *The Journal of Clinical Endocrinology and Metabolism*, 86, 5992.
28. Druce, M. R., Wren, A. M., Park, A. J., Milton, J. E., Patterson, M., Frost, G., et al. (2005). Ghrelin increases food intake in obese as well as lean subjects. *International Journal of Obsterics (London)*, 29, 1130–1136.
29. Tschop, M., Smiley, D. L. & Heiman, M. L. (2000). Ghrelin induces adiposity in rodents. *Nature*, 407, 908–913.
30. Zigman, J. M., Nakano, Y., Coppari, R., Balthasar, N., Marcus, J. N., Lee, C. E., et al. (2005). Mice lacking ghrelin receptors resist the development of diet-induced obesity. *The Journal of Clinical Investigation*, 115, 3564–3572.
31. Theander-Carrillo, C., Wiedmer, P., Cettour-Rose, P., Nogueiras, R., Perez-Tilve, D., Pfluger, P., et al. (2006). Ghrelin action in the brain controls adipocyte metabolism. *The Journal of Clinical Investigation*, 116, 1983–1993.
32. Leidy, H. J., Gardner, J. K., Frye, B. R., Snook, M. L., Schuchert, M. K., Richard, E. L., et al. (2004). Circulating ghrelin is sensitive to changes in body weight during a diet and exercise program in normal-weight young women. *The Journal of Clinical Endocrinology and Metabolism*, 89, 2659–2664.
33. Cummings, D. E., Weigle, D. S., Frayo, R. S., Breen, P. A., Ma, M. K., Dellinger, E. P., et al. (2002). Plasma ghrelin levels after diet-induced weight loss or gastric bypass surgery. *The New England Journal of Medicine*, 346, 1623–1630.
34. Korbonits, M., Gueorguiev, M., O'Grady, E., Lecoecur, C., Swan, D. C., Mein, C. A., et al. (2002). A variation in the ghrelin gene increases weight and decreases insulin secretion in tall, obese children. *The Journal of Clinical Endocrinology and Metabolism*, 87, 4005–4008.
35. Cummings, D. E., Clement, K., Purnell, J. Q., Vaisse, C., Foster, K. E., Frayo, R. S., et al. (2002). Elevated plasma ghrelin levels in Prader Willi syndrome. *Nature Medicine*, 8, 643–644.
36. Zorrilla, E. P., Iwasaki, S., Moss, J. A., Chang, J., Otsuji, J., Inoue, K., et al. (2006). Vaccination against weight gain. *Proceedings of the National Academy of Sciences of the United States of America*, 103, 13226–13231.
37. Moran, T. H. & Kinzig, K. P. (2004). Gastrointestinal satiety signals II. Cholecystokinin. *American Journal of Physiology. Gastrointestinal and Liver Physiology*, 286, G183–188.
38. Beglinger, C. (2002). Overview. Cholecystokinin and eating. *Current Opinion in Investigational Drugs (London, England: 2000)*, 3, 587–588.
39. Brennan, I. M., Little, T. J., Feltrin, K. L., Smout, A. J., Wishart, J. M., Horowitz, M., et al. (2008). Dose-dependent effects of cholecystokinin-8 on antropyloroduodenal motility, gastrointestinal hormones, appetite, and energy intake in healthy men. *American Journal of Physiology. Endocrinology and Metabolism*, 295, E1487–1494.
40. Little, T. J., Horowitz, M. & Feinle-Bisset, C. (2005). Role of cholecystokinin in appetite control and body weight regulation. *Obesity Reviews: An Official Journal of the International Association for the Study of Obesity*, 6, 297–306.
41. West, D. B., Fey, D. & Woods, S. C. (1984). Cholecystokinin persistently suppresses meal size but not food intake in free-feeding rats. *The American Journal of Physiology*, 246, R776–787.
42. Moran, T. H. (2000). Cholecystokinin and satiety: current perspectives. *Nutrition (Burbank, Los Angeles County, California)*, 16, 858–865.
43. Tomasik, P. J., Sztelfko, K. & Starzyk, J. (2004). Cholecystokinin, glucose dependent insulinotropic peptide and glucagon-like peptide 1 secretion in children with anorexia nervosa and simple obesity. *Journal of Pediatric Endocrinology & Metabolism*, 17, 1623–1631.
44. Miyasaka, K., Takiguchi, S. & Funakoshi, A. (2007). Cholecystokinin 1A receptor polymorphisms. *Current Topics in Medicinal Chemistry*, 7, 1205–1210.
45. Baggio, L. L. & Drucker, D. J. (2007). Biology of incretins: GLP-1 and GIP. *Gastroenterology*, 132, 2131–2157.
46. Meier, J. J. & Nauck, M. A. (2004). GIP as a potential therapeutic agent? *Hormone and Metabolic Research. Hormon- und Stoffwechselforschung.*, 36, 859–866.



47. Drucker, D. J. (2005). Biologic actions and therapeutic potential of the proglucagon-derived peptides. *Nature Clinical Practice. Endocrinology & Metabolism*, 1, 22–31.
48. Bell, G. I., Sanchez-Pescador, R., Laybourn, P. J. & Najarian, R. C. (1983). Exon duplication and divergence in the human preproglucagon gene. *Nature*, 304, 368–371.
49. Kieffer, T. J. & Habener, J. F. (1999). The glucagon-like peptides. *Endocrine Reviews*, 20, 876–913.
50. Flint, A., Raben, A., Astrup, A. & Holst, J. J. (1998). Glucagon-like peptide 1 promotes satiety and suppresses energy intake in humans. *The Journal of Clinical Investigation*, 101, 515–520.
51. Verdich, C., Flint, A., Gutzwiller, J. P., Naslund, E., Beglinger, C., Hellstrom, P. M., et al. (2001). A meta-analysis of the effect of glucagon-like peptide-1 (7–36) amide on ad libitum energy intake in humans. *The Journal of Clinical Endocrinology and Metabolism*, 86, 4382–4389.
52. Scrocchi, L. A., Brown, T. J., McClusky, N., Brubaker, P. L., Auerbach, A. B., Joyner, A. L., et al. (1996). Glucose intolerance but normal satiety in mice with a null mutation in the glucagon-like peptide 1 receptor gene. *Nature Medicine*, 2, 1254–1258.
53. Kinnamon, S. C. (2000). A plethora of taste receptors. *Neuron*, 25, 507–510.
54. Jayasena, C. N. & Bloom, S. R. (2008). Role of gut hormones in obesity. *Endocrinology and Metabolism Clinics of North America*, 37, 769–787, xi.
55. Carr, R. D., Larsen, M. O., Jelic, K., Lindgren, O., Vikman, J., Holst, J. J., et al. Secretion and dipeptidyl peptidase-4-mediated metabolism of incretin hormones after a mixed meal or glucose ingestion in obese compared to lean, nondiabetic men. *The Journal of Clinical Endocrinology and Metabolism*, 95, 872–878.
56. Ashrafian, H. & le Roux, C. W. (2009). Metabolic surgery and gut hormones - a review of bariatric entero-humoral modulation. *Physiology & Behavior*, 97, 620–631.
57. Amori, R. E., Lau, J. & Pittas, A. G. (2007). Efficacy and safety of incretin therapy in type 2 diabetes: systematic review and meta-analysis. *The Journal of the American Medical Association*, 298, 194–206.
58. Drucker, D. J. & Nauck, M. A. (2006). The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. *Lancet*, 368, 1696–1705.
59. Bradley, D. P., Kulstad, R. & Schoeller, D. A. Exenatide and weight loss. *Nutrition (Burbank, Los Angeles County, California)*, 26, 243–249.
60. Ahmad, S. R. & Swann, J. (2008). Exenatide and rare adverse events. *The New England Journal of Medicine*, 358, 1970–1971; discussion 1971–1972.
61. Faludi, P., Brodows, R., Burger, J., Ivanyi, T. & Braun, D. K. (2009). The effect of exenatide re-exposure on safety and efficacy. *Peptides*, 30, 1771–1774.
62. Astrup, A., Rossner, S., Van Gaal, L., Rissanen, A., Niskanen, L., Al Hakim, M., et al. (2009). Effects of liraglutide in the treatment of obesity: a randomised, double-blind, placebo-controlled study. *Lancet*, 374, 1606–1616.
63. Ahren, B. (2003). Gut peptides and type 2 diabetes mellitus treatment. *Current Diabetes Reports*, 3, 365–372.
64. Asmar, M. & Holst, J. J. Glucagon-like peptide 1 and glucose-dependent insulinotropic polypeptide: new advances. *Current Opinion in Endocrinology, Diabetes and Obsterics*, 17, 57–62.
65. Beck, B. (1989). Gastric inhibitory polypeptide: a gut hormone with anabolic functions. *Journal of Molecular Endocrinology*, 2, 169–174.
66. Miyawaki, K., Yamada, Y., Ban, N., Ihara, Y., Tsukiyama, K., Zhou, H., et al. (2002). Inhibition of gastric inhibitory polypeptide signaling prevents obesity. *Nature Medicine*, 8, 738–742.
67. Nauck, M. A. & Meier, J. J. (2005). Glucagon-like peptide 1 and its derivatives in the treatment of diabetes. *Regulatory Peptides*, 128, 135–148.
68. Baggio, L. L., Huang, Q., Brown, T. J. & Drucker, D. J. (2004). Oxyntomodulin and glucagon-like peptide-1 differentially regulate murine food intake and energy expenditure. *Gastroenterology*, 127, 546–558.
69. Wynne, K. & Bloom, S. R. (2006). The role of oxyntomodulin and peptide tyrosine-tyrosine (PYY) in appetite control. *Nature Clinical Practice. Endocrinology & Metabolism*, 2, 612–620.

70. Dakin, C. L., Small, C. J., Park, A. J., Seth, A., Ghatei, M. A. & Bloom, S. R. (2002). Repeated ICV administration of oxyntomodulin causes a greater reduction in body weight gain than in pair-fed rats. *American Journal of Physiology. Endocrinology and Metabolism*, 283, E1173–1177.
71. Wynne, K., Park, A. J., Small, C. J., Meeran, K., Ghatei, M. A., Frost, G. S., et al. (2006). Oxyntomodulin increases energy expenditure in addition to decreasing energy intake in overweight and obese humans: a randomised controlled trial. *International Journal of Obsterics (London)*, 30, 1729–1736.
72. Wynne, K., Park, A. J., Small, C. J., Patterson, M., Ellis, S. M., Murphy, K. G., et al. (2005). Subcutaneous oxyntomodulin reduces body weight in overweight and obese subjects: a double-blind, randomized, controlled trial. *Diabetes*, 54, 2390–2395.
73. Schjoldager, B. T., Baldissera, F. G., Mortensen, P. E., Holst, J. J. & Christiansen, J. (1988). Oxyntomodulin: a potential hormone from the distal gut. Pharmacokinetics and effects on gastric acid and insulin secretion in man. *European Journal of Clinical Investigation*, 18, 499–503.
74. Field, B. C., Wren, A. M., Peters, V., Baynes, K. C., Martin, N. M., Patterson, M., et al. PYY3-36 and oxyntomodulin can be additive in their effect on food intake in overweight and obese humans. *Diabetes* 59, 1635–1639.
75. Batterham, R. L., Cowley, M. A., Small, C. J., Herzog, H., Cohen, M. A., Dakin, C. L., et al. (2002). Gut hormone PYY(3-36) physiologically inhibits food intake. *Nature*, 418, 650–654.
76. Grudell, A. B. & Camilleri, M. (2007). The role of peptide YY in integrative gut physiology and potential role in obesity. *Current Opinion in Endocrinology, Diabetes and Obsterics*, 14, 52–57.
77. Batterham, R. L., Heffron, H., Kapoor, S., Chivers, J. E., Chandarana, K., Herzog, H., et al. (2006). Critical role for peptide YY in protein-mediated satiation and body-weight regulation. *Cell Metabolism*, 4, 223–233.
78. Degen, L., Oesch, S., Casanova, M., Graf, S., Ketterer, S., Drewe, J., et al. (2005). Effect of peptide YY3-36 on food intake in humans. *Gastroenterology*, 129, 1430–1436.
79. Batterham, R. L., Cohen, M. A., Ellis, S. M., Le Roux, C. W., Withers, D. J., Frost, G. S., et al. (2003). Inhibition of food intake in obese subjects by peptide YY3-36. *The New England Journal of Medicine*, 349, 941–948.
80. Pfluger, P. T., Kampe, J., Castaneda, T. R., Vahl, T., D'Alessio, D. A., Kruthaupt, T., et al. (2007). Effect of human body weight changes on circulating levels of peptide YY and peptide YY3-36. *The Journal of Clinical Endocrinology and Metabolism*, 92, 583–588.
81. le Roux, C. W., Batterham, R. L., Aylwin, S. J., Patterson, M., Borg, C. M., Wynne, K. J., et al. (2006). Attenuated peptide YY release in obese subjects is associated with reduced satiety. *Endocrinology*, 147, 3–8.
82. Ahituv, N., Kavaslar, N., Schackwitz, W., Ustaszewska, A., Collier, J. M., Hebert, S., et al. (2006). A PYY Q62P variant linked to human obesity. *Human Molecular Genetics*, 15, 387–391.
83. Asakawa, A., Inui, A., Yuzuriha, H., Ueno, N., Katsuura, G., Fujimiya, M., et al. (2003). Characterization of the effects of pancreatic polypeptide in the regulation of energy balance. *Gastroenterology*, 124, 1325–1336.
84. Batterham, R. L., Le Roux, C. W., Cohen, M. A., Park, A. J., Ellis, S. M., Patterson, M., et al. (2003). Pancreatic polypeptide reduces appetite and food intake in humans. *The Journal of Clinical Endocrinology and Metabolism*, 88, 3989–3992.
85. Ueno, N., Inui, A., Iwamoto, M., Kaga, T., Asakawa, A., Okita, M., et al. (1999). Decreased food intake and body weight in pancreatic polypeptide-overexpressing mice. *Gastroenterology*, 117, 1427–1432.
86. Bueter, M. & le Roux, C. W. (2009). Sir David Cuthbertson Medal Lecture. Bariatric surgery as a model to study appetite control. *The Proceedings of the Nutrition Society*, 68, 227–233.
87. Kahn, S. E., Andrikopoulos, S. & Verchere, C. B. (1999). Islet amyloid: a long-recognized but underappreciated pathological feature of type 2 diabetes. *Diabetes*, 48, 241–253.

88. Christopoulos, G., Perry, K. J., Morfis, M., Tilakaratne, N., Gao, Y., Fraser, N. J., et al. (1999). Multiple amylin receptors arise from receptor activity-modifying protein interaction with the calcitonin receptor gene product. *Molecular Pharmacology*, 56, 235–242.
89. Larhammar, D. (1996). Structural diversity of receptors for neuropeptide Y, peptide YY and pancreatic polypeptide. *Regulatory Peptides*, 65, 165–174.
90. Chapman, I., Parker, B., Doran, S., Feinle-Bisset, C., Wishart, J., Strobel, S., et al. (2005). Effect of pramlintide on satiety and food intake in obese subjects and subjects with type 2 diabetes. *Diabetologia*, 48, 838–848.
91. Reinehr, T., de Sousa, G., Niklowitz, P. & Roth, C. L. (2007). Amylin and its relation to insulin and lipids in obese children before and after weight loss. *Obesity (Silver Spring)*, 15, 2006–2011.
92. Edelman, S., Garg, S., Frias, J., Maggs, D., Wang, Y., Zhang, B., et al. (2006). A double-blind, placebo-controlled trial assessing pramlintide treatment in the setting of intensive insulin therapy in type 1 diabetes. *Diabetes Care*, 29, 2189–2195.
93. Hollander, P. A., Levy, P., Fineman, M. S., Maggs, D. G., Shen, L. Z., Strobel, S. A., et al. (2003). Pramlintide as an adjunct to insulin therapy improves long-term glycemic and weight control in patients with type 2 diabetes: a 1-year randomized controlled trial. *Diabetes care*, 26, 784–790.
94. Roth, J. D., Roland, B. L., Cole, R. L., Trevaskis, J. L., Weyer, C., Koda, J. E., et al. (2008). Leptin responsiveness restored by amylin agonism in diet-induced obesity: evidence from nonclinical and clinical studies. *Proceedings of the National Academy of Sciences of the United States of America*, 105, 7257–7262.
95. Huda, M. S., Wilding, J. P. & Pinkney, J. H. (2006). Gut peptides and the regulation of appetite. *Obesity Reviews: An Official Journal of the International Association for the Study of Obesity*, 7, 163–182.
96. Dumoulin, V., Dakka, T., Plaisancie, P., Chayvialle, J. A. & Cuber, J. C. (1995). Regulation of glucagon-like peptide-1-(7-36) amide, peptide YY, and neurotensin secretion by neurotransmitters and gut hormones in the isolated vascularly perfused rat ileum. *Endocrinology*, 136, 5182–5188.
97. Gutzwiller, J. P., Drewe, J., Hildebrand, P., Rossi, L., Lauper, J. Z. & Beglinger, C. (1994). Effect of intravenous human gastrin-releasing peptide on food intake in humans. *Gastroenterology*, 106, 1168–1173.
98. Jensen, R. T., Battey, J. F., Spindel, E. R. & Benya, R. V. (2008). International Union of Pharmacology. LXVIII. Mammalian bombesin receptors: nomenclature, distribution, pharmacology, signaling, and functions in normal and disease states. *Pharmacological Reviews*, 60, 1–42.
99. Ohki-Hamazaki, H., Watase, K., Yamamoto, K., Ogura, H., Yamano, M., Yamada, K., et al. (1997). Mice lacking bombesin receptor subtype-3 develop metabolic defects and obesity. *Nature*, 390, 165–169.
100. Guan, X. M., Chen, H., Dobbelaar, P. H., Dong, Y., Fong, T. M., Gagen, K., et al. Regulation of energy homeostasis by bombesin receptor subtype-3: selective receptor agonists for the treatment of obesity. *Cell Metabolism*, 11, 101–112.
101. Mitchell, J. D., Maguire, J. J. & Davenport, A. P. (2009). Emerging pharmacology and physiology of neuromedin U and the structurally related peptide neuromedin S. *British Journal of Pharmacology*, 158, 87–103.
102. Hanada, R., Teranishi, H., Pearson, J. T., Kurokawa, M., Hosoda, H., Fukushima, N., et al. (2004). Neuromedin U has a novel anorexigenic effect independent of the leptin signaling pathway. *Nature Medicine*, 10, 1067–1073.
103. Drucker, D. J. (2001). Glucagon-like peptide 2. *The Journal of Clinical Endocrinology and Metabolism*, 86, 1759–1764.
104. Vickers, S. P. & Kennett, G. A. (2005). Cannabinoids and the regulation of ingestive behaviour. *Current Drug Targets*, 6, 215–223.
105. de Kloet, A. D. & Woods, S. C. (2009). Minireview: Endocannabinoids and their receptors as targets for obesity therapy. *Endocrinology*, 150, 2531–2536.

106. Dockray, G. J. (2009). Cholecystokinin and gut-brain signalling. *Regulatory Peptides*, 155, 6–10.
107. Trevaskis, J. L., Turek, V. F., Griffin, P. S., Wittmer, C., Parkes, D. G. & Roth, J. D. Multi-hormonal weight loss combinations in diet-induced obese rats: therapeutic potential of cholecystokinin? *Physiology & Behavior*, 100, 187–195.
108. Korner, J. & Leibel, R. L. (2003). To eat or not to eat - how the gut talks to the brain. *The New England Journal of Medicine*, 349, 926–928.
109. Neary, N. M., Small, C. J., Druce, M. R., Park, A. J., Ellis, S. M., Semjonous, N. M., et al. (2005). Peptide YY3-36 and glucagon-like peptide-17-36 inhibit food intake additively. *Endocrinology*, 146, 5120–5127.
110. Wideman, R. D. & Kieffer, T. J. (2009). Mining incretin hormone pathways for novel therapies. *Trends in Endocrinology and Metabolism*, 20, 280–286.



# Chapter 7

## Genes and Human Obesity

R. Arlen Price

### Introduction

Obesity has increased dramatically over the last few decades. Since 1990 the prevalence of obesity has more than tripled and by 2007 approached one-third of the population, with another third that was overweight (CDC, <http://www.cdc.gov/NCCDPHP/dnpa/obesity/trend/index.htm>). Highest rates are found in the United States and the United Kingdom among developed countries, and in the Middle East and Pacific Islands in the developing world (WHO: <http://www.who.int/infobase/comparestart.aspx>). The increase in obesity rates in developing countries has coincided with “westernization” [1–3]. Life in the developed and developing world has become increasingly sedentary while relatively inexpensive, highly palatable food with high caloric content has become widely available. Although many lifestyle factors have been suggested to contribute to the dramatic obesity increase, the primary cause is, as one would expect, excess caloric intake [4, 5]. Diet accounts in part for national differences, but change within countries appears to be driven primarily by overall food availability [6]. Food is readily available and people are overeating.

### Heritability

Gene frequencies do not change over short periods in large populations, and the large secular increases must have an environmental origin. This fact may lead some to wonder whether the heritability of obesity has declined during the same period, but this is not the case. There have been a large number of studies that estimated heritability of body mass index (BMI) and related variables [7], and they are consistent in finding moderate to high heritability. Furthermore, the estimates do not depend

---

R.A. Price (✉)

Pennsylvania Center for Neurobiology and Behavior, Translational Research  
Laboratories, 125 South 31st Street, Philadelphia, PA 19104, USA  
e-mail: [arlen@exchange.upenn.edu](mailto:arlen@exchange.upenn.edu)

on the period of the study. For example, two studies on twins conducted almost 20 years apart found virtually identical estimates of overall heritability of BMI of about .80 [8, 9]. So, while estimates from particular studies vary, there is no trend toward decreasing (or increasing) heritability.

## Gene–Environment Interaction

Bouchard and colleagues completed a series of landmark studies that helped to explain the role of inherited variation in environmentally influenced change. Bouchard's research group studied monozygotic twins exposed to long-term positive or negative energy balance. There were considerable individual differences in weight gain or loss under the different conditions, but changes were similar in the genetically identical co-twins, both in overall weight and visceral fat. The results indicate that genotype mediates response to the environment [10]. In other words, response to environmental change is itself heritable.

The major environmental changes that are credited with causing the obesity pandemic occurred at a population level, but, as with the study on twins, individuals differ in their response. While two-thirds of populations of developed countries are overweight or obese, the remaining third, living in the same environment, are of normal weight or thin. At the least, this implies a behavioral interaction, and, given the heritability of obesity and coordinate changes in twins, gene–environment interaction must play a major mediating role. A few studies have tried to identify environmental interactions with specific genes, focusing on weight gain or loss as phenotypes, and diet or exercise as components of the environment. One review identified some 13 studies that reported associations with some measure of exercise and 15 with diet and/or exercise [11]. However, most associations have not been replicated. The interaction most consistently supported was with the Trp64Arg polymorphism in the adrenergic receptor beta 3 (ADRB3) gene [11]. Limited power due to small sample size may in part contribute to the inconsistency of results. However, in the end most reported associations will be false positives while a few failures to replicate could be false negatives. The pairing of new technologies with larger sample sizes could prove more robust for examining gene–environment interactions, but this possible outcome will depend on the nature and magnitude of the individual interactions.

## Candidate Genes

The phenotypic response in susceptible individuals must be influenced by variability in genes that influence energy balance. Energy homeostasis requires the coordination of appetite and satiety with energy expenditure and storage. A great deal has been learned about how energy homeostasis is maintained [12]. It is a complex process involving genes that regulate appetite, energy metabolism, and fat deposition.

Many genes that lie in associated regulatory pathways have become candidates for weight gain and obesity. These have included Leptin, Leptin Receptor, MC4R, UCPs, PPARG, NPY, and Ghrelin as well as genes in signaling pathways.

Candidate gene studies have identified mutations in humans or introduced them in animal models [13]. The last comprehensive count of human studies identified associations with 127 genes, most with at best mixed records of replication. The positive side of a candidate gene approach is that the genes derive from an emerging understanding of biology. Any associations that are detected with common obesity fit into a preexisting framework. Candidate gene studies have had their successes. Major gene mutations have been associated with obesity. However, they tend to be rare and account for a few cases of extreme obesity [13].

## Common Obesity and Rare Gene Variation

Overall, candidate gene studies have been unsuccessful in explaining common forms of obesity. Genes central to energy balance tend to have low variability, presumably because of strong selection pressure. Even so, some have argued that mutations in a large number of genes may account for most human obesity and other common diseases. This view is sometimes called the common trait rare gene hypothesis (CTRV, [14, 15]), as opposed to the common trait common variant (CTCV) hypothesis.

Substantial progress in finding rare variants has come with a focus on copy number variation (CNV, a variant in a DNA segment of 1 or more kb in length). While major deletions, duplications, and rearrangements of DNA sequence associated with rare diseases have been known for some time, the scale of CNV was not appreciated until the last few years. One whole-genome survey found more than 4,000 variants, affecting more than 600 Mb of genomic DNA sequence [16]. Large-scale screening has identified associations of CNVs with a number of phenotypes [17] including type 1 diabetes, neuropsychiatric conditions [18], and several other common disorders [19].

To date, there have been few studies of CNVs associated with obesity. An association between BMI and a chromosome 10q11 CNV was recently reported in a Chinese cohort [20]. Two genes in this region are *GPRIN2* and *PPYRI*, which are worthy of follow-up studies in larger samples. In other studies, a deletion on 16p11.2 was recently reported to be associated with obesity [21, 22].

We recently completed a genome-wide CNV survey of obese cases and never-overweight control subjects [23]. CNVs larger than 1 Mb were found to be over-represented in obese cases compared with never overweight control subjects (odds ratio (OR) = 1.5), and CNVs larger than 2 Mb were present in 1.3% of the cases but absent in control subjects. When focusing on rare deletions that disrupt genes, even more pronounced effect sizes were observed (OR = 2.7 for CNVs larger than 1 Mb). Interestingly, obese cases that carry these large CNVs have only moderately high BMI. Several CNVs were found to disrupt known candidate genes for obesity, such as a 3.3 Mb deletion disrupting *NAPIL5* and a 2.1 Mb duplication disrupting *UCP1* and *IL15*. Our results suggest that large CNVs, especially rare deletions,



confer risk of obesity in individuals with moderate to extreme obesity. The genes affected by these CNVs thus become candidates for obesity.

## Linkage Studies

One source of motivation for proposing the CTRV hypothesis was that attempts, through linkage and association, to identify common genes had been unsuccessful, at least until recently. The search for common genes has generally taken a genomic approach in which the entire genome is screened without prior hypotheses. Linkage studies were the first to take a whole-genome approach. There have been more than 60 of them for obesity-related traits [13], for example, but the results have been disappointing. A meta-analysis of 37 of these studies found only two regions to be significantly supported at the 1% level [24]: chromosome 13q for BMI and chromosome 12q for obesity (BMI  $\geq 30$ ).

The outcome of the meta-analysis helps to explain why most comprehensive searches for gene associations under linkage peaks have been unsuccessful. Many factors may account for this lack of success, but some are particularly important. Linkage studies tended to be underpowered, often in the extreme, and have had inadequate marker coverage. A particularly unfortunate aspect of low power is that most “significant” results are likely to be false positives, and because of this most studies that follow will fail to replicate. Another difficulty with low power is that even some weak positives may be true and therefore missed.

## Whole-Genome Association Studies

Whole-genome association (WGA) studies made it possible to address the two most serious deficiencies of previous approaches, in that new genotyping technology has been combined with very large sample sizes. Moreover, WGA studies have several advantages over whole-genome linkage scans. The resolution is two to three orders of magnitude greater, 2–5 Mb in linkage studies compared with 10–100 kb with association. Cases and controls are much easier to collect than families, and the sample sizes required while large are much smaller than those required for linkage [25] and well within reach for collaborative groups, if not individual investigators. The advantages of a WGA approach were recognized some time ago [26], but the available technology was insufficient at that time. Circumstances have changed.

Recent GWA studies have depended upon advances in marker identification and genomic technology for high-throughput genotyping. The International HapMap Project (<http://www.hapmap.org/>) has identified more than 4 million single-nucleotide polymorphisms (SNPs) and 550,000 of them provide about 95% coverage of the genome in most populations, with about double that number needed for Africans [27]. High-throughput technology makes it possible to type up to 1 million genotypes in a single pass (Affymetrix and Illumina). Greatly reduced costs have made the

technology widely accessible. Finally, large sample sizes have been developed through the cooperation of investigators at multiple sites.

WGA studies have become widely available only within the past 3 years. Yet, there already have been a number of them related to obesity. The first few had relatively low genome coverage and found no associations that met a genome-wide level of significance ( $2 \times 10^{-7}$ ) [28, 29]. Not surprisingly, replication of the early findings has been mixed at best, for example, the reported association with the gene INSIG2 [30].

The breakthrough for WGA studies came from the Wellcome Trust Case Control Consortium (WTCCC) study that included 490,000 SNPs and a total of almost 39,000 individuals, although the initial phase utilized a much smaller number of cases and controls, about 2,000 and 3,000, respectively [31]. The study was unprecedented in size and in the strength of the association with FTO. An association with MC4R has also been reported based on the WTCCC sample [32]. The association with FTO has been replicated in most studies that followed. MC4R has been replicated as well, although not as consistently. A summary of reported associations with obesity-related traits that reached genome-wide significance is summarized in Table 1, while Table 2 lists those with the strongest support. The amount of variance in BMI accounted for by variants in these genes is disappointingly low, about two-thirds of 1% [30].

**Table 1** Whole-genome association studies for obesity related traits reaching genome-wide significance as of April 2010

Study	N SNPs	Sample size	Genome-wide significance
Fraling et al. 2007 [61]	490k	38,759	FTO
Scuteri et al. 2007 [62]	361k	4,000+	FTO
Liu et al. 2008 [63]	500k	1,000	CTNBL1
Loos et al. 2008 [32]	490	16,876	MC4R (FTO)
Thorleifsson et al. 2009 [64]	306k	38,112	FTO, MC4R, NEGR1, TMEM18, SH2B1, and 6 other loci
Meyre et al. 2009 [65]	300k	2,796	FTO, MC4R, NPC1, MAF, PTER
Willer et al. 2009 [30]	CNV370 Various	32,000	FTO, MC4R, TMEM18, CTD15, GNPDA2, SH2B1, MTCH2, NEGR1
Meta-analysis Lindgren et al. 2009 [66]	Various	38,580 Original 70,689 replications	FTO, MC4R, TFAP2B, MSRA
Meta-analysis Johansson et al. 2010 [67]		3,448 linkage 3,925 associations	MGAT1
Wang et al. 2010 [23]	550k	2,363	FTO

**Table 2** Obesity-related trait gene associations replicated at genome-wide level of significance. Together, these 5 genes account for less than 1% of the variance in BMI, ~0.67%

Gene	Proportion of variance (%)
FTO, fat mass associated gene	0.33
MC4R, melanocortin receptor 4	0.10
TMEM18, transmembrane protein 18	0.13
SH2B1, Src domain homology 2 B adaptor protein 1	0.08
NEGR1, neuronal growth regulator 1	0.03

From Willer et al. 2009 [30].

Larger sample sizes should help to identify more associations and improve replication, however, the effect sizes will only grow smaller. These findings on obesity are consistent with those for stature, a complex trait with an even higher heritability of at least .80. A large GWAS of stature involving some 63,000 subjects found 54 associated genes that accounted for only about 5% of the total variation in height [33–35]. This finding led to much discussion and speculation as to what happened to the so-called “missing heritability” [36]. Suggestions have included gene–environment interaction, as well as epigenetics. As discussed in an earlier section, gene–environment interaction can play an important role in the development of obesity, although it should be borne in mind that this may only complicate things further, as environmental response is itself heritable. Epigenetics will be discussed later in this chapter.

## Disparate Approaches Appear to Converge

GWAS results have demonstrated that there are indeed common variants in genes that increase risk for obesity. This is particularly true for FTO that has been widely replicated. However, the proportion of variance in BMI these common genes account for is quite small, less than 1% [30]. Major gene mutations such as those in leptin, leptin receptor, and POMC have dramatic effects on individuals but are so rare that they account for essentially no common variance. CNVs are much more common than major gene mutations, but they are still relatively rare and account for little variance overall. While there are marked differences in frequency, each approach has been successful. However, the identified variants individually and together account for very little of the overall variance.

Taken on face value, the results from the different approaches suggest polygenic inheritance. The classic polygenic model was devised by R. A. Fisher as a way of incorporating Mendelian inheritance into quantitative variation [37]. For convenience he assumed there were multiple causal genes, each with small and roughly equal effects. The particulars, however, give a somewhat different picture. It turns out there are indeed multiple causal genes, and each variant accounts for little overall variance. However, the variants have a wide range of effects on the individuals that carry them. There is as yet no evidence the effects sum to create the phenotype. Studies published so far show little or no overlap in the genes identified by the different approaches.

## Unanticipated Genes

Whole-genome approaches have the capacity to detect associations with genes that could not have been anticipated based on current knowledge. FTO for example falls outside any of the pathways that were known to affect appetite and energy balance. FTO had been identified previously through large-scale mutagenesis in mice [38] and received the acronym *Fto* because mice having a deletion of this gene had fused toes on the fore limbs. Ironically, it was called “fatso”, not because of an obese phenotype (there was none) but because of a relatively large gene footprint.

## Epigenetic Modification

There has been much discussion of late about the possible effects of epigenetic changes on risk for common disorders [39]. Epigenetic modification refers to changes in gene expression that are heritable, that is, which are maintained during somatic cell division and may in some cases be passed on to offspring.

Genomic imprinting is the most studied form of epigenetic modification, and involves the differential marking of parental chromosomes during gametogenesis. Imprinting appears to occur in all marsupial and placental species, and many of the imprinted genes are related to body size and/or metabolism [40–42]. The conflict theory suggests the association of imprinting with body size arose due to differential parental investment in offspring in polyandrous animals. Males are invested in larger body size of their offspring while females have an equal investment in all offspring regardless of the father. The theory is supported by fetus size in deer mice (*peromyscus*) hybrids of monogamous and polyandrous species [43].

The best known example relating to obesity is the Prader-Willi and Angelman syndromes, which are due to imprinting of the paternal or maternal chromosome, respectively, of region 15q11–13. Another imprinted gene is insulin-like growth factor 2, and paternal expression is strongly related to several measures of fat deposition in pigs [44]. In addition, quantitative trait loci (QTL), inferred genes based on linkage, have been identified in mice. Imprinting is suggested because linkage depends on parent of origin. In one study, five QTL were found, two paternal, two maternal, and one with no parent of origin effect [45].

Parent of origin effects have also been identified in humans. A large survey reported parent of origin-dependent associations of variants in known imprinted regions on chromosomes 7q32 and 11p15 with several complex disorders, including type 2 diabetes [46]. In our own work, we have found parent of origin effects on linkage in chromosome regions 10p12 and 12q24, where the linkage signal is due entirely to maternal transmission [47]. Chromosome 12q24 was one of the best supported linkage results in a meta-analysis [24], which seems to indicate that the linkage signal is detectable even if parent of origin is not modeled in the analyses. The chromosome 10p12 region (19.4–33.3 Mb) is homologous to a largely overlapping segment of mouse chromosome 2A3 (15–23 Mb) that has been predicted to be imprinted based on a machine learning model [48]. Two genes in this region have

previously been associated with obesity, glutamate decarboxylase 2 (GAD2), and G protein receptor 158 (GPR158) [49]. The concordance is intriguing, although imprinting mechanisms remain to be identified through molecular studies. A further suggestion of imprinting effects in humans is our recent finding of a CNV deletion of a region of chromosome 4 including the NAP1L5 gene [23]. The gene is normally expressed only on the paternal chromosome, which is deleted, apparently leading to an absence of gene expression.

Environmentally induced epigenetic modification has been recognized in cancer for some time, but a role in complex disorders such as obesity has only recently begun to be examined at a genomic level. However, indirect evidence demonstrating environmental effects on risk for obesity has been known for some time. For example, an early study found increased rates of obesity in men who had been *in utero* or neonatal during the height of the Dutch famine of 1944–1945 [50]. Other studies of this type also have found that maternal malnutrition contributes to risk for obesity and other aspects of the metabolic syndrome [51]. Another study [52] found that prenatal exposure to maternal diabetes increased the risk for obesity in Pima Indians. Animal studies similarly have shown that maternal exposure to malnutrition, high fat diets, stress, and other factors increase risk for obesity and the metabolic syndrome. It is of some interest that both under- and over-nutrition during fetal development can increase risk [53].

More recent studies have focused on epigenetic changes associated with prenatal exposure. A follow-up study on the Dutch famine cohort, for example, found that exposure indeed led to decreased methylation of the imprinted IGF2 gene [54]. Gene expression differences in monozygotic twins discordant for obesity also suggest the possibility of epigenetic modification [55]. While overall differences in expression could be state dependent, mitochondrial DNA copy number differences in adipose tissue of discordant twins are consistent with epigenetic effects.

The obesity state affects expression of many genes, with perhaps as many as 17,000 transcripts related to BMI in adipose tissue according to one estimate [56]. Gene expression in normal weight animals has also been related to later obesity. Inbred C57BL/6J mice are susceptible to diet-induced obesity, but there is variation in adiposity from an early age and the differences are maintained under both high-fat and restricted low-fat diets [57]. Microarray analysis found parallel pre-obesity differences in the expression of genes in several known metabolic pathways. The causes of the expression differences are unclear but could be due to prenatal or early postnatal environment.

## Applications: Prevention and Therapy

One goal of genetic research, whether stated or implicit, is that findings will eventually make it possible to use genotype to make decisions about appropriate approaches to prevention and therapy. The nature of the genetics of human obesity complicates

its application, particularly in identifying individuals most at risk. Odds ratios for most variants will be even smaller than for FTO (about 1.65) and have been estimated to be in the range of 1.2. Prediction will therefore involve only small increments in risk. In most cases, familial obesity will continue to be the best predictor of risk. This difficulty will not only limit application but can also raise ethical concerns in providing risk assessments to individuals who may never develop obesity or become overweight for different reasons. While overall heritability is substantial, the contribution of individual genes or genotypes is likely to be very small relative to the major environmental influences of diet and lifestyle.

The identification of protective genes may have the earliest application in the form of more individualized pharmacological treatment, for example, identifying individuals with resistance to drug-induced weight gain. To do so, it is not necessary to identify genes involved in etiology, only those genes that directly influence drug effectiveness or side effects. Research in other areas has already made it possible to tailor medication to individual genotype, particularly for cancers. Response to tamoxifen treatment for breast cancer, for example, appears to be ineffective in 5–8% of women with a variant of the CYP2D6 gene [58]. With regard to obesity, several genes have been identified that may influence drug-induced weight gain, for example, due to olanzapine, including PMCH, 5HT2A, ADRA2A, and PKHD1 [59]. In addition, SLC6A2 and GRIN1 have been associated with weight loss in response to norepinephrine/dopamine transporter inhibitors [60]. Further research will be needed before genomic screening is practical on a large scale, but applications may be generally available in the not too distant future.

## What Lies Ahead

Genomic approaches may well detect other previously unknown genes that are common and exert their influence through unanticipated pathways. Whole-genome sequencing will permit the identification of new variants, particularly CNVs that are individually rare but have larger effects than common SNP alleles on obesity phenotypes. Environmental influences can be better understood by the identification of interactions with specific, measured genotypes. New genes will provide additional targets for pharmacological intervention. Genotypes at these loci may be used in therapeutic interventions through knowledge of their influence on drug effectiveness or side effects.

## References

1. Sugarman JR, White LL, Gilbert TJ. Evidence for a secular change in obesity, height, and weight among Navajo Indian schoolchildren. *Am J Clin Nutr* 1990;52(6):960–6.
2. Hodge AM, Dowse GK, Toelue P, Collins VR, Imo T, Zimmet PZ. Dramatic increase in the prevalence of obesity in western Samoa over the 13 year period 1978–1991 [published erratum appears in *Int J Obes Relat Metab Disord* 1995 Sep;19(9):689]. *Int J Obes Relat Metab Disord* 1994;18(6):419–28.

3. Price RA, Charles MA, Pettitt DJ, Knowler WC. Obesity in Pima Indians: large increases among post-World War II birth cohorts. *Am J Phys Anthropol* 1993;92(4):473–9.
4. Bleich S, Cutler D, Murray C, Adams A. Why is the developed world obese? *Annu Rev Public Health* 2008;29:273–95.
5. Kumanyika SK. Global calorie counting: a fitting exercise for obese societies. *Annu Rev Public Health* 2008;29:297–302.
6. Silventoinen K, Sans S, Tolonen H, Monterde D, Kuulasmaa K, Kesteloot H, et al. Trends in obesity and energy supply in the WHO MONICA Project. *Int J Obes Relat Metab Disord* 2004;28(5):710–18.
7. Maes HH, Neale MC, Eaves LJ. Genetic and environmental factors in relative body weight and human adiposity. *Behav Genet* 1997;27(4):325–51.
8. Malis C, Rasmussen EL, Poulsen P, Petersen I, Christensen K, Beck-Nielsen H, et al. Total and regional fat distribution is strongly influenced by genetic factors in young and elderly twins. *Obes Res* 2005;13(12):2139–45.
9. Stunkard AJ, Foch TT, Hrubec Z. A twin study of human obesity. *Journal of the American Medical Association* 1986;256:51–4.
10. Bouchard C, Tremblay A. Genetic influences on the response of body fat and fat distribution to positive and negative energy balances in human identical twins. *J Nutr* 1997;127(5 Suppl):943S–47S.
11. Lu Qi YAC. Gene-environment interaction and obesity. *Nutrition Reviews* 2008;66(12):684–94.
12. Farooqi S, O’Rahilly S. Genetics of obesity in humans. *Endocr Rev* 2006;27(7):710–18.
13. Rankinen T, Zuberi A, Chagnon YC, Weisnagel SJ, Argyropoulos G, Walts B, et al. The Human Obesity Gene Map: The 2005 Update. *Obesity* 2006;14(4):529–644.
14. Bodmer W, Bonilla C. Common and rare variants in multifactorial susceptibility to common diseases. *Nat Genet* 2008;40(6):695–701.
15. Iyengar SK, Elston RC. The genetic basis of complex traits: rare variants or “common gene, common disease”? *Methods Mol Biol* 2007;376:71–84.
16. Cooper GM, Nickerson DA, Eichler EE. Mutational and selective effects on copy-number variants in the human genome. *Nat Genet* 2007;39(7 Suppl):S22–9.
17. Wong KK, deLeeuw RJ, Dosanjh NS, Kimm LR, Cheng Z, Horsman DE, et al. A comprehensive analysis of common copy-number variations in the human genome. *Am J Hum Genet* 2007;80(1):91–104.
18. Cook Jr EH, Scherer SW. Copy-number variations associated with neuropsychiatric conditions. *Nature* 2008;455(7215):919–23.
19. Estivill X, Armengol Ls. Copy Number Variants and Common Disorders: Filling the Gaps and Exploring Complexity in Genome-Wide Association Studies. *PLoS Genetics* 2007;3(10):e190.
20. Sha BY, Yang TL, Zhao LJ, Chen XD, Guo Y, Chen Y, et al. Genome-wide association study suggested copy number variation may be associated with body mass index in the Chinese population. *J Hum Genet* 2009;54(4):199–202.
21. Walters RG, Jacquemont S, Valsesia A, de Smith AJ, Martinet D, Andersson J, et al. A new highly penetrant form of obesity due to deletions on chromosome 16p11.2. *Nature* 2010;463(7281):671–5.
22. Bochukova EG, Huang N, Keogh J, Henning E, Purmann C, Blaszczyk K, et al. Large, rare chromosomal deletions associated with severe early-onset obesity. *Nature* 2010;463(7281):666–70.
23. Wang K, Li WD, Glessner JT, Grant SFA, Hakonarson H, Price RA. Large copy number variations are enriched in cases with moderate to extreme obesity. *Diabetes* 2010.
24. Saunders CL, Chiodini BD, Sham P, Lewis CM, Abkevich V, Adeyemo AA, et al. Meta-analysis of genome-wide linkage studies in BMI and obesity. *Obesity (Silver Spring)* 2007;15(9):2263–75.
25. Sham PC, Cherny SS, Purcell S, Hewitt JK. Power of Linkage versus Association Analysis of Quantitative Traits, by Use of Variance-Components Models, for Sibship Data. *Am J Hum Genet* 2000;66(5):1616–30.



26. Risch N, Merikangas K. The future of genetic studies of complex human diseases. *Science* 1996;273:1516–17.
27. A second generation human haplotype map of over 3.1 million SNPs. *Nature* 2007;449(7164):851–61.
28. Fox CS, Heard-Costa N, Cupples LA, Dupuis J, Vasan RS, Atwood LD. Genome-wide association to body mass index and waist circumference: the Framingham Heart Study 100K project. *BMC Med Genet* 2007;8 Suppl 1:S18.
29. Herbert A, Gerry NP, McQueen MB, Heid IM, Pfeufer A, Illig T, et al. A common genetic variant is associated with adult and childhood obesity. *Science* 2006;312(5771):279–83.
30. Willer CJ, Speliotes EK, Loos RJ, Li S, Lindgren CM, Heid IM, et al. Six new loci associated with body mass index highlight a neuronal influence on body weight regulation. *Nat Genet* 2009;41(1):25–34.
31. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature* 2007;447(7145):661–78.
32. Loos RJ, Lindgren CM, Li S, Wheeler E, Zhao JH, Prokopenko I, et al. Common variants near MC4R are associated with fat mass, weight and risk of obesity. *Nat Genet* 2008;40(6):768–75.
33. Gudbjartsson DF, Walters GB, Thorleifsson G, Stefansson H, Halldorsson BV, Zusmanovich P, et al. Many sequence variants affecting diversity of adult human height. *Nat Genet* 2008;40(5):609–15.
34. Lettre G, Jackson AU, Gieger C, Schumacher FR, Berndt SI, Sanna S, et al. Identification of ten loci associated with height highlights new biological pathways in human growth. *Nat Genet* 2008;40(5):584–91.
35. Weedon MN, Lango H, Lindgren CM, Wallace C, Evans DM, Mangino M, et al. Genome-wide association analysis identifies 20 loci that influence adult height. *Nat Genet* 2008;40(5):575–83.
36. Visscher PM. Sizing up human height variation. *Nat Genet* 2008;40(5):489–90.
37. Fisher RA. The correlation between relatives on the supposition of mendelian inheritance. *Transactions of the Royal Society of Edinburg* 1918;52:399–433.
38. Peters T, Ausmeier K, Ruther U. Cloning of Fatso (Fto), a novel gene deleted by the Fused toes (Ft) mouse mutation. *Mamm Genome* 1999;10(10):983–6.
39. Feinberg AP. Phenotypic plasticity and the epigenetics of human disease. *Nature* 2007;447(7143):433–40.
40. Reik W, Walter J. Genomic imprinting: parental influence on the genome. *Nat Rev Genet* 2001;2(1):21–32.
41. Wilkinson LS, Davies W, Isles AR. Genomic imprinting effects on brain development and function. *Nat Rev Neurosci* 2007;8(11):832–43.
42. Wood AJ, Oakey RJ. Genomic imprinting in mammals: emerging themes and established theories. *PLoS Genet* 2006;2(11):e147.
43. Vrana PB, Guan XJ, Ingram RS, Tilghman SM. Genomic imprinting is disrupted in interspecific *Peromyscus* hybrids. *Nat Genet* 1998;20(4):362–5.
44. Nezer C, Moreau L, Brouwers B, Coppieters W, Detilleux J, Hanset R, et al. An imprinted QTL with major effect on muscle mass and fat deposition maps to the IGF2 locus in pigs. *Nat Genet* 1999;21(2):155–6.
45. de Koning DJ, Rattink AP, Harlizius B, van Arendonk JA, Brascamp EW, Groenen MA. Genome-wide scan for body composition in pigs reveals important role of imprinting. *Proc Natl Acad Sci U S A* 2000;97(14):7947–50.
46. Kong A, Steinthorsdottir V, Masson G, Thorleifsson G, Sulem P, Besenbacher S, et al. Parental origin of sequence variants associated with complex diseases. *Nature* 2009;462(7275):868–74.
47. Dong C, Li WD, Geller F, Lei L, Li D, Gorlova OY, et al. Possible genomic imprinting of three human obesity-related genetic loci. *Am J Hum Genet* 2005;76(3):427–37.
48. Luedi PP, Hartemink AJ, Jirtle RL. Genome-wide prediction of imprinted murine genes. *Genome Res* 2005;15(6):875–84.



49. Bell CG, Walley AJ, Froguel P. The genetics of human obesity. *Nat Rev Genet* 2005;6(3):221–34.
50. Ravelli GP, Stein ZA, Susser MW. Obesity in young men after famine exposure in utero and early infancy. *N Engl J Med* 1976;295(7):349–53.
51. Hales CN, Barker DJ. The thrifty phenotype hypothesis. *Br Med Bull* 2001;60:5–20.
52. Pettitt DJ, Baird HR, Aleck KA, Bennett PH, Knowler WC. Excessive obesity in offspring of Pima Indian women with diabetes during pregnancy. *N Engl J Med* 1983;308(5):242–5.
53. Tamashiro KL, Terrillion CE, Hyun J, Koenig JJ, Moran TH. Prenatal stress or high-fat diet increases susceptibility to diet-induced obesity in rat offspring. *Diabetes* 2009;58(5):1116–25.
54. Heijmans BT, Tobi EW, Stein AD, Putter H, Blauw GJ, Susser ES, et al. Persistent epigenetic differences associated with prenatal exposure to famine in humans. *Proc Natl Acad Sci U S A* 2008;105(44):17046–9.
55. Pietilainen KH, Naukkarinen J, Rissanen A, Saharinen J, Ellonen P, Keranen H, et al. Global transcript profiles of fat in monozygotic twins discordant for BMI: pathways behind acquired obesity. *PLoS Med* 2008;5(3):e51.
56. Chen Y, Zhu J, Lum PY, Yang X, Pinto S, MacNeil DJ, et al. Variations in DNA elucidate molecular networks that cause disease. *Nature* 2008;452(7186):429–35.
57. Koza RA, Nikonova L, Hogan J, Rim JS, Mendoza T, Faulk C, et al. Changes in gene expression foreshadow diet-induced obesity in genetically identical mice. *PLoS Genet* 2006;2(5):e81.
58. Weinshilboum R. Pharmacogenomics of endocrine therapy in breast cancer. *Adv Exp Med Biol* 2008;630:220–31.
59. Muller DJ, Kennedy JL. Genetics of antipsychotic treatment emergent weight gain in schizophrenia. *Pharmacogenomics* 2006;7(6):863–87.
60. Spraggs CF, Pillai SG, Dow D, Douglas C, McCarthy L, Manasco PK, et al. Pharmacogenetics and obesity: common gene variants influence weight loss response of the norepinephrine/dopamine transporter inhibitor GW320659 in obese subjects. *Pharmacogenet Genomics* 2005;15(12):883–9.
61. Frayling TM. Genome-wide association studies provide new insights into type 2 diabetes aetiology. *Nat Rev Genet* 2007;8(9):657–62.
62. Scuteri A, Sanna S, Chen WM, Uda M, Albai G, Strait J, et al. Genome-wide association scan shows genetic variants in the FTO gene are associated with obesity-related traits. *PLoS Genet* 2007;3(7):e115.
63. Liu YJ, Liu XG, Wang L, Dina C, Yan H, Liu JF, et al. Genome-wide association scans identified CTNBL1 as a novel gene for obesity. *Hum Mol Genet* 2008;17(12):1803–13.
64. Thorleifsson G, Walters GB, Gudbjartsson DF, Steinthorsdottir V, Sulem P, Helgadóttir A, et al. Genome-wide association yields new sequence variants at seven loci that associate with measures of obesity. *Nat Genet* 2009;41(1):18–24.
65. Meyre D, Delplanque J, Chevre JC, Lecoeur C, Lobbens S, Gallina S, et al. Genome-wide association study for early-onset and morbid adult obesity identifies three new risk loci in European populations. *Nat Genet* 2009;41(2):157–9.
66. Lindgren CM, Heid IM, Randall JC, Lamina C, Steinthorsdottir V, Qi L, et al. Genome-wide association scan meta-analysis identifies three Loci influencing adiposity and fat distribution. *PLoS Genet* 2009;5(6):e1000508.
67. Johansson A, Marroni F, Hayward C, Franklin CS, Kirichenko AV, Jonasson I, et al. Linkage and genome-wide association analysis of obesity-related phenotypes: association of weight with the MGAT1 gene. *Obesity (Silver Spring)* 2010;18(4):803–8.

# Chapter 8

## Classical Hormones Linked to Obesity

Hyeong-Kyu Park and Rexford S. Ahima

### Introduction

Hyperinsulinism, hypercortisolism, hypothyroidism, polycystic ovarian syndrome, and growth hormone deficiency are often associated with obesity. Insulin is a potent anabolic hormone. Treatment with insulin or some antidiabetic drugs results in weight gain through several mechanisms. Insulinoma is a rare cause of hyperinsulinism associated with hypoglycemia, hunger, and rapid weight gain. The etiology of polycystic ovary syndrome (PCOS) is complex and multifactorial. PCOS is often associated with central obesity, insulin resistance, and hyperandrogenemia. Excessive glucocorticoid exposure, such as Cushing's syndrome, results in central obesity, sarcopenia, osteoporosis, hypertension, and hyperlipidemia. The local production of active glucocorticoids in adipose tissue by 11 $\beta$ -hydroxysteroid dehydrogenase type 1 has been implicated in obesity, insulin resistance, and hypertension. Hypothyroidism increases body weight by decreasing thermogenesis, and increasing fluid retention and interstitial accumulation of glycosaminoglycans. Hypothyroidism also increases cholesterol synthesis and impairs insulin sensitivity. Growth hormone deficiency in adults decreases lean tissue mass and increases fat. Although primary (common) obesity is associated with hyperinsulinemia and changes in adrenal, thyroid, and sex hormones, the roles of these hormones in the pathogenesis of obesity and related metabolic diseases are unclear. This chapter reviews the differential diagnosis of secondary obesity resulting from excessive exposure to insulin and glucocorticoids, and thyroid and growth hormone deficiencies.

---

R.S. Ahima (✉)

Division of Endocrinology, Diabetes and Metabolism, and the Institute for Diabetes, Obesity and Metabolism, University of Pennsylvania School of Medicine, 415 Curie Boulevard, 712A Clinical Research Building, Philadelphia, PA 19104, USA  
e-mail: ahima@mail.med.upen.edu

## Insulin

Insulin is secreted by pancreatic islet  $\beta$  cells of the pancreas in response to hyperglycemia, amino acids, and nonesterified fatty acids (NEFAs). Insulin is the primary regulator of the blood glucose level. During the postprandial period, elevated blood glucose stimulates pancreatic  $\beta$  cells to secrete insulin, which promotes glucose storage in the liver and skeletal muscle, and lipid storage in adipose tissue and liver. The action of insulin is initiated by binding to its cell-surface receptor, which consists of two  $\alpha$  subunits and two  $\beta$  subunits that form a  $\alpha_2\beta_2$  heterotetrameric complex. Insulin binds to the extracellular  $\alpha$  subunits, transmitting a signal across the plasma membrane that activates the intracellular tyrosine kinase domain of the  $\beta$  subunit. Insulin binding to the external component of its receptor results in activation of receptor tyrosine kinase. The activated insulin receptor (IR) kinase phosphorylates its substrate proteins on tyrosine residues. IR substrates include IRS (IR substrate) proteins, Shc, Cbl, APS, Gab-1 (Grb2-associated binder-1). The insulin signaling network involves three major pathways, the phosphatidylinositol 3-kinase (PI 3-kinase), the mitogen-activated protein kinase (MAPK), and Cbl/CAP pathways [1]. A pathway leading to activation of MAPK mediates the growth-promoting effects of insulin by phosphorylating transcription factors leading to activation of gene expression, whereas the PI 3-kinase and Cbl/CAP pathways, triggered by insulin, generate a diverse array of biologic responses [2, 3]. The major metabolic pathways stimulated by insulin are glycolysis, glycogen synthesis, lipogenesis, and protein synthesis. The pathways inhibited by insulin are gluconeogenesis, glycogen breakdown, lipolysis, fatty acid oxidation, and protein degradation.

Insulin has been known to increase glucose utilization by enhancing glucose uptake to skeletal muscle and fat. Insulin increases the rate of glycolysis by increasing glucose transport and the activities of hexokinase and 6-phospho-fructokinase in muscle. Glycogen synthase is the key regulating enzyme for glycogen synthesis and is activated by insulin. When glycogen store in muscle is replete, the glucose taken up is converted to lactate. Lactate, produced and released by muscle and adipose tissue, is taken up by liver and converted to glucose. Conversion of glucose to lactate occurs in several tissues, but only the muscle and adipose tissues are sensitive to insulin. Lactate is converted to pyruvate, which is a precursor for acetyl-CoA [4]. During fasting, the fall in insulin and increase in counter-regulatory hormones, e.g. glucagon, epinephrine, glucocorticoids, and growth hormone, stimulate glycogenolysis and gluconeogenesis in the liver, leading to glucose release to ensure adequate fuel supply to the brain and other vital organs.

Insulin also plays an important role in lipid metabolism. Adipose tissue triglycerides (TGs) represent the major source of stored fuel available for mobilization when energy requirements are increased or when glucose availability is reduced. Plasma NEFA is derived from lipolysis of adipose tissue TGs by hormone-sensitive lipase (HSL). Elevated insulin levels suppress adipose tissue lipolysis through its inhibition of the HSL activity, thereby reducing the release of NEFAs and glycerol. Insulin resistance attenuates lipolysis, especially upper body or visceral fat, in obesity.

Thus, obese individuals with a predominance of intraabdominal fat have higher rates of NEFA mobilization and greater resistance to the antilipolytic effects of insulin when compared with individuals with lower body obesity [5].

Insulin stimulates *de novo* lipogenesis from glucose in the liver and adipose tissue. In adipose tissue, insulin increases glucose uptake, thereby increasing the supply of lipogenic substrate. Insulin is a strong activator of lipogenesis pathway through increased expression of lipogenic enzymes such as fatty acid synthase and acetyl-CoA carboxylase. Insulin also stimulates reesterification of fatty acids in adipose tissue and liver storage in the form of TGs. Insulin increases lean and muscle mass, by decreasing proteolysis and activating protein synthesis [6].

### ***Insulin and Obesity***

The prevalence of obesity and diabetes continues to escalate, and these two conditions are closely related. Weight gain occurs in both type 1 and type 2 diabetes. Because most patients with type 2 diabetes are overweight at the time of diagnosis, iatrogenic weight gain is not only unwelcome, but represents an important clinical issue that can become a barrier to successful management. Unfortunately, most insulin and other anti-diabetic drugs increase weight. After 1 year of treatment, a study showed that patients using thiazolidinediones gained the most weight (5.0 kg), followed by those using insulin (3.3 kg), and then sulfonylureas (1.8 kg). Those using metformin lost a mean of 2.4 kg [7]. In the United Kingdom Prospective Diabetes Study (UKPDS), increased weight gain was associated with improved glycemic control and intensification of therapy. However, on average, patients in the intensive intervention cohort gained 5 kg (~3 kg more than conventionally treated patients), during the 10-year follow-up period, with most of this increase occurring in the first 12 months. Weight gain was seen with all pharmacotherapies used for intensive intervention, with the exception of metformin, but was greatest in insulin-treated patients who gained a mean of 6.5 kg [8, 9].

Although weight gain in type 1 diabetes is often perceived as desirable, overweight or obesity can become a problem with intensive insulin therapy. The Diabetes Control and Complications Trial (DCCT) showed that insulin-associated weight gain was greater in patients receiving intensified treatment compared to conventional treatment (5.1 vs. 3.7 kg, during the first 12 months of therapy), but the mean weight of both groups increased to values beyond ideal. After 12 months of therapy, the intensively treated cohort had a body weight that was, on average, 10% above ideal. After 8 years, body weight continued to increase every year in both groups – more so in the intensively treated cohort. After an average 6 years of follow-up, patients in the intensively treated group had gained a mean of nearly 5 kg more than their conventionally treated counterparts [10–12].

Weight gain is often a major concern for many diabetic patients and commonly observed when pharmacotherapies with insulin and/or oral hypoglycemic agents are intensified. How does chronically elevated insulin result in weight gain? A possible explanation is a defensive or unconscious increase in calorie intake

caused by the fear or experience of hypoglycemia. In fact, individuals may increase their intake of carbohydrates episodically or chronically in response to a perceived threat or experience of hypoglycemia. As discussed earlier, insulin stimulates adipogenesis and lipid storage. Moreover, weight gain may result from the correction of glycosuria. Once glucose control improves, less energy is lost in the urine, and if food intake calorie intake is not reduced, this will result in a net gain in weight [13]. It is also possible that subcutaneous administration of insulin contributes to weight gain. When insulin is given subcutaneously, the absorbed insulin first circulates systemically, so muscle and adipose tissues are “over-insulinized” and the liver “under-insulinized.” This leads to an increase in fat accumulation and insulin resistance, which in turn necessitates an increase in insulin requirements [12].

Although rare, insulinoma is the most common functioning islet cell tumor of the pancreas. Patients with an insulinoma present with symptoms of hypoglycemia secondary to excessive and uncontrolled secretion of insulin. The symptoms are typically episodic, and range from intense hunger, palpitations, and sweating to neuropsychiatric manifestations, such as anxiety, confusion, and coma. Symptoms usually occur in the morning after an overnight fast, and often precipitated by exercise. Patients with an insulinoma learn to avoid symptoms by eating frequent small meals and sugary snacks, with resultant weight gain. The diagnosis is established with the determination of fasting hyperinsulinemia and hypoglycemia. Increased C-peptide and proinsulin levels distinguish insulinoma from factitious insulin therapy. Several options are available for imaging and localizing these tumors including ultrasonography, computed tomography, and intra-arterial calcium stimulation with venous sampling. Surgical resection is the treatment of choice and offers the only chance of cure [14, 15].

## Glucocorticoids

Cortisol is the principal, active glucocorticoid (GC) in humans, and an important regulator of many physiological pathways, particularly at times of stress or illness. Secretion of GCs by the adrenal cortex is normally regulated by the hypothalamo-pituitary-adrenal (HPA) axis. Activation of the HPA axis starts with the secretion of hypothalamic corticotropin releasing hormone (CRH), the activation of pituitary pro-opiomelanocortin (POMC) gene transcription in response to CRH, secretion of the POMC-encoded adrenocorticotrophic hormone (ACTH), and stimulation of adrenal GC synthesis and secretion. GCs, in turn, inhibit CRH gene expression and secretion at the hypothalamic level, and POMC transcription and ACTH-secretion in the anterior pituitary, thereby establishing a regulatory feedback loop [16, 17]. GCs mediate their physiologic effects by binding to a specific, intracellular receptor, the GC receptor. The GC receptor represents a member of the hormone receptor subclass of the nuclear receptor superfamily of transcription factors. Upon GC binding in the cytosol, the GC receptor translocates into the nucleus where it serves as a DNA sequence-specific transcriptional regulator of distinct GC-responsive target genes [18].

The increase of GC from exogenous treatment, e.g. for asthma and inflammatory conditions, or an exposure from endogenous overproduction of GC (due to pituitary adenomas, ectopic ACTH-producing tumors, or adrenal tumors), results centripetal obesity, sarcopenia, insulin resistance, dyslipidemia, fatty liver, hypertension, and immunodeficiency. Many of the complications of GC excess (Cushing's syndrome) resemble the metabolic syndrome associated with idiopathic (primary) obesity [19].

*Clinical manifestations of Cushing's syndrome.* Cushing's syndrome can be classified into (i) ACTH-dependent Cushing's syndrome, in which inappropriately high plasma ACTH concentrations stimulate the adrenal cortex to produce excessive amounts of cortisol, (ii) ACTH-independent Cushing's syndrome, in which excessive production of cortisol by abnormal adrenocortical tissue causes the syndrome and suppresses the secretion of both CRH and ACTH. Rarely, Cushing's syndrome may be caused by ectopic CRH secretion, bilateral primary pigmented nodular adrenal hyperplasia, and macronodular adrenal hyperplasia, and adrenocortical hyperfunction associated with McCune-Albright syndrome and Carney's complex [20].

ACTH-dependent Cushing's syndrome accounts for ~85% of endogenous cases of hypercortisolism. Of the latter, autonomous pituitary ACTH secretion, called Cushing's disease, is responsible for 80%; the rest are caused by ectopic ACTH or, rarely, CRH secretion. Benign cortisol-secreting adenomas or adrenocortical carcinomas are responsible for about 15% of endogenous cases [21]. The incidence of pituitary-dependent Cushing's disease and adrenal adenomas in women is three to four times that of men. The usual symptoms and signs of Cushing's syndrome include a rapid increase in weight, central obesity, mooning, and plethora of the face, dorsocervical fat pad (buffalo hump) and supraclavicular fat pad, hypertension, glucose intolerance, oligomenorrhea or amenorrhea, decreased libido in men, and spontaneous ecchymoses, proximal muscle wasting and weakness, and the development of multiple purple striae wider than 1 cm on the abdomen or proximal extremities. Depression and insomnia often occur at the same time as other symptoms. Patients with Cushing's disease may have mild hirsutism and acne, but severe androgenization, e.g. hirsutism and especially virilization, strongly suggest an adrenal carcinoma. Cutaneous hyperpigmentation is unusual, except in patients with the ectopic ACTH syndrome in whom plasma ACTH concentrations are markedly elevated. Thinning of the skin and osteoporosis, with low back pain and vertebral collapse, tend to be more common in older patients [20, 22].

*Laboratory diagnosis of Cushing's syndrome.* The cardinal biochemical features comprise excess endogenous secretion of cortisol, loss of the normal feedback of the HPA axis, and disturbance of the normal circadian rhythm of cortisol secretion. The determination of 24-h excretion of cortisol in urine (UFC; urinary free cortisol) is a reliable practical index of cortisol secretion. UFC integrates the plasma-free cortisol concentrations during the entire day, with a raised level being consistent with Cushing's syndrome. The upper normal range in most assays is 220–330 nmol/24 h (80–120 mg/24 h) [20]. In a patient thought to have Cushing's syndrome, cortisol should be measured in two or three consecutive 24-h urine specimens. Occasionally, cortisol production in Cushing's syndrome fluctuates, ranging from days to months. This relatively rare phenomenon of periodic, cyclic, or episodic

hypercortisolism may require several UFC determinations for a period of 3–6 months to finally establish the diagnosis [21].

An overnight 1 mg dexamethasone suppression test (DST) is a simple screening test for endogenous hypercortisolism. The test involves the oral administration of 1 mg dexamethasone between 11 pm and midnight, after which a plasma cortisol sample is obtained between 8 and 9 a.m. the next morning. A cortisol concentration of 3.6  $\mu\text{g/dL}$  or less achieves high sensitivity; however, up to 30% of false-positive may occur as a result of primary obesity, chronic illness, psychiatric disorders, and even normal individuals [23]. The two-day, low-dose DST (0.5 mg every 6 h for 2 days) identifies patients with Cushing's syndrome. Measuring morning serum cortisol after low-dose DST and a cut-off value for suppression of 1.8  $\mu\text{g/dL}$  has a sensitivity of 98% for the diagnosis of Cushing's syndrome. The next challenge is to identify the source of excess cortisol. Immunoradiometric assays (IRMA) provide highly reproducible and sensitive ACTH measurement. Plasma ACTH concentrations  $<5\text{--}10$  pg/mL suggest an adrenal source of cortisol. Normal or elevated ACTH concentrations indicate a pituitary or an ectopic source of ACTH. The standard 2-day, high-dose DST (2 mg every 6 h for 2 days), distinguishes Cushing's disease, in which there is only relative resistance to GC negative feedback, from the ectopic ACTH syndrome, in which there is usually complete resistance. The high-dose DST is performed on 24 h collections of urine for the measurement of UFC, calculating the degree of suppression from day 1 to day 3 after the administration of oral dexamethasone. Suppression of UFC by 90% results in 100% specificity and 83% sensitivity for the diagnosis of pituitary disease [24]. As an alternative, a single 8-mg dose of dexamethasone is given orally at 11 pm, and plasma cortisol is measured at 8 am before and after dexamethasone administration. This test has a sensitivity ranging from 57% to 92% and a specificity ranging from 57% to 100% [20]. The most direct way to demonstrate pituitary hypersecretion of ACTH corticotropin is to document a central-to-peripheral-vein gradient in blood draining the tumor [22].

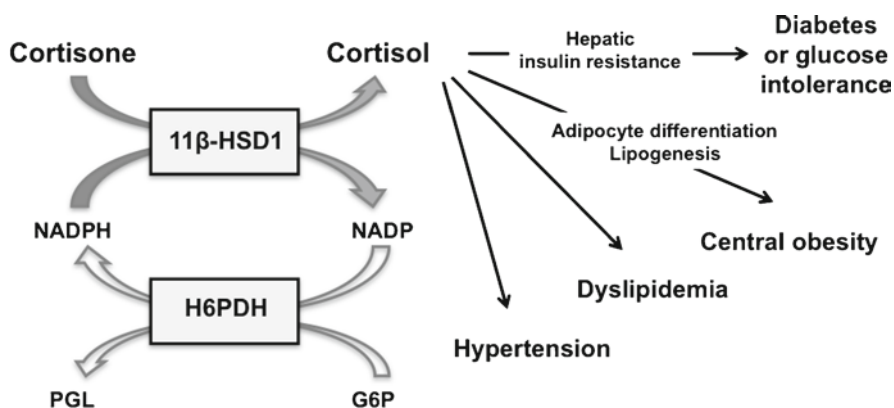
*Pseudo-Cushing's syndrome.* Patients with certain nonendocrine disorders may exhibit some of the clinical or biochemical manifestations of Cushing's syndrome. The differentiation between mild Cushing's syndrome and pseudo-Cushing's syndrome can prove extremely difficult. As many as 80% of patients with major depressive disorder have abnormal cortisol secretion. Their hormonal abnormalities presumably result from hyperactivity of the HPA axis and disappear with the remission of depression [25]. Chronic alcoholism can mimic Cushing's syndrome; however, liver dysfunction is prominent, and the hormonal abnormalities disappear rapidly during abstinence from alcohol as their liver function returns to normal. The mechanism of the hypercortisolism in chronic alcoholism may involve either increased CRH secretion or impaired hepatic metabolism of cortisol [22].

The dexamethasone-CRH test distinguishes patients with pseudo-Cushing's syndrome from those with Cushing's syndrome. The test is performed with low-dose DST followed by CRH (1  $\mu\text{g/kg}$  body weight) stimulation and cortisol measurements. In patients with pseudo-Cushing's, the pituitary corticotroph is appropriately suppressed by GCs and does not respond to CRH, while in Cushing's syndrome the corticotroph tumor is generally resistant to dexamethasone and



responds to CRH. Therefore, plasma cortisol level at 15 min after CRH injection is greater than 1.4  $\mu\text{g/dL}$  supports the diagnosis of Cushing's syndrome, while lower values are seen in normal individuals and those with pseudo-Cushing states. Measurement of plasma cortisol at midnight can also be used, with 95% diagnostic accuracy using a cut-point of  $>7.5 \mu\text{g/dL}$  to diagnose Cushing's syndrome. The circadian rhythmicity of cortisol is preserved in pseudo-Cushing states but disrupted in Cushing's syndrome [23]. True hypercortisolism will persist and the symptoms worsen, whereas hypercortisolism associated with pseudo-Cushing's states typically resolve spontaneously, or following definitive therapy, e.g. antidepressant treatment or abstinence from alcohol [21].

*Linking cortisol and metabolic syndrome.* Although there is a striking resemblance between the physical and biochemical features of Cushing's syndrome and the metabolic syndrome associated with primary obesity, plasma cortisol levels tend to be normal or reduced in the latter. This paradox was explained by the discovery that intracellular GC reactivation occurs in adipose tissue and liver of obese rodents and humans.  $11\beta$ -hydroxysteroid dehydrogenase type 1 ( $11\beta$ -HSD1) is the enzyme that mediates the adipose conversion of inactive cortisone to active cortisol in humans (Fig. 1), and deoxycorticosterone to corticosterone in rodents.  $11\beta$ -HSD1 is located within the endoplasmic reticulum and is highly expressed in liver and adipose tissue [26]. Transgenic mice overexpressing  $11\beta$ -HSD1 in adipose tissue exhibited elevated intra-adipose and portal, but not systemic corticosterone levels, abdominal obesity, insulin resistance, hyperglycemia, hyperlipidemia, and hypertension [27, 28]. Overexpression of  $11\beta$ -HSD1 in the liver produced mild insulin resistance, fatty liver, hyperlipidemia, and hypertension, but not obesity or glucose intolerance [29]. In contrast,  $11\beta$ -HSD1 knock-out ( $11\beta$ -HSD1 $^{-/-}$ ) mice had



**Fig. 1** Role of  $11\beta$ -hydroxysteroid dehydrogenase type 1 in the metabolic syndrome.  $11\beta$ -hydroxysteroid dehydrogenase type 1 ( $11\beta$ -HSD1) generates cortisol from cortisone, using the cofactor NADPH donated by hexose 6-phosphate dehydrogenase (H6PDH). Enhanced activity of  $11\beta$ -HSD1 in adipose tissue has been implicated in central obesity, insulin resistance, type 2 diabetes, dyslipidemia, and atherogenic cardiovascular disease. G6P, glucose 6-phosphate; PGL, 6-phosphogluconolactonate

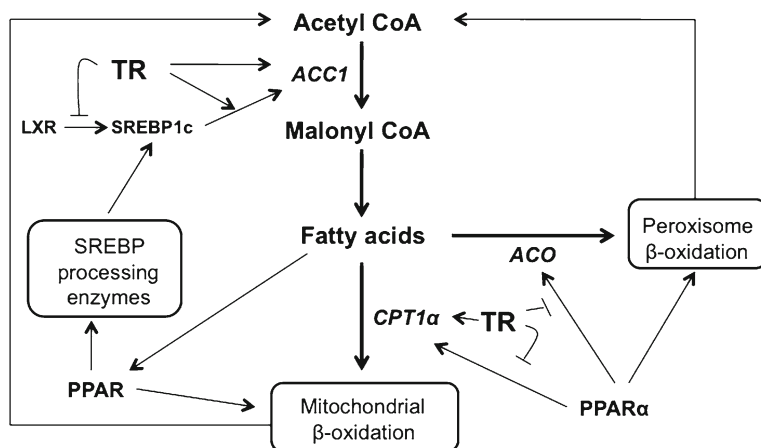


improved glucose tolerance, improved lipid profile, and reduced weight and visceral fat when fed a high-fat diet [30, 31]. The role of  $11\beta$ -HSD1 in human obesity, metabolic syndrome, and type 2 diabetes has been inconsistent, perhaps as a reflection of variability of subjects, and different ethnic populations and methods. The decrease in hepatic  $11\beta$ -HSD1 activity that occurs in simple obesity is not observed in type 2 diabetes [32]. Perhaps reduced  $11\beta$ -HSD1 activity is a compensatory mechanism to preserve insulin sensitivity and decrease hepatic glucose output. Failure to suppress  $11\beta$ -HSD1 may lead to elevated GC levels in adipose and other tissues, contributing to obesity, insulin resistance, and hyperglycemia. Many studies have demonstrated increased  $11\beta$ -HSD1 expression and activity in subcutaneous and omental adipose tissue in human obesity.  $11\beta$ -HSD1 inhibitors have been tested in rodents and shown therapeutic effects to reduce adiposity, enhance insulin sensitivity, and improve lipid profile. GC receptor antagonists have also resulted in favorable metabolic effects in rodents. However, any benefits have to be weighed against detrimental effects on the HPA axis [26, 31].

## Thyroid Hormone

Thyroid hormone is required for the normal function of nearly all tissues, with major effects on oxygen consumption and metabolic rate. Thyroid hormone also plays critical roles during embryogenesis and early life, and has profound effects in adult life, including changes in protein, carbohydrate, and lipid metabolism [33]. The synthesis and secretion of thyroid hormone are regulated by a feedback system, the HPT axis. Thyrotropin-releasing hormone (TRH) is synthesized in the paraventricular nucleus of the hypothalamus, and transported via axons to the median eminence, where it is released into the portal capillary plexus and stimulates TSH synthesis and secretion by the anterior pituitary [34]. TSH stimulates thyroxine ( $T_4$ ) and triiodothyronine ( $T_3$ ) synthesis.  $T_4$  is more abundant but less potent than  $T_3$ . Plasma and cellular  $T_3$  levels are mainly derived from  $T_4$  conversion by type 1 (D1) and type 2 (D2) 5'-deiodinases. D1 is located on the cell membrane and generates circulating  $T_3$ . D2 is expressed in the cytoplasm, and rapidly produces  $T_3$ . The adrenergic system stimulates D2 activity. A high level of D2 is expressed in the hypothalamus and pituitary, and produces  $T_3$  that mediates the negative feedback regulation of TSH and TRH [35, 36]. Only 0.03% of the total serum  $T_4$  is free or unbound, with the remainder bound to carrier proteins such as thyroxine-binding globulin (TBG), albumin, and thyroid-binding prealbumin. Approximately 0.3% of the total serum  $T_3$  is free, with the remainder bound to TBG and albumin. It is the free thyroid hormone that enters target cells and generates a biological response.

Thyroid hormone acts mainly through its nuclear receptors, thyroid hormone receptor (TR)  $\alpha$  and  $\beta$ . TR forms a heterodimeric complex with retinoid X receptor (RXR), which binds to a thyroid hormone response element (TRE) to regulate the expression of genes involved in the metabolism of lipids, carbohydrates, bile acids, and other processes. The binding of  $T_3$  to TR stimulates gene expression, while



**Fig. 2** Effects of thyroid hormone on fatty acid metabolism in the liver. The ACC1 promoter contains a thyroid hormone receptor response element (TRE) and sterol regulating element binding protein response element (SRE). Thyroid hormone directly stimulates the synthesis of ACC1 which catalyzes the formation of fatty acids. Thyroid hormone increases fatty acid oxidation by upregulating expression of CPT-1 $\alpha$ . Unliganded thyroid hormone receptor (TR) blocks stimulation of CPT1 $\alpha$  and ACO by PPAR $\alpha$ . ACC, acetyl-CoA carboxylase; ACO, acetyl-CoA oxidase; CPT, carnitine palmitoyltransferase

unliganded TR binds to a TRE and represses gene expression. Thyroid hormone increases basal metabolic rate via Na/K ATPase, and also interacts with the adrenergic nervous system to produce heat in response to cold exposure [37]. This process, termed adaptive thermogenesis, occurs in rodent brown adipose tissue, requires both TR $\alpha$  and TR $\beta$ , and involves uncoupling protein (UCP)-1 expression. In addition, T3 stimulates lipolysis in adipose tissue and fatty acid oxidation in the liver (Fig. 2), and reduces cholesterol by increasing the expression of low-density lipoprotein receptor. Glucose metabolism is modulated by thyroid hormone. Excess thyroid hormone stimulates hepatic gluconeogenesis and glucose production, increases glucose transporter (GLUT4) in skeletal muscle, and reduces insulin levels, partly by accelerating insulin degradation [38]. T3 stimulates carbohydrate response element binding protein (ChREBP), a transcription factor that increases glycolysis and *de novo* lipogenesis in the liver [37].

*Clinical manifestations of hypothyroidism.* Weight gain is a common complaint in patients with hypothyroidism. The commonest cause of hypothyroidism in developed countries is autoimmune thyroiditis. Radioiodine ablation or surgical thyroidectomy as treatment for hyperthyroidism or thyroid cancer can also lead to hypothyroidism if thyroxine replacement is inadequate. Hypothyroidism may be drug-induced (e.g. lithium, amiodarone), or result from disorders of the pituitary (secondary) or hypothalamus (tertiary). In the United States, hypothyroidism develops in ~5% of the population, and is especially common in women older than 60 years. Anti-thyroid peroxidase (TPO) antibodies are associated with

hypothyroidism, and more common in women than in men and increase with age [39]. In addition to modest weight gain, other features of hypothyroidism include a general slowing down, mental depression, cold intolerance, constipation, dryness of the skin, and brittleness of the hair. As the disorder becomes more fully established, the classic features of non-pitting edema (myxedema) of the skin, periorbital edema, hoarseness, sinus bradycardia, hypothermia, and delayed relaxation of the deep tendon reflexes appear [40].

The serum TSH is the most sensitive test for detecting early thyroid failure. An increase in TSH precedes an a decline of serum free  $T_4$  by many months and sometimes years. Serum  $T_3$  concentration is often normal. Adults presenting with symptomatic hypothyroidism often have a TSH level in excess of 10 mU/L coupled with a reduction in the serum free or total  $T_4$  concentration below the reference range. Some adults have less severe hypothyroidism, with a serum TSH that is increased (typically between 5 and 10 mU/L), but a serum  $T_4$  concentration within the reference range. This is termed "subclinical hypothyroidism," and in many patients represents a state of compensated or mild thyroid failure [40]. Subclinical hypothyroidism increases with age and is more common in women. However, after the sixth decade, the prevalence in men approaches that of women, with a combined prevalence of 10%. Antithyroid antibodies can be detected in 80% of patients with subclinical hypothyroidism, and 80% of patients with subclinical hypothyroidism have a serum TSH less than 10 mU/L. Patients with subclinical hypothyroidism have a high rate of progression to clinically overt hypothyroidism, ~2.6% each year if TPO antibodies are absent, and 4.3% if they are present. A TSH level greater than 10 mIU/L predicts a higher rate of progression of hypothyroidism [41]. Laboratory investigation of hypothyroidism may reveal a mild anemia, increased creatine phosphokinase concentrations suggesting myopathy, and an abnormal lipid profile with increased total and low-density lipoprotein cholesterol and decreased high-density lipoprotein cholesterol concentrations [42].

Central hypothyroidism is a rare cause of hypothyroidism characterized by a defect of thyroid hormone production due to an insufficient stimulation by TSH of an otherwise normal thyroid gland. Secondary hypothyroidism can be congenital or acquired in the case of lesions affecting either the pituitary (secondary hypothyroidism) or the hypothalamus (tertiary hypothyroidism). The diagnosis is usually made on a biochemical basis showing reduced serum free or total  $T_4$  concentration associated with an inappropriately low TSH level. TRH testing may help in the differential diagnosis between tertiary (hypothalamic) and secondary (pituitary) hypothyroidism. In the latter, TSH response may be absent or impaired, whereas tertiary hypothyroidism is characterized by normal, exaggerated, or delayed TSH responses to TRH injection [43].

*Hypothyroidism and obesity.* There are multiple potential explanations for weight gain in hypothyroidism. Thyroid hormone is required for the normal regulation of resting energy expenditure (REE). In hypothyroid patients receiving long-term  $T_4$  treatment who maintained a euthyroid state, small changes in the daily dose to ensure that serum free  $T_4$  concentrations moved within the normal range were associated with detectable changes in REE. Serum TSH, the most sensitive

marker of thyroid hormone action, is inversely associated with REE [44]. Moreover, spontaneous fluctuations in free T4 concentration have been associated with significant changes in REE [45, 46]. Several studies have demonstrated a positive cross-sectional association between serum TSH levels and body mass index. Change in serum TSH levels over time, although within the reference range, was strongly and linearly associated with weight gain [47–49].

Hypothyroidism is associated with cardiac wall stiffness, bradycardia, and depressed myocardial contractility, which account for reduced cardiac output [50]. A low cardiac output and a decrease in renal blood flow and glomerular filtration rate lead to impaired renal water excretion, which contributes to edema and weight gain [51]. Hypothyroidism also causes generalized interstitial deposition of glycosaminoglycans, which in turn leads to fluid and sodium retention. Hyaluronan, an abundant non-sulfated glycosaminoglycan, accumulates in many tissues including the skin, myocardium, kidney, and vasculature in severe, long-standing hypothyroidism due to a reduced clearance rate and increased synthetic rate. Hyaluronan exhibits a remarkable avidity for water, thus causing the tissues to expand greatly [52, 53].

## Growth Hormone

Growth hormone (GH) is produced by the somatotroph cells of the anterior pituitary. GH secretion is stimulated by GH-releasing hormone (GHRH) and inhibited by somatostatin. GH secretion is also regulated by metabolic cues: insulin, glucose, and fatty acids inhibit GH secretion, while arginine stimulates secretion [54]. GH secretion is pulsatile, and the amplitude of the pulses is highest at night. The 24-h GH secretion is maximal during puberty and declines gradually thereafter in both women and men. GH binds and activates receptors on hepatocytes and other cells, leading to the tyrosine phosphorylation and association with JAK2. Several of the proteins phosphorylated and activated by the GH receptor through JAK2 serve as adapters, linking GH signaling to a variety of signal transduction pathways. IRS-1, IRS-2, Shc, and the EGF receptor all have been implicated as GH-regulated docking proteins, providing connections to the PI3 kinase and MAP kinase pathways [55, 56]. GH is the main regulator of insulin-like growth factor (IGF)-1. The liver is a major target tissue of GH action and produces IGF-1 and IGF binding protein-3 (IGFBP-3) in response to GH. IGFBP-3 prolongs the half-life of IGF-I. Unbound IGF-1 mediates a negative feedback control of GH secretion by acting directly on the somatotroph and on hypothalamic GHRH and somatostatin neurons [54].

*Metabolic complications of GH deficiency.* Growth hormone deficiency (GHD) may be isolated or occur as part of multiple hormone deficiencies. GHD often results from damage to the pituitary gland or hypothalamus, caused by a tumor in the area or following surgical resection or radiotherapy. The syndrome associated with GHD includes metabolic and cardiovascular complications, osteopenia and osteoporosis, and reduced quality of life. Patients with GHD typically have

increased abdominal fat, reduced exercise capacity, and elevated levels of total and low-density lipoprotein cholesterol. Triglycerides may be elevated and high-density lipoprotein cholesterol reduced in GHD [57]. Studies have shown that the changes in lipid profile contribute to the increased coronary risk in GHD patients, particularly in females. Central adiposity in GHD is associated with elevated fasting insulin levels and insulin resistance [58].

GH secretion is pulsatile and has a short half-life, therefore, serum GH may be undetectable in normal subjects, and a single random GH measurement cannot identify GHD. Serum IGF-1 concentrations below the normal range are suggestive of GHD, but do not rule out the diagnosis. Moreover, reduced IGF-1 levels are seen in several conditions, e.g. starvation, chronic liver and kidney diseases, hypothyroidism, and diabetes. GHD is evaluated using provocative dynamic tests. Insulin tolerance test (ITT), considered the “gold standard,” is reproducible and if plasma glucose concentration less than 2.2 mmol/L (40 mg/dL) is attained. A peak GH response to hypoglycemia of less than 3  $\mu\text{g/L}$  measured by polyclonal competitive radioimmunoassay, or less than 5.1  $\mu\text{g/L}$ , measured by immunochemiluminescent two-site assay, has sufficient specificity and sensitivity for the diagnosis of GHD in adults. However, it is important to be aware that insulin resistance in severe obesity can attenuate the hypoglycemic effect of ITT, and thus diminish the GH response. The ITT is contraindicated in patients with ischemic heart disease, cerebrovascular disease, or seizure disorders. Precautions should be taken if ITT is done in patients older than 60 years. Alternative tests to ITT include the combined administration of GHRH + arginine, GHRH, glucagon, propranolol. For the GHRH + arginine test, a GH peak of less than 4.1  $\mu\text{g/L}$  indicates GH deficiency [59, 60].

The goal for GH replacement is to correct the abnormalities associated with GHD syndrome. GH dosing regimens should be individualized, at a starting dose of 300  $\mu\text{g/day}$ , and an increase in daily dosing of 100–200  $\mu\text{g/day}$  for every 1 or 2 months. A typical median maintenance dose is 400  $\mu\text{g/day}$ . It is recommended that GH be administered in the evening to mimic the greater secretion of GH at night. GH treatment is titrated according to clinical response, side effects, and IGF-I levels. It should also take age, sex, and estrogen status into account. Patients should be monitored at 1–2 month intervals during the dose titration, and then at 6 month intervals during the maintenance phase. As with other hormonal replacement therapies, the GH dose may vary over time, and should be monitored and adjusted. Patients with GHD receiving GH replacement should be managed by an endocrinologist or internist with expertise in pituitary disease [61, 62].

## Conclusions

Obesity can be a manifestation of hypothyroidism, hyperinsulinism, hypercortisolism or growth hormone deficiency, and is often associated with glucose intolerance or diabetes, dyslipidemia, hypertension, and increased risk of atherogenic cardiovascular disease. Secondary obesity resulting from abnormal regulation of

classical hormones tends to have a rapid onset and progression, and be associated with symptoms and signs of the underlying diseases. Understanding the pathogenesis, clinical features, and laboratory evaluation of endocrinopathies enables a specific treatment strategy that often cures obesity and related metabolic disorders.

**Acknowledgments** This work was supported by grants RO1-DK62348 and PO1-DK49210 from the National Institutes of Health.

## References

1. Pessin, J. E., & Saltiel, A. R. (2000). Signaling pathways in insulin action: Molecular targets of insulin resistance. *Journal of Clinical Investigation*, 106(2), 165–169.
2. Avruch, J., Khokhlatchev, A., Kyriakis, J. M., Luo, Z., Tzivion, G., Vavvas, D., et al. (2001). Ras activation of the Raf kinase: Tyrosine kinase recruitment of the MAP kinase cascade. *Recent Progress in Hormone Research*, 56, 127–155.
3. Khan, A. H., & Pessin, J. E. (2002). Insulin regulation of glucose uptake: A complex interplay of intracellular signalling pathways. *Diabetologia*, 45(11), 1475–1483.
4. Rossetti, L., & Giaccari, A. (1990). Relative contribution of glycogen synthesis and glycolysis to insulin-mediated glucose uptake. A dose-response euglycemic clamp study in normal and diabetic rats. *Journal of Clinical Investigation*, 85(6), 1785–1792.
5. Guo, Z., Hensrud, D. D., Johnson, C. M., & Jensen, M. D. (1999). Regional postprandial fatty acid metabolism in different obesity phenotypes. *Diabetes*, 48(8), 1586–1592.
6. Capeau, J. (2008). Insulin resistance and steatosis in humans. *Diabetes & Metabolism*, 34(6 Pt 2), 649–657.
7. Nichols, G. A., & Gomez-Caminero, A. (2007). Weight changes following the initiation of new anti-hyperglycaemic therapies. *Diabetes, Obesity & Metabolism*, 9(1), 96–102.
8. UK Prospective Diabetes Study (UKPDS) Group. (1998). Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *Lancet*, 352(9131), 837–853.
9. UK Prospective Diabetes Study (UKPDS) Group. (1998). Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). *Lancet*, 352(9131), 854–865.
10. DCCT Research Group. (1988). Weight gain associated with intensive therapy in the diabetes control and complications trial. *Diabetes Care*, 11(7), 567–573.
11. DCCT Research Group. (2001). Influence of intensive diabetes treatment on body weight and composition of adults with type 1 diabetes in the Diabetes Control and Complications Trial. *Diabetes Care*, 24(10), 1711–1721.
12. Russell-Jones, D., & Khan, R. (2007). Insulin-associated weight gain in diabetes—Causes, effects and coping strategies. *Diabetes, Obesity & Metabolism*, 9(6), 799–812.
13. Carver, C. (2006). Insulin treatment and the problem of weight gain in type 2 diabetes. *Diabetes Educator*, 32 (6), 910–917.
14. Tucker, O. N., Crotty, P. L., & Conlon, K. C. (2006). The management of insulinoma. *British Journal of Surgery*, 93(3), 264–275.
15. Grant, C. S. (2005). Insulinoma. *Best Practice & Research. Clinical Gastroenterology*, 19(5), 783–798.
16. Malkoski, S. P., & Dorin, R. I. (1999). Composite glucocorticoid regulation at a functionally defined negative glucocorticoid response element of the human corticotropin-releasing hormone gene. *Molecular Endocrinology*, 13(10), 1629–1644.
17. Watts, A. G. (2005). Glucocorticoid regulation of peptide genes in neuroendocrine CRH neurons: A complexity beyond negative feedback. *Frontiers in Neuroendocrinology*, 26(3–4), 109–130.

18. Tata, J. R. (2002). Signalling through nuclear receptors. *Nature Reviews. Molecular Cell Biology*, 3(9), 702–710.
19. Vegiopoulos, A., & Herzig, S. (2007). Glucocorticoids, metabolism and metabolic diseases. *Molecular and Cellular Endocrinology*, 275(1–2), 43–61.
20. Newell-Price, J., Trainer, P., Besser, M., & Grossman, A. (1998). The diagnosis and differential diagnosis of Cushing's syndrome and pseudo-Cushing's states. *Endocrine Reviews*, 19(9), 647–672.
21. Tsigos, C., & Chrousos, G. P. (1996). Differential diagnosis and management of Cushing's syndrome. *Annual Review of Medicine*, 47, 443–461.
22. Orth, D. N. (1995). Cushing's syndrome. *New England Journal of Medicine*, 332(12), 791–803.
23. Nieman, L. K. (2002). Diagnostic tests for Cushing's syndrome. *Annals of the New York Academy of Sciences*, 970, 112–118.
24. Flack, M. R., Oldfield, E. H., Cutler, G. B., Jr., Zweig, M. H., Malley, J.D., Chrousos, G. P., et al. (1992). Urine free cortisol in the high-dose dexamethasone suppression test for the differential diagnosis of the Cushing syndrome. *Annals of Internal Medicine*, 116(3), 211–217.
25. Gold, P. W., Loriaux, D. L., Roy, A., Kling, M. A., Calabrese, J. R., Kellner, C. H., et al. (1986). Responses to corticotropin-releasing hormone in the hypercortisolism of depression and Cushing's disease. Pathophysiologic and diagnostic implications. *New England Journal of Medicine*, 314(21), 1329–1335.
26. Tomlinson, J. W., & Stewart, P. M. (2007). Modulation of glucocorticoid action and the treatment of type-2 diabetes. *Best Practice & Research. Clinical Endocrinology & Metabolism*, 21, 607–619.
27. Masuzaki, H., Paterson, J., Shinyama, H., Morton N. M., Mullins, J.J., Seckl, J. R., et al. (2001). A transgenic model of visceral obesity and the metabolic syndrome. *Science*, 294(5549), 2166–2170.
28. Masuzaki, H., Yamamoto, H., Kenyon, C. J., Elmquist, J. K., Morton, N. M., Paterson, J. M., et al. (2003). Transgenic amplification of glucocorticoid action in adipose tissue causes high blood pressure in mice. *Journal of Clinical Investigation*, 112(1), 83–90.
29. Paterson, J. M., Morton, N. M., Fievet, C., Kenyon, C. J., Holmes, M. C., Staels, B., et al. (2004). Metabolic syndrome without obesity: Hepatic overexpression of 11beta-hydroxysteroid dehydrogenase type 1 in transgenic mice. *Proceedings of the National Academy of Sciences of the United States of America*, 101(18), 7088–7093.
30. Kotelevtsev, Y., Holmes, M. C., Burchell, A., Houston, P. M., Schmolli, D., Jamieson, P., et al. (1997). 11Beta-hydroxysteroid dehydrogenase type 1 knockout mice show attenuated glucocorticoid-inducible responses and resist hyperglycemia on obesity or stress. *Proceedings of the National Academy of Sciences of the United States of America*, 94(26), 14924–14929.
31. Wake, D. J., & Walker, B. R. (2006). Inhibition of 11beta-hydroxysteroid dehydrogenase type 1 in obesity. *Endocrine*, 29(1), 101–108.
32. Valsamakis, G., Anwar, A., Tomlinsen, J. W., Shackleton, C. H., McTernan, P. G., Chetty, R., et al. (2004). 11Beta-hydroxysteroid dehydrogenase type 1 activity in lean and obese males with type 2 diabetes mellitus. *Journal of Clinical Endocrinology and Metabolism*, 89(9), 4755–4761.
33. Yen, P. M. (2001). Physiological and molecular basis of thyroid hormone action. *Physiological Review*, 81(3), 1097–1142.
34. Shupnik, M. A., Ridgway, E. C., & Chin, W. W. (1989). Molecular biology of thyrotropin. *Endocrine Reviews*, 10(4), 459–475.
35. Bianco, A. C., Salvatore, D., Gereben, B., Berry, M. J., & Larsen, P. R. (2002). Biochemistry, cellular and molecular biology, and physiological roles of the iodothyronine selenodeiodinases. *Endocrine Reviews*, 23(1), 38–89.
36. Oetting, A., & Yen, P. M. (2007). New insights into thyroid hormone action. *Best Practice & Research. Clinical Endocrinology & Metabolism*, 21(2), 193–208.
37. Liu, Y. Y., & Brent, G. A. (2009). Thyroid hormone crosstalk with nuclear receptor signaling in metabolic regulation. *Trends in Endocrinology and Metabolism*, 21(3), 166–173.
38. Potenza, M., Via, M. A., & Yanagisawa, R. T. (2009). Excess thyroid hormone and carbohydrate metabolism. *Endocrine Practice*, 15, 254–262.



39. Hollowell, J. G., Staehling, N. W., Flanders, W. D., Hannon, W. H., Gunter, E. W., Spencer, C. A., et al. (2002). Serum TSH, T(4), and thyroid antibodies in the United States population (1988 to 1994): National Health and Nutrition Examination Survey (NHANES III). *Journal of Clinical Endocrinology and Metabolism*, 87(2), 489–499.
40. Vaidya, B., & Pearce, S. H. (2008). Management of hypothyroidism in adults. *BMJ*, 337, a801.
41. Fatourechì, V. (2009). Subclinical hypothyroidism: An update for primary care physicians. *Mayo Clinic Proceedings*, 84(1), 65–71.
42. Woeber, K. A. (2000). Update on the management of hyperthyroidism and hypothyroidism. *Archives of Internal Medicine*, 160(8), 1067–1071.
43. Lania, A., Persani, L., & Beck-Peccoz, P. (2008). Central hypothyroidism. *Pituitary*, 11(2), 181–186.
44. Al-Adsani, H., Hoffer, L. J., & Silva, J. E. (1997). Resting energy expenditure is sensitive to small dose changes in patients on chronic thyroid hormone replacement. *Journal of Clinical Endocrinology and Metabolism*, 82(4), 1118–1125.
45. Boivin, M., Camirand, A., Carli, F., Hoffer, L. J., & Silva, J. E. (2000). Uncoupling protein-2 and -3 messenger ribonucleic acids in adipose tissue and skeletal muscle of healthy males: Variability, factors affecting expression, and relation to measures of metabolic rate. *Journal of Clinical Endocrinology and Metabolism*, 85(5), 1975–1983.
46. Silva, J. E. (2003). The thermogenic effect of thyroid hormone and its clinical implications. *Annals of Internal Medicine*, 139(3), 205–213.
47. Knudsen, N., Laurberg, P., Rasmussen, L. B., Bulow, I., Perrild, H., Ovesen, L., et al. (2005). Small differences in thyroid function may be important for body mass index and the occurrence of obesity in the population. *Journal of Clinical Endocrinology and Metabolism*, 90(7), 4019–4024.
48. Nyren, A., Jorde, R., & Sundsfjord, J. (2006). Serum TSH is positively associated with BMI. *International Journal of Obesity*, 30(1), 100–105.
49. Fox, C. S., Pencina, M. J., D'Agostino, R. B., Murabito, J.M., Seely, E. W., Pearce, E. N., et al. (2008). Relations of thyroid function to body weight: Cross-sectional and longitudinal observations in a community-based sample. *Archives of Internal Medicine*, 168(6), 587–592.
50. Fazio, S., Palmieri, E. A., Lombardi, G., & Biondi, B. (2004). Effects of thyroid hormone on the cardiovascular system. *Recent Progress in Hormone Research*, 59, 31–50.
51. Montenegro, J., Gonzalez, O., Saracho, R., Aguirre, R., & Martinez, I. (1996). Changes in renal function in primary hypothyroidism. *American Journal of Kidney Diseases*, 27(2), 195–198.
52. Smith, T. J., Murata, Y., Horwitz, A. L., Philipson, L., & Refetoff, S. (1982). Regulation of glycosaminoglycan synthesis by thyroid hormone in vitro. *Journal of Clinical Investigation*, 70(5), 1066–1073.
53. Gianoukakis, A. G., Jennings, T. A., King, C. S., Sheehan, C. E., Hoa, N., Heldin, P., et al. (2007). Hyaluronan accumulation in thyroid tissue: Evidence for contributions from epithelial cells and fibroblasts. *Endocrinology*, 148(1), 54–62.
54. Meinhardt, U. J., & Ho, K. K. (2006). Modulation of growth hormone action by sex steroids. *Clinical Endocrinology*, 65(4), 413–422.
55. Vance, M. L., & Mauras, N. (1999). Growth hormone therapy in adults and children. *New England Journal of Medicine*, 341(16), 1206–1216.
56. Woelfle, J., Chia, D. J., Massart-Schlesinger, M. B., Moyano, P., & Rotwein, P. (2005). Molecular physiology, pathology, and regulation of the growth hormone/insulin-like growth factor-I system. *Pediatric Nephrology*, 20(3), 295–302.
57. Shalet, S. M., Toogood, A., Rahim, A., & Brennan, B. M. (1998). The diagnosis of growth hormone deficiency in children and adults. *Endocrine Reviews*, 19(2), 203–223.
58. Carroll, P. V., Christ, E. R., Bengtsson, B. A., Carlsson, L., Christiansen, J. S., Clemmons, D., et al. (1998). Growth hormone deficiency in adulthood and the effects of growth hormone replacement: A review. Growth Hormone Research Society Scientific Committee. *Journal of Clinical Endocrinology and Metabolism*, 83(2), 382–395.
59. Gasco, V., Corneli, G., Rovere, S., Croce, C., Beccuti, G., Mainolfi, A., et al. (2008). Diagnosis of adult GH deficiency. *Pituitary*, 11(2), 121–128.



60. Casanueva, F. F., Castro, A. I., Micic, D., Kelestimur, F., & Dieguez, C. (2009). New guidelines for the diagnosis of growth hormone deficiency in adults. *Hormone Research*, 71(Suppl 1), 112–115.
61. Ho, K. K. (2007). Consensus guidelines for the diagnosis and treatment of adults with GH deficiency II: A statement of the GH Research Society in association with the European Society for Pediatric Endocrinology, Lawson Wilkins Society, European Society of Endocrinology, Japan Endocrine Society, and Endocrine Society of Australia. *European Journal of Endocrinology*, 157(6), 695–700.
62. Johannsson, G. (2009). Treatment of growth hormone deficiency in adults. *Hormone Research*, 71(Suppl 1), 116–122.

## Chapter 9

# Inflammation and Adipose Dysfunction

Rachana Shah and Muredach P. Reilly

### Systemic Activation of Inflammatory Pathways

While inflammatory changes in obesity have been recognized for many years, the pathophysiology underlying these alterations is still being elucidated. In fact, the primary causal mechanisms by which obesity results in activation of immune pathways are not yet fully understood. Some of the prominent theories are explored here.

Overnutrition and impaired metabolic homeostasis can elicit a systemic stress response in which the hypothalamic-pituitary-adrenal axis and the autonomic nervous system play a critical role. Both circulating catecholamines and steroids can adversely affect adipose tissue insulin sensitivity and lipid metabolism [1]. Furthermore, dysregulation of local adrenocorticoid action is suggested by 11 $\beta$ -hydroxysteroid dehydrogenase type 1 (11 $\beta$ -HSD1) modulation of human obesity and type 2 diabetes [2]. This enzyme catalyzes conversion of inactive cortisone to active cortisol, controlling local action of glucocorticoid hormones in adipose. Mice lacking 11 $\beta$ -HSD1 are protected from diet-induced obesity and insulin resistance [3].

Systemic activation of innate immunity, via toll-like receptors (TLRs), triggers the development of adipose inflammation and insulin resistance and may contribute chronically to obesity and its complications. Agwunobi and colleagues [4] were the first to show impaired insulin sensitivity in humans, using euglycemic clamps during experimental administration of endotoxin (lipopolysaccharide; LPS), the classic pathogen ligand for TLR4. Mehta et al. [5] recently demonstrated that endotoxemia activated the hypothalamic-pituitary-adrenal axis and modulated adipose inflammatory and insulin signaling pathways prior to the induction of systemic insulin resistance. In a related work, Shah et al. [6] characterized adipose mRNA changes before and after endotoxemia and revealed marked upregulation of adipose

---

M.P. Reilly (✉)

Cardiovascular Institute and the Institute for Diabetes, Obesity and Metabolism, and the Institute for Translational Medicine and Therapeutics, Departments of Medicine and Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, PA, USA  
e-mail: muredach@spirit.gerc.upenn.edu

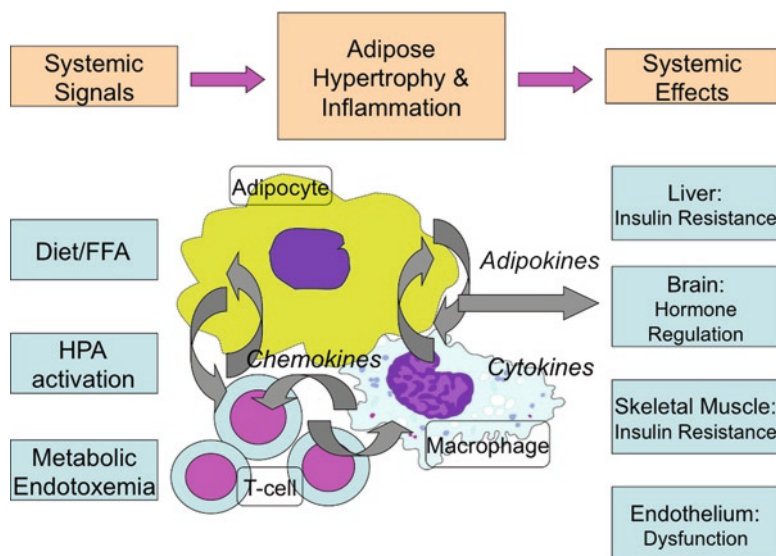
inflammatory genes, including many related to macrophage and inflammatory T cell activation, recruitment and retention.

Remarkably, “metabolic endotoxemia” may activate TLR4 signaling and provide a link between diet, obesity, low-grade inflammation and insulin resistance. In contrast to mice with a normal gut microbiota, germ-free animals are protected against diet-induced obesity [7]. In murine models and humans, LPS is constantly produced within the gut by the death of Gram-negative bacteria and transported to the vasculature. Furthermore, mice fed a high-fat diet have increased gut and plasma LPS concentrations. Plasma LPS has been found to correlate with energy intake and diet composition in healthy human subjects, with high-fat, high-carbohydrate diets leading to increased plasma LPS and mononuclear cell TLR and suppressor of cytokine signaling (SOCS) expression [8]. Remarkably, endogenous non-pathogen TLR ligands, including dietary fats, modified lipoproteins and adipocyte-derived free fatty acids (FFA), are also increased in obesity and insulin resistance. Such ligands can activate TLR4 and trigger innate and adaptive immune responses thus modulating insulin signaling [9]. These data provide a direct link between the Western lifestyle and systemic inflammatory responses that may chronically modulate insulin-sensitive tissues.

## **Adipose-Specific Activation of Immune Pathways**

In humans, obesity results in hypertrophy of adipocytes and leukocyte infiltration. Pro-inflammatory cytokines, chemokines and adipokines are secreted by adipose tissue, either by adipocytes themselves or leukocytes and stromal cells. These factors, through paracrine and endocrine pathways, result in decreased insulin sensitivity, altered lipid metabolism and atherogenesis. There are several mechanisms by which obesity is proposed to result in increased adipose inflammation. Processes specific to adipose tissue include direct effects of adipocyte hypertrophy, exhaustion of local oxygen supply, hypoperfusion and adipose hypoxia, and endoplasmic reticulum stress. Recent studies provide evidence for leukocyte recruitment and accumulation in human adipose in obesity and demonstrate the role of chemokine signaling in these events [10]. Adipocyte–inflammatory cell interactions may increase inflammatory adipocytokines, alter lipid homeostasis and suppress normal adipocyte endocrine functions.

An increase in adipocyte size due to increased triglyceride storage is a fundamental process in obesity [11]. Adipocyte hypertrophy leads to altered signaling through the c-Jun N-terminal kinase (JNK) and nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) inflammatory pathways, which likely leads to pro-inflammatory adipocytokine overproduction. Increased adipocyte volume, independent of fat mass, is also associated with impaired systemic insulin sensitivity, increased circulating inflammatory markers and increased macrophage number in adipose tissue while caloric restriction leads to decreased adipocyte lipid storage



**Fig. 1** Overview of systemic and adipose tissue inflammation and resultant whole-body metabolic dysregulation. Systemic sources of inflammation, including dietary excess and FFA overload, HPA axis activation and endogenous innate antigens generate circulating factors that influence adipose tissue inflammation. In adipose, secretion of chemokines leads to recruitment and inflammatory activation of macrophages and T cells, and a cycle of escalating inflammation. Adipokines and cytokines are then released into the systemic circulation leading to insulin resistance in liver and skeletal muscle, endothelial activation and atherosclerotic changes, and alteration in central nervous system hormonal and neuronal circuitry affecting energy expenditure and appetite. *HPA* hypothalamic-pituitary axis, *FFA* free fatty acids

and smaller adipocytes with improved whole body insulin sensitivity [12]. The precise mechanisms by which hypertrophied adipocytes induce adipose inflammation and insulin resistance remain to be established. Several leading hypotheses are outlined below and in Fig. 1.

**Hypoxia.** Adipose tissue hypoxia, hypothesized to be due to relative underperfusion in a rapidly expanding fat mass, is another proposed link between obesity and adipose inflammation. In *ob/ob* mice, weight gain was associated with decline in oxygen partial pressure ( $PO_2$ ) to levels 40–60% lower than in lean mice. Induction of hypoxia in vivo in *ob/ob* mice and in vitro in 3T3-L1 adipocytes, lowered levels of insulin signaling proteins, decreased insulin-stimulated glucose uptake, reduced FFA uptake and increased lipolysis [13]. In cultured adipocytes, hypoxia decreased mRNA levels of adiponectin, while increasing those of pro-inflammatory genes (PAI-1, leptin, MIF-1, TNF $\alpha$ , IL-1, IL-6, MCP-1 and TGF- $\beta$ ), together with those of hypoxia response genes [hypoxia inducible factor 1-alpha (HIF-1 $\alpha$ ), glucose transporter 1, VEGF] [14]. These changes were dependent in part on activation of NF $\kappa$ B pathways. Increased HIF-1 $\alpha$  expression and adipose tissue hypoxia have also been demonstrated in human obesity [15].

**Cellular Stress.** Obesity, as a state of energy excess with overabundance of fatty acids and glucose, results in metabolic, oxidative and endoplasmic reticulum (ER) stress in cells. Increased levels of oxidized proteins are present in adipose tissue of obese mice. In humans, nicotinamide adenine dinucleotide phosphate:quinone oxidoreductase 1 (NQO1), an enzyme involved in the protection against oxidative stress is highly expressed in human adipose tissue of obese individuals and correlates with parameters of obesity and insulin resistance. ER stress can be elicited by hypoxia or the inundation of the cell and ER with nutrients, activating the unfolded protein response cascade which results in transcriptional induction of a number of genes involved in the assembly, folding, modification and degradation of proteins [16]. Cellular stress leads to activation of stress-responsive signaling pathways, including the “stress-activated protein kinases” p38MAPK and JNK in omental fat. These kinases increase the production of pro-inflammatory cytokines such as IL-6, TNF $\alpha$  and MCP-1.

**Free Fatty Acids.** The combination of increased dietary intake and decreased insulin inhibition of lipolysis seen in obesity leads to increased circulating FFA. As noted above, fatty acids can signal via TLR4 to activate innate immune responses. In the adipocyte, FFA activation of TLR4 can directly induce adipose inflammation and alter adipocytokine secretion [17]. This, coupled with pro-inflammatory leukocyte activation by FFA via TLR4, is likely to create a feed-forward loop that amplifies adipose inflammation in obesity.

## Adipose Leukocyte Infiltration

A major discovery in the past decade is that obesity is associated with macrophage and T cell infiltration of adipose, and that a paracrine loop between adipocytes and leukocytes fuels adipose inflammation and dysfunction [10]. Recent studies suggest a role for T lymphocytes in the earliest stages of diet-induced adipose dysfunction and recruitment of inflammatory macrophages [18].

**Adipose Tissue Macrophages.** Infiltration and activation of monocyte/macrophages plays an important role in adipose inflammation and insulin resistance [10]. Bone marrow cell transplantation experiments in irradiated mice demonstrated that adipose tissue macrophages (ATMs) arise from bone marrow [10]. While constitutive or resident ATMs assist with homeostasis and tissue remodeling, recruited ATMs may be involved in adipocyte inflammation, promote adipose neovascularization and interfere with insulin signaling [19]. Recruitment and activation of macrophages is largely influenced by the expression of chemokines [monocyte chemoattractant protein 1 (MCP-1 or CCL2), macrophage inhibitory factor] [20] and their receptors, which are upregulated in visceral and subcutaneous adipose tissue in obese humans. However, other factors such as hypoxia, FFA [21] and adipocyte cell death [22], may also, directly or indirectly (via upregulation of chemokines), contribute to macrophage recruitment.

In vitro data suggest that macrophages may modulate adipocyte inflammation via the NF $\kappa$ B pathway. When mixed with macrophage media, adipocytes

demonstrate upregulation of inflammatory genes, monocyte adhesion, increased NF $\kappa$ B activity and decreased insulin-stimulated glucose uptake [23]. Mice fed high-fat diet have greater numbers of infiltrating macrophages. While MCP-1 elevation often accompanies this change, it is not attenuated in CCL2 $-/-$  mice, suggesting other factors are also involved [24]. In humans, macrophage infiltration is correlated with both adipocyte size and BMI and is reduced after surgery-induced weight loss in morbidly obese subjects. Expression of macrophage markers in adipose tissue was an independent negative predictor of whole-body insulin sensitivity in obese Pima Indians [25]. With weight loss through dietary intervention, microarray mRNA profiling of adipose tissue in obese women demonstrated downregulation of 511 macrophage markers. These same markers were upregulated during a preceding short phase of severe energy restriction, suggesting that in the long term, more gradual changes in weight may be necessary to decrease macrophage infiltration of adipose tissue [26].

Obesity also results in a phenotypic switch in macrophage activation state. Macrophages can be converted from a resting state (M0) to “classically activated” (M1) via LPS and/or interferon gamma (IFN $\gamma$ ) treatment, or “alternatively activated” (M2) phenotypes via IL-4 or IL-13 treatment. These macrophage subsets possess pro-inflammatory or anti-inflammatory activities, with M1 macrophages secreting high levels of TNF $\alpha$  and IL-6, while M2 cells secrete predominantly IL-10 and IL-1Ra [27]. The macrophage subtypes have divergent effects on adipocytes. When conditioned media from M1, M2 and control M0 ATMs was placed on 3T3-L1 adipocytes, the M1 media inhibited insulin-stimulated glucose uptake and prevented normal differentiation of preadipocytes.

In mice, obesity induces a definite change in ATMs from a predominantly M2 phenotype that protects against insulin resistance to an M1 inflammatory state that confers sensitivity to TNF $\alpha$ -induced insulin resistance. Mice fed a high-fat diet have higher levels of circulating M1 monocytes and increased macrophage recruitment and retention in adipose tissue [28]. The numbers of both M1 and M2 macrophages were elevated in mice fed a high-fat diet [29]. While M2 ATMs localized to interstitial spaces between adipocytes in lean mice, diet-induced obesity led to additional M1 cells in clusters surrounding necrotic adipocytes. This phenomenon was attenuated in mice lacking the MCP-1 receptor (CCR2 $-/-$ ) [30]. In rats, treatment with a PPAR- $\gamma$  agonist, rosiglitazone, shifted macrophage markers towards a higher M2 to M1 ratio [29].

In human obesity, ATMs may possess a more complex phenotype with some M2 markers and M1-like pro-inflammatory cytokine production. Though typically described as “anti-inflammatory”, the M2 macrophage subtype has been shown under certain conditions to secrete significant amounts of inflammatory cytokines. In contrast, a study in obese humans portrayed a phenomenon similar to that seen in mice. Examination of adipose tissue in obese women demonstrated a significant increase in CD40(+) M1 macrophages with higher body-mass index (BMI), with the difference being more pronounced in visceral compared to subcutaneous adipose tissue. Furthermore, the ratio of M1 to M2 cells decreased after gastric bypass surgery and weight loss [31].

***T Cell Modulation of Adipose Function.*** Although macrophages are considered the primary adipose tissue leukocyte, recent evidence suggests that T lymphocyte alterations accompany, and may even precede, macrophage modulation of adipose [32]. Initial studies indicated high numbers of T cells in adipose tissue of diet-induced obese insulin-resistant mice [33] with T cell infiltration, particularly CD4+ T cells, in early obesity even prior to the recruitment of macrophages. TH1 cytokines, in particular the pro-inflammatory IFN $\gamma$  [35], stimulate macrophages towards an M1 phenotype. IFN $\gamma$  is secreted by various T cell subtypes, including natural killer cells, CD4+ and CD8+ cells, as part of the innate and adaptive immune response. T cell chemokines secreted from adipose tissue as a result of inflammation, including RANTES [33], IP-10 (CXCL10) and SDF-1, appear to be critical triggers for T cell recruitment although the metabolic or inflammatory adipose signals that initiate this process have yet to be defined.

T cell classifications are intricate, involving distinctions based on cell-surface markers and on secretory capabilities of various cell types. Generally, cells are divided into CD4+ or CD8+ categories, though some cells can express both surface markers. CD8+ cells are considered “effector” or cytotoxic T cells and tend to have pro-inflammatory activity. CD4+ lymphocytes can differentiate to either TH1 cells secreting classic inflammatory modulators IFN $\gamma$  and TNF $\alpha$ , or TH2 anti-inflammatory cells expressing IL-10 and IL-4. Thus, T cell populations and secreted factors may be integral to deciding macrophage activation states in adipose tissue [34]. Additional T cell types include the Foxp3+ anti-inflammatory regulatory T cells (Treg), systemic deficiency of which leads to severe immune-mediated disease, CD8+ natural killer cells, and CD4+ IL-17-secreting TH-17 cells.

Rocha et al. [34] identified a specific role for IFN $\gamma$  in diet-induced adipose inflammation, obesity and glucose intolerance. In their studies, visceral adipose tissue of diet-induced obese mice had higher CD4+ and CD8+ T cells than lean controls, while obese IFN $\gamma$  deficient mice had reduced expression of adipose inflammatory genes, decreased ATM and T cell accumulation, and improved glucose sensitivity. Recently, McGillicuddy et al. [36] showed that primary human adipocytes treated with IFN $\gamma$  also demonstrated decreased glucose uptake and downregulation of insulin signaling genes and proteins. In diet-induced obese mice, insulin resistance occurred at 5 weeks and was accompanied by a marked T cell infiltration in visceral adipose tissue, whereas macrophages were not detected until 10 weeks [18].

Further studies have explored phenotypic differences in adipose tissue lymphocytes with obesity and insulin resistance. Obese mice demonstrated a striking decrease in adipose tissue CD4+ Treg cells, coincident with insulin resistance [37]. Winer et al. [38] found that diet-induced obese Rag1-deficient mice, which have lymphocyte deficiency, developed more severe insulin resistance than control mice. Transfer of CD4+, but not CD8+, T cells normalized glucose tolerance, implying a protective role of this subtype. The attenuation of insulin resistance was dependent on anti-inflammatory TH2 factor IL-10, suggesting regulation of the activity of macrophages. High-fat diet fed mice were found to have increased numbers of CD8+ effector cells and decreased CD4+ helper and Treg cells in perigonadal

adipose tissue. These changes preceded macrophage infiltration and depletion or inhibition of action of the CD8+ T cells attenuated macrophage accumulation, adipose inflammation and systemic insulin resistance [39].

Studies to validate this pathophysiology in humans are limited. In type 2 diabetic patients, subcutaneous adipose contained T cells (mainly CD4+) and macrophages with markers suggesting IFN $\gamma$  activation; the T cell counts correlated positively with waist circumference [18]. Duffaut et al. demonstrated that T cell number by flow cytometry correlated positively with BMI and was greater in visceral than subcutaneous fat. They also found the T cell chemokine CCL20 to be upregulated with increasing BMI, and its receptor (CCR6) was present in adipose tissue lymphocytes [40].

Overall, these data suggest that modulation of T cells plays an important role in obesity-induced adipose tissue inflammation, macrophage recruitment and activation, and subsequent insulin resistance. Obesity is associated with a shift from predominantly anti-inflammatory TH2 and Treg lymphocytes to pro-inflammatory TH1 and CD8+ lymphocytes. Further work is clearly required to elucidate the physiology and determine its role in human obesity and insulin resistance. Data on the role of other types of T lymphocytes in adipose, including CD8+ natural killer cells and CD4+ IL-17-secreting TH-17 cells, are minimal. In human bone marrow mesenchymal stem cells, IL-17A was found to inhibit adipocyte differentiation and increase mRNA and protein secretion of IL-6 and IL-8 via COX-2 induction [41].

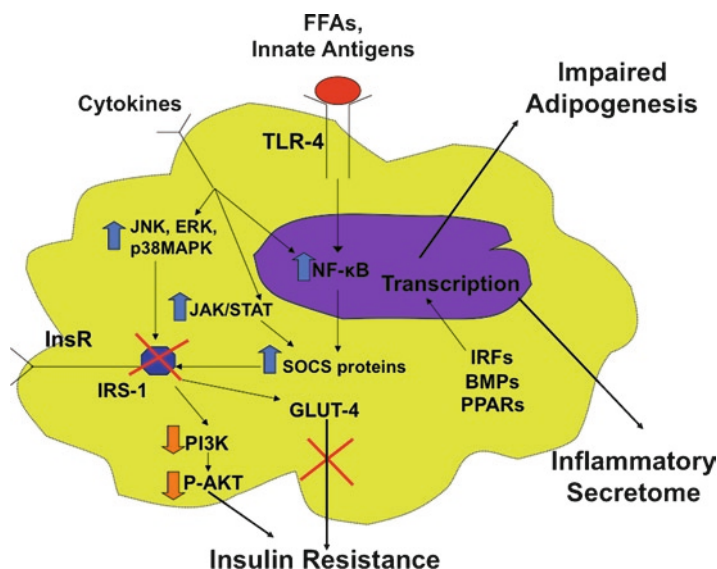
## **Adipose Inflammation Attenuates Insulin Signaling**

Adipose inflammation induces insulin resistance by direct and indirect effects on the insulin signaling pathway. The insulin receptor (InsR), a transmembrane dimeric protein with intrinsic tyrosine kinase activity, recruits insulin receptor substrate (IRS) proteins upon insulin binding. Tyrosine phosphorylation of IRS proteins activates phosphatidylinositol-3-kinase (PI3K) leading to v-akt murine thymoma viral oncogene (AKT) phosphorylation and glucose transporter 4 (GLUT-4) mobilization. Each of these steps can be targeted by adipose-derived inflammatory signals both locally in adipocytes and remotely across insulin-sensitive tissues. These processes are depicted in Fig. 2.

## **Endotoxemia and Adipose TLR Signaling**

Rodent models and in vitro studies have proven that activation of innate immunity, via the TLR4 receptor, induces adipose tissue inflammation coincident with local and systemic insulin resistance. TLR4 is the endogenous receptor for endotoxin that transduces downstream cytokine expression [42]. TLR4 can signal via the adaptor protein myeloid differentiation primary response gene 88 (MyD88) to





**Fig. 2** Effects of inflammation on insulin signaling and adipocyte function. Binding of free fatty acids (FFAs) and innate antigens to Toll-like receptors (TLRs) on adipocytes activates the transcription factor NFκB. This upregulates SOCS proteins that block tyrosine phosphorylation of IRS-1, attenuating PI3K and AKT phosphorylation and GLUT-4 translocation, ultimately resulting in insulin resistance. Cytokine binding to adipocyte receptors similarly effects InsR signaling, through (1) the NFκB pathway, (2) activation of kinases (JNK, ERK, p38MAPK) that serine phosphorylate IRS-1 and the InsR or (3) the JAK/STAT pathway that lead to upregulation of SOCS proteins and downregulation of adipocyte differentiation. Adipose inflammation modulates transcription factors (PPARs, IRFs, BMPs) that impair adipocyte differentiation, promoting a pro-inflammatory, insulin-resistant preadipocyte phenotype. These inflammatory adipocytes secrete cytokines, chemokines and adipokines (the “inflammatory secretome”), which further drives the escalating inflammation, systemic insulin resistance, endothelial dysfunction and target organ dysfunction. *FFA* free fatty acids, *NFκB* Nuclear factor kappa-light-chain-enhancer of activated B cells, *SOCS* suppressor of cytokine signaling, *IRS-1* insulin receptor substrate-1, *PI3K* phosphoinositide 3-kinase, *AKT* v-akt murine thymoma viral oncogene homolog 1, *GLUT-4* glucose transporter 4, *InsR* Insulin receptor, *JNK* c-Jun N-terminal kinase, *ERK* extracellular signal-regulated kinase, *p38MAPK* p38 mitogen activated protein kinase, *JAK/STAT* janus tyrosine kinases/signal transducers and activators of transcription, *PPAR* peroxisome proliferator-activated receptor, *IRF* interferon regulated factor, *BMP* bone morphogenetic protein

activate NFκB signaling or via a MyD88-independent pathway to induce interferon-regulatory genes. In adipocytes, activation of TLR4 induces NFκB target genes and decreases AKT and glycogen synthase kinase 3β (GSK3β) phosphorylation, the key mediators of insulin signaling and glucose uptake. Mice with loss-of-function mutations in TLR4 are protected against inflammatory changes, insulin resistance and obesity. Infusion or injection of LPS in mice resulted in increased whole-body and adipose tissue weight gain, insulin resistance and inflammatory adipokine gene expression [43]. Endotoxemia in a rat model also decreases tyrosine

phosphorylation of IRSs and decreases activation of PI3K. Similarly, human endotoxemia leads to downregulation of IRS-1 protein and mRNA [5].

## The Adipose Inflammatory Secretome

Cytokines and chemokines produced by adipocytes, leukocytes and stromal cells in adipose tissue lead to exacerbation of the adipose inflammatory state.

### *Cytokines*

TNF $\alpha$  was the first inflammatory factor discovered to be secreted from adipose tissue, and is now considered a primary contributor to adipose dysfunction in obesity. Both systemic and adipose tissue TNF $\alpha$  levels are upregulated in obesity and insulin resistance. This cytokine, acting through NF $\kappa$ B and JNK, upregulates in vivo and in vitro adipose expression and secretion of pro-inflammatory mediators while downregulating adiponectin. In adipocytes and skeletal muscle, TNF $\alpha$  inhibits tyrosine phosphorylation of IRS-1, while TNF $\alpha$  receptor deficiency protects against insulin resistance. In obese mice, neutralization of TNF $\alpha$  caused a significant increase in the peripheral insulin-stimulated glucose uptake whereas in humans, TNF $\alpha$  infusion decreases insulin sensitivity and increases phosphorylation of serine 312 on IRS-1 [43].

IL-6 is elevated in obesity and is increased in the portal circulation, thereby stimulating hepatic production of acute-phase reactants such as C-reactive protein. Human adipose is a major source of circulating IL-6 [44]. The IL-6 receptor belongs to the class I family of cytokine receptors, which uses Janus kinases (JAKs) as intracellular signaling pathways. In mice fed a high-fat diet, the increased production of IL-6 by adipose induced hepatic insulin resistance. This hepatic insulin resistance could be mediated, in part, by the increased expression of SOCS-3, a protein that binds and inhibits the insulin receptor and also targets IRS proteins for proteosomal degradation.

While in vitro work suggests that IL-1 plays a role in insulin resistance, in vivo studies have shown contradictory results. IL-1 $\beta$  treatment of 3T3-L1 cells led, via decreased IRS-1, to impaired GLUT4 expression and translocation and impaired insulin signaling [45]. Surprisingly, knockout of IL-1 receptor in mice led to obesity and insulin resistance [46] while loss of the IL-1 receptor antagonist (IL-1RA) actually led to weight loss and elevated metabolic rate [47]. In humans, association studies reveal elevated circulating levels and adipose tissue expression of IL-1 $\beta$  and IL-1RA in obesity [48]. As noted above, recent studies suggest that IFN $\gamma$  attenuates adipocyte differentiation and insulin signaling in vitro [36] and promotes diet-induced obesity in vivo. Substantial work remains, however, to define specific actions of diverse pro- and anti-inflammatory cytokines, generated systemically

and locally by macrophages, T cells, adipocytes and stromal cells, on insulin signaling in insulin-sensitive tissues.

## ***Chemokines***

Chemokines and their receptors are critical in the recruitment of monocyte/macrophage and T cells to adipose tissue. For example, MCP-1 (CCL2) is a major contributor of macrophage recruitment and adipose remodeling [49]. In mice, MCP-1 deficiency or inhibition leads to increased insulin sensitivity and decreased number of ATMs. In mice fed a high-fat diet, knockout of CCR2 (the MCP-1 receptor) had a similar phenotype with decreased macrophage infiltration of adipose tissue, increased insulin sensitivity, elevated adiponectin, and lower inflammatory cytokines [30]. In contrast, mice overexpressing adipose tissue MCP-1 had the opposite phenotype. Circulating and adipose tissue levels of MCP-1 are elevated in obesity.

Notably, many other CC and CXC chemokines are upregulated in adipose of obesity and have been implicated in recruitment of inflammatory T cell and monocyte recruitment [50]. Fewer data are available for other MCPs such as MCP-2, -3 and -4 (CCL-8, -7, and -13) but these also appear to be elevated in obese patients. RANTES (regulated on activation, normal T cell expressed and secreted) or CCL5 is a chemokine important in T cell chemotaxis that is elevated in the adipose tissue of obese mice [33] and humans. Levels of both CXCL8 (IL-8) and CXCL 10 (interferon  $\gamma$ -induced protein) are increased in human obesity [51].

## **Role of Intracellular Kinases**

TLR4 ligands and inflammatory cytokines activate diverse kinases that modulate adipose insulin signaling. NF $\kappa$ B drives transcription of inflammatory cytokines and contributes to insulin resistance in the setting of obesity and a high-fat diet. NF $\kappa$ B is normally attenuated by inhibitor of nuclear factor kappa B alpha (I $\kappa$ B $\alpha$ ) which traps NF $\kappa$ B in the cytosol but inflammatory stimuli activate inhibitor of kappa light polypeptide gene enhancer in B cells, kinase beta (I $\kappa$ K $\beta$ ) (a serine kinase) which phosphorylates and degrades I $\kappa$ B $\alpha$  allowing NF $\kappa$ B to enter the nucleus. I $\kappa$ K $\beta$  also directly interferes with insulin signaling by serine phosphorylation of IRS-1. Overexpression of I $\kappa$ K $\beta$  increases NF $\kappa$ B activity and decreases insulin signaling, whereas deficiency improves insulin sensitivity [5, 43].

The mitogen-activated protein kinase (MAPK) family is composed of JNK, p38 MAPK and extracellular signal-regulated kinase (ERK). JNK is activated through TNF $\alpha$  signaling, and leads to serine phosphorylation of IRS-1. ERKs also phosphorylate IRS-1 at serine residues, while p38 MAPK decreases expression of genes involved in insulin signaling, including GLUT-4 and phosphoinositide phosphatase. The MAPK family kinases are induced in visceral adipose during obesity, suggesting depot-specific

roles in insulin resistance. ERK1 knockout mice are resistant to high-fat diet induced obesity and insulin resistance, as well as decreased adipocytes and impaired adipogenesis, linking this protein to adipocyte differentiation, adiposity and adverse effects of nutrient overload [52]. ERK activity is also essential in the inflammatory effects of IL-1 $\beta$  [46]. Treatment of mice with an inhibitor of p38 prevented the loss of GLUT4 protein expression in insulin-resistant adipocytes without improving insulin receptor substrate 1 (IRS-1) protein levels or insulin signaling [53].

Other kinases, including protein kinase C, interleukin-1 receptor-associated kinase 1 (IRAK-1), and the Janus tyrosine kinases (JAK)/signal transducers and activators of transcription (STAT), serine phosphorylate IRS-1 and attenuate insulin signaling. In particular, the JAK/STAT pathway potently induces suppressor of cytokine signaling molecules 1 and 3 (SOCS 1 and 3), which have been implicated in TNF $\alpha$ -induced insulin resistance in adipocyte and hepatic insulin resistance in vitro and in vivo. The JAK/STAT pathway also plays a crucial role in transducing the effects of IFN $\gamma$ , a potent T cell secreted cytokine. Inflammatory T cells, which secrete IFN $\gamma$ , have been implicated in the earliest stages of diet- and obesity-induced adipose inflammation and insulin resistance. Notably, IFN $\gamma$  induced insulin resistance and dedifferentiation of human adipocytes likely via JAK1/STAT1 [36]. These recent data support an important role for inflammatory T cell secretion of IFN $\gamma$  with adipocyte JAK/STAT activation in adipose inflammation and insulin resistance. Mammalian target of rapamycin (mTOR) and its effector S6 Kinase 1 (S6K1) are also involved in the integration of nutrient signals and insulin signaling. Rapamycin, which inhibits mTOR, blocks TNF $\alpha$ -induced attenuation of IRS-1 tyrosine phosphorylation and also protects against IRS-1 proteosomal degradation. Additionally, mice deficient in S6K1 exhibit decreased obesity and insulin resistance in conditions of overnutrition [54].

## Suppressor of Cytokine Signaling Proteins

As noted, SOCS family proteins are induced by multiple cytokines and kinases. These proteins target cytokine tyrosine kinase receptor signaling in a negative feedback loop. SOCS proteins are elevated in insulin-resistant tissues and attenuate signaling via the insulin receptor, itself a tyrosine kinase, by binding directly to IRSs and blocking InsR-mediated tyrosine phosphorylation and further by promoting ubiquitination and degradation of IRSs. Concomitant with insulin resistance, SOCS proteins are upregulated in adipose tissue of humans after endotoxemia [5].

## Modulation of Transcription Factors

Beyond kinase-dependent modulation of insulin signaling pathway proteins, transcription factors that play critical roles in the regulation of adipose differentiation and insulin sensitivity are an important integrative target of multiple inflammatory

signals. PPAR- $\gamma$ , the master regulator of adipogenesis, is regulated by serine phosphorylation and is attenuated in insulin-resistant states and by activation of inflammatory pathways [55]. GATA2, another transcription factor regulated by serine phosphorylation, and FOXO1 inhibit adipogenesis, partially via inhibition of PPAR- $\gamma$  function, and may be modulated by stress and nutrient load. Recently, several additional transcription factor families that are regulated by inflammation, including bone morphogenic proteins (BMPs) and interferon regulatory factors (IRFs) have been identified as important regulators in adipose tissue. Various BMPs act at different stages of differentiation, in a dose-dependent manner to promote formation of adipocytes. In particular, BMP-4 and BMP-7 are essential for early commitment of progenitor cells, while BMP-2 acts on preadipocytes via enhancement of PPAR- $\gamma$  transcription and function [56]. Several of the IRFs, which are upregulated by IFNs and other inflammatory stimuli, bind to promoters of adipocyte genes and repress differentiation [57].

Overall, inflammatory stimuli modulate transcription factors and result in impaired adipogenesis of pre-adipocytes and de-differentiation of mature adipocytes. This results in adipose tissue dysfunction, including impaired lipid storage, reduced insulin signaling and increased secretion of adipocytokines. Specifically, adipocytes exposed to inflammatory stimuli, including TNF $\alpha$  and IFN $\gamma$ , demonstrate decreased levels of PPAR- $\gamma$ , adiponectin, lipoprotein lipase and fatty acid synthase as well as decreased lipid accumulation [36]. Given that immature adipocytes possess increased inflammatory capability, lack of appropriate differentiation results in further amplification of the pro-inflammatory state.

## Gene Expression Profiling and Proteomics

Genetic profiling of human and rodent adipose tissue has led to identification of many novel genes involved in obesity and insulin resistance. For example, microarray analysis of visceral fat from obese subjects compared to non-obese controls revealed over-representation of genes involved in lipid and glucose metabolism, membrane transport and cell cycle regulation [58]. Examination of the omental adipose in obese males showed that obesity was associated with upregulation of MAPKs and downregulation of lipolysis-inducing genes and growth factors [59].

Using microarray analysis of adipocytes cultured *ex vivo* from subcutaneous adipose of obese vs. lean Pima Indians, Nair et al. [60] identified 218 total modulated genes, generally revealing an upregulation of inflammatory genes in the preadipocyte stromal fraction of obese subjects. A recent study in which microarray analysis was performed on subcutaneous adipose tissue of healthy humans before and after endotoxemia revealed many modulated genes involved in adipose inflammation, macrophage and T cell activation and migration, and metabolic regulation, as well as a variety of novel factors. Validation studies determined that many of these genes were present and regulated by inflammation in adipocytes and macrophages, suggesting these cell types as primary source of inflammatory mediators in adipose [6].

Recent studies have explored the proteomics of adipose and adipocytes, focusing on depot-specific differences and progressive changes during differentiation [61, 62]. Together, these discovery-based studies serve as an important tool in elucidating the pathophysiology of obesity-related adipose tissue inflammation and metabolic dysregulation. Particularly in humans, they provide clinically relevant targets for mechanistic follow up as well as in prioritization of novel biomarkers and therapeutic opportunities.

## **Therapeutic Implications**

Implication of inflammation in the epidemic of human obesity and its complications has created an attractive target for therapeutic intervention. Numerous strategies are available for exploration, directed at either specific targets or systemic pathways.

## **Modulation of Systemic inflammation**

### ***Existing Therapies***

Weight loss, via lifestyle modifications or bariatric surgery, is the cornerstone of obesity management. Effects of the different methods of weight loss, and the quantity and duration of the loss, on inflammation can vary. Given the greater overall weight loss observed in bariatric surgery, this modality expectedly leads to greater improvement in the inflammatory milieu [63].

Various pharmacologic treatments are used for human insulin resistance, diabetes and metabolic syndrome, though the effects of these therapies on systemic and adipose tissue inflammation are not fully known. Several studies have examined the effect of PPAR- $\gamma$  agonists. In addition to its known effects on adipose tissue differentiation, a recent study in mice found that deficiency of PPAR- $\gamma$  in macrophages favored expression of M1 (vs. M2) macrophage markers and inflammatory changes in adipose tissue. In type 2 diabetics, rosiglitazone (but not Metformin) treatment decreased expression of many adipose tissue inflammatory genes [64]. Statins (HMG-CoA reductase inhibitors) are another drug with potential favorable effects on the inflammatory profile. In obese mice, treatment with pravastatin or pitavastatin led to attenuation of MCP-1 and IL-6 expression in adipose.

### ***Novel Strategies***

Specific cytokine blockade in systemic inflammatory disease presents a unique opportunity for exploring anti-inflammatory therapies in human metabolism and

disease. Treatment of rheumatoid arthritis patients with the anti-TNF $\alpha$  agent infliximab acutely reduced serum insulin levels and insulin/glucose index. Though this highlights an advantage of infliximab therapy in this patient population, the risks and benefits would need to be carefully examined before offering treatment for obesity-related complications in patients without inflammatory diseases. Similarly, IL-1 $\beta$  is being studied as a target of cytokine blockade in the treatment of obesity-related complications. Larsen et al. [65] found that type 2 diabetic patients treated with recombinant IL-1RA for 13 weeks had sustained improvement in glycemia and reduced circulating inflammatory markers compared to placebo. These effects, however, were at least partially due to improved beta-cell function.

Modulation of kinases involved in the inflammatory cascade presents an alternate approach to treatment. Salicylic acid, a compound long associated with anti-diabetic effects, functions as an I $\kappa$ B inhibitor. Early studies using high-dose aspirin in type 2 diabetics not only found favorable effects on glycemia, but also risk for serious side effects [66]. More recently, an open-label study in type 2 diabetics found that 1 month of treatment with salsalate (a prodrug of salicylic acid) improved glycemia, increased adiponectin and lowered FFA without safety concerns [67]. Fleischman et al. also noted that in obese humans, 1 month of salsalate improved glycemia, increased adiponectin and lowered C-reactive protein levels compared to placebo [68]. In addition, several inflammatory kinases including P38 MAPK, JNK and JAK-STATs represent tractable targets for therapeutic development particularly because small molecule inhibitors already exist or are in development.

The concept of metabolic endotoxemia, with changes in gut microbiota and circulating pathogen and non-pathogen TLR ligands in obesity, opens another interesting avenue of therapeutic potential. In obese mice, antibiotic treatment led to decreased LPS levels, altered gut flora, and reduction in systemic and adipose specific inflammation and insulin sensitivity. Probiotic treatments to increase favorable intestinal bacteria reduced high-fat-diet induced metabolic dysregulation in mice. Antibiotic treatment also reduced weight gain, insulin resistance and inflammatory adipokine gene expression in mice infused with LPS [69].

## Modulation of Leukocyte Infiltration in Adipose

Therapeutic approaches to prevent inflammatory macrophage and/or T cell infiltration into adipose tissue may have beneficial effects on obesity-linked inflammatory response and metabolic abnormalities. This has been shown to be effective, as targeted ablation of CD11c+ macrophages in obese mice markedly decreases local and systemic inflammatory markers and attenuates insulin resistance [70]. Blockade of the key macrophage chemoattractant MCP-1 and/or its receptor was initially considered an attractive target. In obese mice, deficiency or pharmacologic blockade of MCP-1 or its receptor reduced adipose tissue inflammatory markers and macrophage content and improved systemic insulin sensitivity [30]. However, other chemokines are also involved, and upregulation of one may in fact compensate for loss of another.



T cell immunomodulatory therapy may also provide promise. Because obesity and insulin resistance has been associated with increased IFN $\gamma$ -secreting TH1 cells and decreased FOXP3 $^{+}$  Treg cells in adipose tissue, reversal of these proportions could favorably affect metabolic pathways. In obese mice, brief immunotherapy with CD3-specific antibody reduces the predominance of TH1 cells over Foxp3 $^{+}$  cells, reversing insulin resistance for months, despite continuation of a high-fat diet [38].

## Summary

Inflammation is a crucial link between obesity and associated metabolic complications. Clearly, nutrient overload, via systemic or tissue-specific pathways, leads to activation of immune pathways that alter metabolism particularly in adipose. While the mechanisms involved are myriad and not fully elucidated, ongoing study is leading to the identification of novel targets for future diagnostic and therapeutic approaches to this epidemic problem.

**Acknowledgments** R.S. is supported by a K12 award from the University of Pennsylvania Clinical and Translational Science Award (UL1RR024134) from the National Center for Research Resources (NCRR). R.S. and M.P.R. receive support from the Diabetes and Endocrine Research Center (P30-DK 019525) at the University of Pennsylvania. M.P.R. is supported by RO1-DK071224 and P50 HL-083799-SCCOR from the National Institutes of Health.

## References

1. Buren, J. & Eriksson, J. W. (2005). Is insulin resistance caused by defects in insulin's target cells or by a stressed mind? *Diabetes/Metabolism Research and Reviews*, 21(6), 487–494.
2. Valsamakis, G., Anwar, A., Tomlinson, J. W., et al. (2004). 11 $\beta$ -hydroxysteroid dehydrogenase type 1 activity in lean and obese males with type 2 diabetes mellitus. *The Journal of Clinical Endocrinology and Metabolism*, 89(9), 4755–4761.
3. Kotelevtsev, Y., Holmes, M. C., Burchell, A., et al. (1997). 11 $\beta$ -hydroxysteroid dehydrogenase type 1 knockout mice show attenuated glucocorticoid-inducible responses and resist hyperglycemia on obesity or stress. *Proceedings of the National Academy of Sciences of the United States of America*, 94(26), 14924–14929.
4. Agwunobi, A. O., Reid, C., Maycock, P., Little, R. A., & Carlson, G. L. (2000). Insulin resistance and substrate utilization in human endotoxemia. *The Journal of Clinical Endocrinology and Metabolism*, 85(10), 3770–3778.
5. Mehta NN, McGillicuddy FC, Anderson PD, et al. Experimental Endotoxemia Induces Adipose Inflammation and Insulin Resistance in Humans. *Diabetes*. 2010 59(1):172–81.
6. Shah, R., Lu, Y., Hinkle, C. C., et al. (2009). Gene profiling of human adipose tissue during evoked inflammation in vivo. *Diabetes*, 58(10), 2211–2219.
7. Backhed, F., Manchester, J. K., Semenkovich, C. F., & Gordon, J. I. (2007). Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. *Proceedings of the National Academy of Sciences of the United States of America*, 104(3), 979–984.
8. Ghanim, H., Abuaysheh, S., Sia, C. L., et al. (2009). Increase in plasma endotoxin concentrations and the expression of Toll-like receptors and suppressor of cytokine signaling-3 in



- mononuclear cells after a high-fat, high-carbohydrate meal: implications for insulin resistance. *Diabetes Care*, 32(12), 2281–2287.
9. Song, M. J., Kim, K. H., Yoon, J. M., Kim, J. B. (2006). Activation of Toll-like receptor 4 is associated with insulin resistance in adipocytes. *Biochemical and Biophysical Research Communications*, 346(3), 739–745.
  10. Weisberg, S. P., McCann, D., Desai, M., Rosenbaum, M., Leibel, R. L., & Ferrante, A. W., Jr. (2003). Obesity is associated with macrophage accumulation in adipose tissue. *The Journal of Clinical Investigation*, 112(12), 1796–1808.
  11. Spalding, K. L., Arner, E., Westermark, P. O., et al. (2008). Dynamics of fat cell turnover in humans. *Nature*, 453(7196), 783–787.
  12. Larson-Meyer, D. E., Heilbronn, L. K., Redman, L. M., et al. (2006). Effect of calorie restriction with or without exercise on insulin sensitivity, beta-cell function, fat cell size, and ectopic lipid in overweight subjects. *Diabetes Care*, 29(6), 1337–1344.
  13. Yin, J., Gao, Z., He, Q., Zhou, D., Guo, Z., & Ye, J. (2009). Role of hypoxia in obesity-induced disorders of glucose and lipid metabolism in adipose tissue. *American Journal of Physiology. Endocrinology and Metabolism*, 296(2), E333–342.
  14. Hosogai, N., Fukuhara, A., Oshima, K., et al. (2007). Adipose tissue hypoxia in obesity and its impact on adipocytokine dysregulation. *Diabetes*, 56(4), 901–911.
  15. Cencello, R., Henegar, C., Viguier, N., et al. (2005). Reduction of macrophage infiltration and chemoattractant gene expression changes in white adipose tissue of morbidly obese subjects after surgery-induced weight loss. *Diabetes*, 54(8), 2277–2286.
  16. Bluher, M. (2009). Adipose tissue dysfunction in obesity. *Experimental and Clinical Endocrinology & Diabetes: Official Journal, German Society of Endocrinology [and] German Diabetes Association*, 117(6), 241–250.
  17. Shi, H., Kokoeva, M. V., Inouye, K., Tzameli, I., Yin, H., & Flier, J. S. (2006). TLR4 links innate immunity and fatty acid-induced insulin resistance. *The Journal of Clinical Investigation*, 116(11), 3015–3025.
  18. Kintscher, U., Hartge, M., Hess, K., et al. (2008). T-lymphocyte infiltration in visceral adipose tissue: a primary event in adipose tissue inflammation and the development of obesity-mediated insulin resistance. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 28(7), 1304–1310.
  19. Lumeng, C. N., Deyoung, S. M., Bodzin, J. L., & Saltiel, A. R. (2007). Increased inflammatory properties of adipose tissue macrophages recruited during diet-induced obesity. *Diabetes*, 56(1), 16–23.
  20. Verschuren, L., Kooistra, T., Bernhagen, J., et al. (2009). MIF deficiency reduces chronic inflammation in white adipose tissue and impairs the development of insulin resistance, glucose intolerance, and associated atherosclerotic disease. *Circulation Research*, 105(1), 99–107.
  21. Yeop Han C, Kargi AY, Omer M, et al. Differential effect of saturated and unsaturated free fatty acids on the generation of monocyte adhesion and chemotactic factors by adipocytes: dissociation of adipocyte hypertrophy from inflammation. *Diabetes*. 2010;59(2):386–96.
  22. Cinti, S., Mitchell, G., Barbatelli, G., et al. (2005). Adipocyte death defines macrophage localization and function in adipose tissue of obese mice and humans. *Journal of Lipid Research*, 46(11), 2347–2355.
  23. Permana, P. A., Menge, C., & Reaven, P. D. (2006). Macrophage-secreted factors induce adipocyte inflammation and insulin resistance. *Biochemical and Biophysical Research Communications*, 341(2), 507–514.
  24. Inouye, K. E., Shi, H., Howard, J. K., et al. (2007). Absence of CC chemokine ligand 2 does not limit obesity-associated infiltration of macrophages into adipose tissue. *Diabetes*, 56(9), 2242–2250.
  25. Ortega Martinez de Victoria, E., Xu, X., Koska, J., et al. (2009). Macrophage content in subcutaneous adipose tissue: associations with adiposity, age, inflammatory markers, and whole-body insulin action in healthy Pima Indians. *Diabetes*, 58(2), 385–393.
  26. Capel, F., Klimcakova, E., Viguier, N., et al. (2009). Macrophages and adipocytes in human obesity: adipose tissue gene expression and insulin sensitivity during calorie restriction and weight stabilization. *Diabetes*, 58(7), 1558–1567.

27. Mantovani, A., Sica, A., Sozzani, S., Allavena, P., Vecchi, A., & Locati, M. (2004). The chemokine system in diverse forms of macrophage activation and polarization. *Trends in Immunology*, 25(12), 677–686.
28. Lumeng, C. N., Bodzin, J. L., & Saltiel, A. R. (2007). Obesity induces a phenotypic switch in adipose tissue macrophage polarization. *The Journal of Clinical Investigation*, 117(1), 175–184.
29. Fujisaka, S., Usui, I., Bukhari, A., et al. (2009). Regulatory mechanisms for adipose tissue M1 and M2 macrophages in diet-induced obese mice. *Diabetes*, 58(11), 2574–2582.
30. Weisberg, S. P., Hunter, D., Huber, R., et al. (2006). CCR2 modulates inflammatory and metabolic effects of high-fat feeding. *The Journal of Clinical Investigation*, 116(1), 115–124.
31. Aron-Wisnewsky, J., Tordjman, J., Poitou, C., et al. (2009). Human adipose tissue macrophages: m1 and m2 cell surface markers in subcutaneous and omental depots and after weight loss. *The Journal of Clinical Endocrinology and Metabolism*, 94(11), 4619–4623.
32. Alexander, W. S. (2002). Suppressors of cytokine signalling (SOCS) in the immune system. *Nature Reviews Immunology*, 2(6), 410–416.
33. Wu, H., Ghosh, S., Perrard, X. D., et al. (2007). T-cell accumulation and regulated on activation, normal T cell expressed and secreted upregulation in adipose tissue in obesity. *Circulation*, 115(8), 1029–1038.
34. Rocha, V. Z., Folco, E. J., Sukhova, G., et al. (2008). Interferon-gamma, a Th1 cytokine, regulates fat inflammation: a role for adaptive immunity in obesity. *Circulation Research*, 103(5), 467–476.
35. Lumeng, C. N., Maillard, I., & Saltiel, A. R. (2009). T-ing up inflammation in fat. *Nature Medicine*, 15(8), 846–847.
36. McGillicuddy, F. C., Chiquoine, E. H., Hinkle, C. C., et al. (2009). Interferon gamma attenuates insulin signaling, lipid storage, and differentiation in human adipocytes via activation of the JAK/STAT pathway. *The Journal of Biological Chemistry*, 284(46), 31936–31944.
37. Feuerer, M., Herrero, L., Cipolletta, D., et al. (2009). Lean, but not obese, fat is enriched for a unique population of regulatory T cells that affect metabolic parameters. *Nature Medicine*, 15(8), 930–939.
38. Winer, S., Chan, Y., Paltser, G., et al. (2009). Normalization of obesity-associated insulin resistance through immunotherapy. *Nature Medicine*, 15(8), 921–929.
39. Nishimura, S., Manabe, I., Nagasaki, M., et al. (2009). CD8+ effector T cells contribute to macrophage recruitment and adipose tissue inflammation in obesity. *Nature Medicine*, 15(8), 914–920.
40. Duffaut, C., Zakaroff-Girard, A., Bourlier, V., et al. (2009). Interplay between human adipocytes and T lymphocytes in obesity: CCL20 as an adipochemokine and T lymphocytes as lipogenic modulators. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 29(10), 1608–1614.
41. Shin, J. H., Shin, D. W., & Noh, M. (2009). Interleukin-17A inhibits adipocyte differentiation in human mesenchymal stem cells and regulates pro-inflammatory responses in adipocytes. *Biochemical Pharmacology*, 77(12), 1835–1844.
42. Cani, P. D., Amar, J., Iglesias, M. A., et al. (2007). Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes*, 56(7), 1761–1772.
43. Shah, A., Mehta, N., & Reilly, M. P. (2008). Adipose inflammation, insulin resistance, and cardiovascular disease. *JPEN. Journal of Parenteral and Enteral Nutrition*, 32(6), 638–644.
44. Mohamed-Ali, V., Goodrick, S., Rawesh, A., et al. (1997). Subcutaneous adipose tissue releases interleukin-6, but not tumor necrosis factor-alpha, in vivo. *The Journal of Clinical Endocrinology and Metabolism*, 82(12), 4196–4200.
45. Jager, J., Gremeaux, T., Cormont, M., Le Marchand-Brustel, Y., Tanti, J. F. (2007). Interleukin-1beta-induced insulin resistance in adipocytes through down-regulation of insulin receptor substrate-1 expression. *Endocrinology*, 148(1), 241–251.
46. Garcia, M. C., Wernstedt, I., Berndtsson, A., et al. (2006). Mature-onset obesity in interleukin-1 receptor I knockout mice. *Diabetes*, 55(5), 1205–1213.
47. Somm, E., Henrichot, E., Pernin, A., et al. (2005). Decreased fat mass in interleukin-1 receptor antagonist-deficient mice: impact on adipogenesis, food intake, and energy expenditure. *Diabetes*, 54(12), 3503–3509.

48. Juge-Aubry, C. E., Somm, E., Giusti, V., et al. (2003). Adipose tissue is a major source of interleukin-1 receptor antagonist: upregulation in obesity and inflammation. *Diabetes*, 52(5), 1104–1110.
49. Dahlman, I., Kaaman, M., Olsson, T., et al. (2005). A unique role of monocyte chemoattractant protein 1 among chemokines in adipose tissue of obese subjects. *The Journal of Clinical Endocrinology and Metabolism*, 90(10), 5834–5840.
50. Sell, H., & Eckel, J. (2009). Chemotactic cytokines, obesity and type 2 diabetes: in vivo and in vitro evidence for a possible causal correlation? *The Proceedings of the Nutrition Society*, 68(4), 378–384.
51. Straczkowski, M., Dzienis-Straczowska, S., Stepień, A., Kowalska, I., Szelachowska, M., & Kinalska, I. (2002). Plasma interleukin-8 concentrations are increased in obese subjects and related to fat mass and tumor necrosis factor- $\alpha$  system. *The Journal of Clinical Endocrinology and Metabolism*, 87(10), 4602–4606.
52. Bost, F., Aouadi, M., Caron, L., et al. (2005). The extracellular signal-regulated kinase isoform ERK1 is specifically required for in vitro and in vivo adipogenesis. *Diabetes*, 54(2), 402–411.
53. Carlson, C. J., & Rondinone, C. M. (2005). Pharmacological inhibition of p38 MAP kinase results in improved glucose uptake in insulin-resistant 3T3-L1 adipocytes. *Metabolism: Clinical and Experimental*, 54(7), 895–901.
54. Um, S. H., Frigerio, F., Watanabe, M., et al. (2004). Absence of S6K1 protects against age- and diet-induced obesity while enhancing insulin sensitivity. *Nature*, 431(7005), 200–205.
55. Lehrke, M., & Lazar, M. A. (2005). The many faces of PPAR $\gamma$ . *Cell*, 123(6), 993–999.
56. Schulz, T. J., & Tseng, Y. H. (2009). Emerging role of bone morphogenetic proteins in adipogenesis and energy metabolism. *Cytokine & Growth Factor Reviews*, 20(5–6), 523–531.
57. Lefterova, M. I., & Lazar, M. A. (2009). New developments in adipogenesis. *Trends in Endocrinology and Metabolism: TEM*, 20(3), 107–114.
58. Baranova, A., Collantes, R., Gowder, S. J., et al. (2005). Obesity-related differential gene expression in the visceral adipose tissue. *Obesity Surgery: The Official Journal of the American Society for Bariatric Surgery and of the Obesity Surgery Society of Australia and New Zealand*, 15(6), 758–765.
59. Gomez-Ambrosi, J., Catalan, V., Diez-Caballero, A., et al. (2004). Gene expression profile of omental adipose tissue in human obesity. *The FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology*, 18(1), 215–217.
60. Nair, S., Lee, Y. H., Rousseau, E., et al. (2005). Increased expression of inflammation-related genes in cultured preadipocytes/stromal vascular cells from obese compared with non-obese Pima Indians. *Diabetologia*, 48(9), 1784–1788.
61. Alvarez-Llamas, G., Szalowska, E., de Vries, M. P., et al. (2007). Characterization of the human visceral adipose tissue secretome. *Molecular & Cellular Proteomics: MCP*, 6(4), 589–600.
62. Zvonic, S., Lefevre, M., Kilroy, G., et al. (2007). Secretome of primary cultures of human adipose-derived stem cells: modulation of serpins by adipogenesis. *Molecular & Cellular Proteomics: MCP*, 6(1), 18–28.
63. Forsythe, L. K., Wallace, J. M., & Livingstone, M. B. (2008). Obesity and inflammation: the effects of weight loss. *Nutrition Research Review*, 21(2), 117–133.
64. Kolak, M., Yki-Jarvinen, H., Kannisto, K., et al. (2007). Effects of chronic rosiglitazone therapy on gene expression in human adipose tissue in vivo in patients with type 2 diabetes. *The Journal of Clinical Endocrinology and Metabolism*, 92(2), 720–724.
65. Larsen, C. M., Faulenbach, M., Vaag, A., Ehses, J. A., Donath, M. Y., & Mandrup-Poulsen, T. (2009). Sustained effects of interleukin-1 receptor antagonist treatment in type 2 diabetes. *Diabetes Care*, 32(9), 1663–1668.
66. Hundal, R. S., Petersen, K. F., Mayerson, A. B., et al. (2002). Mechanism by which high-dose aspirin improves glucose metabolism in type 2 diabetes. *The Journal of Clinical Investigation*, 109(10), 1321–1326.
67. Goldfine, A. B., Silver, R., Aldhahi, W., et al. (2008). Use of salsalate to target inflammation in the treatment of insulin resistance and type 2 diabetes. *Clinical and Translational Science*, 1(1), 36–43.

68. Fleischman, A., Shoelson, S. E., Bernier, R., & Goldfine, A. B. (2008). Salsalate improves glycemia and inflammatory parameters in obese young adults. *Diabetes Care*, 31(2), 289–294.
69. Cani, P. D., Bibiloni, R., Knauf, C., et al. (2008). Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. *Diabetes*, 57(6), 1470–1481.
70. Patsouris, D., Li, P. P., Thapar, D., Chapman, J., Olefsky, J. M., & Neels, J. G. (2008). Ablation of CD11c-positive cells normalizes insulin sensitivity in obese insulin resistant animals. *Cell Metabolism*, 8(4), 301–309.



# Chapter 10

## Insulin Resistance in the Metabolic Syndrome

Sudha B. Biddinger and Brice Emanuelli

### Introduction

In 1988, Gerald Reaven coined the term “Syndrome X” to describe a complex of metabolic abnormalities, including glucose intolerance, hypertriglyceridemia and reduced levels of HDL-cholesterol, present in individuals at increased risk for cardiovascular disease [1]. Since then, attempts to quantify cardiovascular disease risk have led to the development of clinical criteria for the diagnosis of this syndrome, now known as the “metabolic syndrome” or “insulin resistance syndrome”. Although these criteria continue to evolve, those put forth by the National Cholesterol Education Program (NCEP), World Health Organization (WHO), European Group for the Study of Insulin Resistance (EGIR), International Diabetes Federation (IDF) and American Association of Clinical Endocrinologists (AACE), all include hyperglycemia, hypertriglyceridemia, low HDL-cholesterol and hypertension (reviewed in [2]) (Table 1). It is clear now that the metabolic syndrome is associated with many diseases in addition to cardiovascular disease. These include cholesterol gallstones, non-alcoholic fatty liver disease, which ranges from benign steatosis to non-alcoholic steatohepatitis (NASH), polycystic ovary disease (PCOS) and neurodegenerative disease.

The prevalence of the metabolic syndrome has risen at an alarming rate; more than one in three adults and increasing numbers of children now carry the diagnosis of the metabolic syndrome [3]. Despite the prevalence of metabolic syndrome, and the serious morbidity and mortality associated with it, the underlying pathophysiology of this disorder remains unclear. Dr. Reaven postulated in 1988 that insulin resistance plays a central role in the metabolic syndrome [1]. Since then, a great deal of data has shown a strong association between insulin resistance and the different components of the metabolic syndrome, but proving a causal role has been difficult [4].

---

S.B. Biddinger (✉)

Division of Endocrinology, Children’s Hospital Boston, MA, Boston, USA  
e-mail: sudha.biddinger@childrens.harvard.edu

**Table 1** Criteria for the diagnosis of the metabolic syndrome (NCEP:ATPIII, 2001).

Metabolic syndrome (three or more of the following)

Abdominal obesity

Men: waist circumference &gt;40 in.

Women: waist circumference &gt;35 in.

Fasting plasma glucose  $\geq 110$  mg/dlBlood pressure  $\geq 130/80$  mmHgTriglycerides  $\geq 140$  mg/dl

High-density lipoprotein cholesterol

Men &lt;40 mg/dl

Women &lt;35 mg/dl

Insulin resistance is only one of the multiple derangements in the hormonal and metabolic milieu which occur in the metabolic syndrome. Some of these derangements could be synergistic with insulin resistance whereas others could be antagonistic. Some changes are so intricately linked to insulin resistance—e.g., hyperglycemia is not only secondary to insulin resistance, but it also appears to exacerbate insulin resistance, resulting in a feed-forward cycle—that it is difficult to study one in isolation of the other. Finally, some of the genetic and dietary factors that induce the metabolic syndrome, like excess dietary fat, could not only act by promoting insulin resistance, but could also act independent of insulin resistance to alter metabolism.

Nonetheless, defining the role of insulin resistance is a fundamental problem with important clinical implications. If insulin resistance does not play a pathogenic role in the metabolic syndrome, one should identify and treat the individual components of the metabolic syndrome, as has been advocated by some experts [4]. For example, dyslipidemia and hypertension should be identified and treated before they progress to cardiovascular disease. If insulin resistance is the central driver of this disorder, we should identify and treat insulin resistance itself, potentially even before the development of dyslipidemia and hypertension, let alone cardiovascular disease.

Over the past 20 years, we have learned a great deal about the mechanisms of insulin signaling. To comprehend how these findings shape our understanding of insulin resistance in the metabolic syndrome, we will review the better known components of the insulin signaling pathway, how defects in the insulin signaling pathway could contribute to the metabolic syndrome phenotype and how such defects arise.

## Clinical Versus molecular definitions of insulin resistance

Clinically, insulin resistance is defined as the failure of insulin to maintain normal serum glucose levels. Thus, the hyperinsulinemic euglycemic clamp is the gold standard for the measurement of insulin resistance, and surrogate measurements involving serum insulin and glucose levels, like the homeostatic model assessment

(HOMA), are also based on glucose metabolism. Such definitions of insulin resistance, however, fail to acknowledge the underlying complexities of insulin signaling in two respects. First, insulin regulates many processes within the cell. It increases glucose uptake, promotes glycogen synthesis and suppresses hepatic glucose production. However, it also stimulates lipogenesis and triglyceride secretion, increases salt and water retention, and regulates many other processes including bile acid metabolism, growth and differentiation. The clinical definitions of insulin resistance imply that all of these processes become resistant in parallel with glucose metabolism, though this is unlikely to be true.

In fact, Dr. Reaven postulated that some pathways remain sensitive to insulin in the metabolic syndrome, while the pathways by which insulin stimulates glucose uptake, particularly by the muscle and fat, become resistant [1]. The resulting hyperglycemia stimulates insulin secretion from the pancreatic  $\beta$ -cell, leading to hyperinsulinemia, which then over-stimulates those pathways that are still sensitive to insulin. For example, hyperinsulinemia triggers excessive lipogenesis and triglyceride secretion, resulting in hypertriglyceridemia and hepatic steatosis, salt and water retention which produces hypertension, and excessive androgen synthesis, resulting in PCOS [5].

The definitions of insulin resistance based on disturbances in glucose homeostasis fail to recognize the intrinsic complexities of the insulin signaling cascade. The insulin signaling pathway (described below) consists of multiple nodes, with many nodes represented by multiple isoforms with seemingly redundant capabilities. Clinical definitions of glucose intolerance suggest that insulin resistance is a homogenous phenomenon, which can only vary quantitatively. In contrast, the presence of so many signaling components suggests that insulin resistance could be a heterogeneous phenomenon, i.e., the phenotype produced by insulin resistance could vary depending on the components affected.

Therefore, it is also useful to consider the concept of molecular insulin resistance, defined as specific defects in one or more components of the insulin signaling pathway. Molecular insulin resistance could be present even in the absence of abnormalities in glucose homeostasis, and conversely, it is possible that multiple forms of molecular insulin resistance could produce abnormal glucose homeostasis. Molecular insulin resistance is not a concept that can, at present, be used in the clinical setting, but our hope is that it will clarify our understanding of the metabolic syndrome, and provide insights into its therapy.

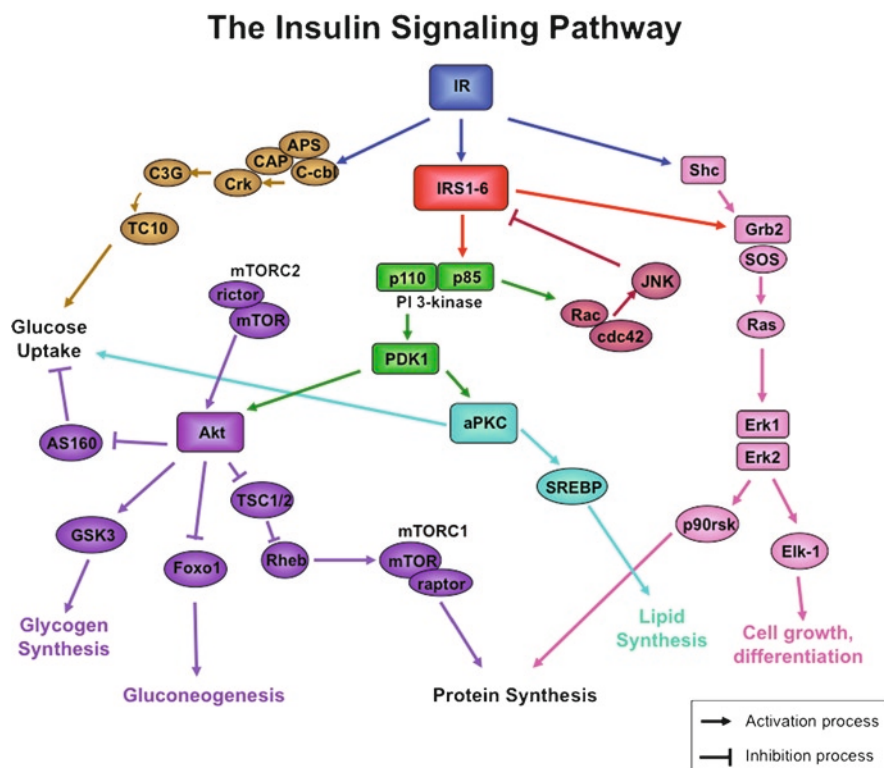
## Insulin Signaling Pathway

Insulin elicits a complex cascade of signaling events, involving multiple nodes. Although we shall present the insulin signaling pathway as a linear chain, it is important to acknowledge the great deal of complexity underlying each node. At most nodes, there are numerous isoforms, which are theoretically capable of responding to and generating subtly different signals. In addition, there is crosstalk



among the different components of the cascade. Here, we will review some of the better studied nodes of the insulin signaling pathway (Fig. 1).

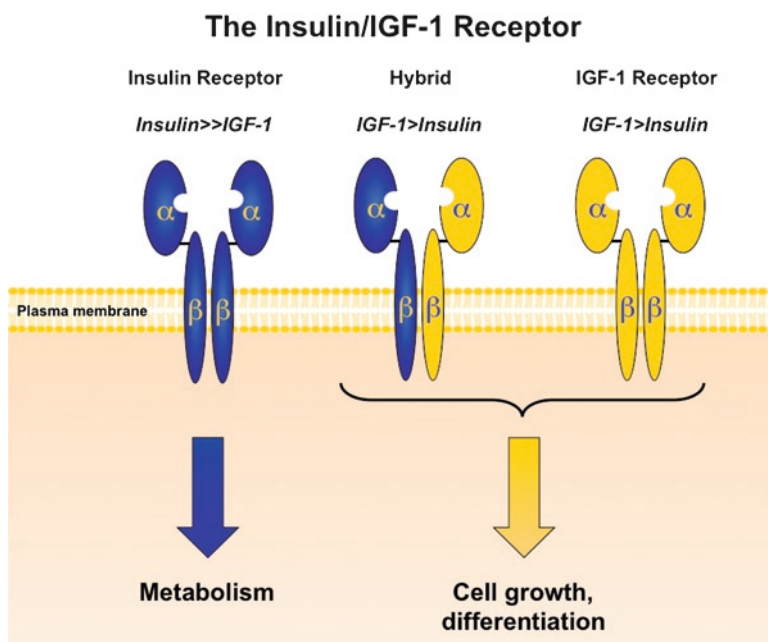
**Insulin Receptor.** The insulin receptor binds insulin and triggers a complex cascade of signaling events. The insulin receptor gene encodes a single chain precursor protein which is post-translationally processed into an  $\alpha$ -subunit and a  $\beta$ -subunit, which are covalently linked by disulfide bonds. The  $\alpha$ -subunit is extra-cellular and binds insulin, whereas the  $\beta$ -subunit is intracellular and contains a tyrosine kinase domain. The  $\alpha/\beta$  subunit complexes dimerize with one another to form the insulin receptor. Upon binding insulin, the  $\beta$ -subunits phosphorylate one another and their intracellular substrates.



**Fig. 1** The insulin signaling network. Upon binding insulin, the insulin receptor (IR) activates the IRS proteins and initiates a complex cascade of signaling events. Here, we show the major branches of this signaling network. Many of the effects of insulin are mediated by PI 3-kinase, which activates Akt, the atypical PKCs (aPKCs) and the stress kinase JNK. Akt in particular mediates many of insulin's metabolic effects by regulating gluconeogenesis, glycogen synthesis and protein synthesis, whereas the aPKCs activate lipid synthesis. In contrast, the proliferative effects of insulin are largely mediated by ERK1 and ERK2, independent of PI 3-kinase. Finally, insulin promotes glucose uptake through Akt, the aPKCs, and the CAP/Cbl complex. Plain arrows indicate stimulation and blocked arrows indicate inhibition

At this signaling node, heterogeneity is generated by alternative splicing and cross-talk with the insulin-like growth factor (IGF)-1 signaling pathway. Alternative splicing of exon 11 yields two isoforms,  $IR_A$  which lacks the 12 amino acids encoded by this exon and  $IR_B$  which contains them. The 12 amino acids encoded by exon 11 are contained in the  $\alpha$ -subunit and increase the affinity of the receptor for the related growth factor, IGF-2 [6]. In addition, the two isoforms activate different downstream events under certain conditions [7].

The insulin receptor is a member of a subfamily of receptor tyrosine kinases which also includes the IGF-1 receptor and the insulin receptor related receptor (IRR). The  $\alpha/\beta$  subunit complex encoded by the IGF-1 receptor gene not only dimerizes with itself to form the IGF receptor, but also dimerizes with the  $\alpha/\beta$  subunit complex of the insulin receptor to generate hybrid receptors. The insulin receptor, IGF receptor and hybrid receptor all bind insulin and IGF-1 with varying affinities (Fig. 2). Although there is a great deal of overlap between insulin and IGF signaling, insulin tends to regulate metabolism whereas IGF tends to regulate growth and proliferation. Hyperinsulinemia in the metabolic syndrome could



**Fig. 2** The insulin /IGF-1 receptor. Both the insulin receptor and the IGF receptor are encoded by single genes which are processed into an  $\alpha$ -chain and  $\beta$ -chain that remain linked by disulfide bonds. These  $\alpha/\beta$  complexes can either homodimerize to form insulin receptors or IGF receptors, or heterodimerize to form hybrid receptors. Insulin binds preferentially to the insulin receptor whereas IGF-1 binds preferentially to the IGF-1 and hybrid receptors. Although there is a great deal of overlap in their function, the insulin receptor is more closely linked with metabolic effects whereas the hybrid receptor and IGF receptor are more closely linked with proliferation

potentially lead to the activation of the hybrid receptor or IGF receptor, driving cell growth and proliferation. This has been implicated in the pathogenesis of acanthosis nigricans, the thickening of the skin, particularly in the neck and axilla, present in insulin resistant individuals, the increased risk of cancer in patients with the metabolic syndrome, pseudoacromegaly and PCOS. However, other factors, including changes in the IGF binding proteins which alter IGF activity, could also play a role in these processes.

***Insulin Receptor Substrate Proteins.*** Upon binding insulin, the insulin receptor phosphorylates and activates its numerous substrates. At least 11 substrates of the insulin receptor kinase have been identified, with the most prominent being the 6 members of the Insulin Receptor Substrate (IRS 1-6) family of proteins [8]. The IRS proteins share a similar structure: the N-terminal region contains a pleckstrin homology (PH) domain which mediates protein–lipid and protein–protein interactions and a phosphotyrosine binding (PTB) domain; the remainder of the molecule contains numerous tyrosine, serine and threonine residues which could potentially undergo phosphorylation. Phosphorylation of the IRS proteins on tyrosine residues activates these proteins, enabling them to recruit and activate their downstream targets [8]. In contrast, phosphorylation of the IRS proteins on serine residues appears to impair insulin signaling as discussed below.

The reason for the existence of so many IRS proteins is still unclear. The fact that IRS-4 is expressed primarily in embryonic tissues or cell lines, suggests that it may be important in producing tissue-specific responses to insulin [8]. IRS-1 and IRS-2, on the other hand, are widely distributed and though largely redundant, may have subtle distinctions in their functions. For example, IRS-1 may play the more important role in mediating insulin-stimulated glucose uptake in muscle [8] whereas IRS-2 may be more important in mediating glucose transport in brown adipocytes [9] and maintaining  $\beta$ -cell mass; [8] in the liver, IRS-1 and IRS-2 may play different roles in the regulation of glucose versus lipid metabolism [8].

The subtle differences in IRS-1 and IRS-2 function could be due to structural differences between the proteins. For example, IRS-2, but not IRS-1, possesses a Kinase Regulatory Loop Binding (KRLB) domain that appears to impair its ability to be tyrosine phosphorylated in response to insulin; [8, 10] the absence of this domain could contribute to preferential signaling through IRS-1 versus IRS-2. Alternatively, the fact that IRS-1 is associated with the low-density microsome (LDM) fraction, whereas IRS-2 is found in both the cytosol and LDM fraction, suggests that the two proteins differ in their subcellular distribution [8]. IRS-1 and IRS-2 expression levels could vary independently of one another, as they appear to be regulated by different mechanisms. For example, prolonged exposure of hepatocytes to insulin leads to a decrease in IRS-2 but not IRS-1 [11]. Consistent with this, hepatic IRS-2 expression is highest in the fasted state [12]. Ultimately, these differences in structure, subcellular localization and expression could yield differences in the downstream signals produced. For example, the Abl tyrosine kinase and the phosphatase SHP2 binds to IRS1, but not IRS-2, while the proteins Grb2, Crk and phospholipase C $\gamma$  bind to IRS1 with a greater affinity than IRS2 [8]. Similarly, IRS-1 binds to 14-3-3 $\epsilon$  protein and PKC $\alpha$ , leading to the formation of a complex that modulates insulin signaling in fibroblasts [13].

Additional targets of the insulin receptor tyrosine kinase include Src-homology-2 containing protein (Shc) which ultimately promotes proliferation; Cas-Br-M (murine) ectopic retroviral transforming sequence homologue (Cbl) which initiates glucose uptake;  $\beta$ -arrestin-2, a member of the  $\beta$ -arrestin family of adaptor proteins originally discovered as desensitizers of G-protein coupled receptors, which complexes Akt to the insulin receptor; [14] and Grb-2 associated binder 1 (Gab1) [8]. Gab1, in contrast to the others, appears to be a negative regulator of insulin signaling since knockout of Gab1 in the liver improves insulin sensitivity and signaling through IRS-1 and IRS-2 [15].

**PI 3-Kinase.** The class Ia phosphatidylinositol 3-kinase (PI 3-kinase) is a lipid kinase which plays a central role in insulin signaling (reviewed in [16]). Active PI 3-kinase phosphorylates phosphatidylinositol-4,5-bisphosphate ( $\text{PIP}_2$ ) to generate phosphatidylinositol-3,4,5-triphosphate ( $\text{PIP}_3$ ). The PI 3-phosphates bind the pleckstrin homology domains of other signaling molecules, activating them or altering their subcellular location. PI 3-kinase thereby activates PDK1 (PI dependent kinase 1), which is in turn able to activate Akt and the atypical forms of protein kinase C, which are critical mediators of insulin action.

PI 3-kinase consists of a catalytic subunit, of which there are three isoforms, and a regulatory subunit, of which there are eight isoforms. As in the case of the IRS proteins, the roles of these different isoforms are not clear. Knockout of the catalytic subunits produces glucose intolerance [17]. However, partial reductions in the regulatory subunits, for example by deleting a single isoform such as  $\text{p85}\alpha$  alone, surprisingly improve glucose tolerance [18]. Thus, either the stoichiometry of the regulatory to catalytic subunits is important or the regulatory subunits have some negative role in insulin signaling.

The importance of PI 3-kinase in insulin signaling is highlighted by studies showing that virtually all of insulin's metabolic effects, including glucose transport, lipogenesis and glycogenesis, are abolished by either inhibitors or dominant negative mutants of PI 3-kinase [19, 20].

**MAP Kinases.** The main members of the mitogen activated protein (MAP) kinase family involved in insulin signaling are the extracellular signal-regulated kinases, ERK1 and ERK2, and the stress kinase c-Jun NH2-terminal kinase (JNK). These proteins have different roles in both propagating and terminating the insulin signal. Insulin stimulates the binding of a complex containing the Src homology 2 (SH2)-containing adaptor protein Grb2 and the guanyl nucleotide exchange factor SOS to phosphotyrosines on the IRS proteins, Shc and Gab-1. This binding triggers the sequential activation of the small GTPase Ras, the kinase Raf, the dual specificity kinases MAPK/Erk Kinase (MEK)-1 and -2, and, ultimately ERK1 and ERK2. Activated ERK1 and ERK2 phosphorylate p90 ribosomal protein S6 kinase (p90RSK), transcription factors such as Elk1 and other targets [8].

ERK1 and ERK2 are mainly involved in mediating cell growth, survival and differentiation. Thus, pharmacological inhibitors and dominant negative mutants of these proteins inhibit the stimulation of cell growth by insulin, but do not alter insulin's metabolic or anabolic effects [16]. Although ERK1 and ERK2 have similar functions, ERK1, but not ERK2, has been shown to be required for adipogenesis both in vitro and in vivo [8].

Three JNK-coding genes (JNK1-3) have been described in mammals, each with multiple splice variants. JNK1 and JNK2 are expressed ubiquitously, whereas JNK3 expression is restricted to neuronal tissues [8]. Insulin has been shown to activate JNK1 and -2 in various cellular systems by a mechanism that may involve Rac and cdc42 [9, 21]. JNK takes part in a negative feedback loop of insulin action by phosphorylating IRS1 on serine residues, impairing the ability of IRS-1 to be activated by insulin. The fact that genetic deletion or downregulation by siRNA, expression of dominant negative mutant or endogenous inhibitory proteins of JNK, especially JNK1, improves insulin sensitivity and many metabolic functions in obese mice suggests that this may be the primary role of JNK in insulin signaling [22]. ERK1 and ERK2 may also have negative roles in insulin signaling [8].

**The CAP/Cbl Pathway.** Insulin also appears to be able to induce glucose transport independent of PI 3-kinase, by assembling signaling platforms that emanate from lipid rafts. This process is initiated by phosphorylation of the proto-oncogene c-cbl and the formation of a multiprotein complex at the plasma membrane, composed of c-cbl, c-Cbl associated protein (CAP) and the adaptor protein APS. CAP contains a sorbin homology domain which appears to bind the scaffolding protein flotillin, and localize the complex to the lipid raft. There, tyrosine-phosphorylated c-cbl is able to recruit CrkII, via its SH2 domain, and activate the guanyl nucleotide exchange protein C3G, which in turn activates the G-protein TC10. Once activated, TC10 promotes the formation of new signaling complexes that inhibit the rab31 GTPase [8]. Together, these events appear to facilitate the translocation, docking and fusion of the glucose transporter Glut4 at the plasma membrane.

**Akt.** In response to insulin, PI 3-kinase activates the serine/threonine kinase Akt (also known as protein kinase B, or PKB). There are three isoforms of Akt: Akt1 is ubiquitously expressed; Akt 2 is expressed predominantly in insulin-sensitive tissues, such as liver, fat and muscle; and Akt3 is expressed primarily in the brain. PI 3-kinase activates Akt through several mechanisms [20, 23]. First, the generation of PI 3-phosphates, particularly  $PIP_3$ , activates PDK1.  $PIP_2$  and  $PIP_3$  also recruit Akt to the plasma membrane through the pleckstrin homology domain of Akt, thus bringing it into proximity with its kinase PDK1. Additionally, binding of  $PIP_3$  to the pleckstrin homology domain of Akt induces a conformational change that allows Thr 308 to be phosphorylated by PDK1. Akt activation also requires phosphorylation on Ser473 by mTORC2, a protein complex which includes the protein kinase mTOR (mammalian target of rapamycin) and the regulatory protein, rictor (rapamycin-insensitive companion of mTOR) [24]. Phosphorylation of Thr308 and Ser473 results in Akt activation. Akt plays a key role in mediating the effects of insulin on glucose transport, protein synthesis, glycogen synthesis and gene expression as described below.

**Atypical PKCs.** PI 3-kinase also activates the atypical PKCs, PKC $\zeta$  (zeta) and PKC $\lambda$  (lambda)/ $\iota$  (iota) PKC $\lambda$  is the mouse ortholog of PKC $\iota$ , which is present in humans. The atypical PKCs (aPKCs) differ from the conventional PKCs [ $\alpha$  (alpha),  $\beta$  (beta)I,  $\beta$  (beta)II,  $\gamma$  (gamma)] and the novel PKCs [ $\delta$  (delta),  $\epsilon$  (epsilon),  $\eta$  (eta),  $\theta$  (theta),  $\mu$  (mu)] in that they do not require diacylglycerol (DAG) for activation. More importantly, conventional and novel PKCs appear to be negative regulators of

insulin signaling, whereas the atypical PKCs are important mediators of insulin action. In muscle and fat, the aPKCs stimulate glucose transport in response to insulin. Overexpression of PKC $\zeta$  or PKC $\lambda$  results in increased translocation of the insulin-sensitive glucose transporter GLUT4 to the plasma membrane [25, 26]. Conversely, dominant negative mutants of PKC $\lambda$  inhibit insulin-stimulated glucose uptake [27]. In the liver, however, the aPKCs appear to stimulate lipogenesis.

**mTOR.** Another important downstream target of insulin is the mTORC1 complex [8]. mTORC1, like mTORC2, contains the protein kinase mTOR. However, the two complexes are functionally distinct, and mTORC1 complex contains the regulatory protein raptor (regulatory associated protein of TOR), instead of rictor. Akt activates mTORC1 by phosphorylating and inhibiting tuberlin, or tuberous sclerosis complex-2 (TSC2), which is in a complex with hamartin, or TSC1 [28]. The TSC1/TSC2 complex inhibits the GTPase Ras homologue enriched in brain (Rheb) [29]. Rheb is an activator of mTORC1. Thus, activation of Akt by insulin results in the dis-inhibition of Rheb and the activation of mTORC1. mTORC1 promotes protein synthesis by phosphorylating eukaryotic translation initiation factor 4E binding protein 1 (4EBP1). 4EBP1 inhibits translation by binding eukaryotic translation initiation factor 4E (eIF4E), a limiting component of the translation initiation complex. Phosphorylation of 4EBP1 allows eIF4E to dissociate, and thereby increases translation. In addition, mTORC1 phosphorylates and activates p70 ribosomal S6 kinase (S6K), which increases ribosome biosynthesis.

**GSK3.** In the liver, insulin is a key signal to promote glycogen synthesis. There are two isoforms of glycogen synthase kinase 3 (GSK3), GSK3 $\alpha$  and GSK3 $\beta$ , encoded by two different genes. GSK3 $\alpha$  and GSK3 $\beta$  phosphorylate and inhibit glycogen synthase, the enzyme catalyzing the final step in glycogen synthesis. Akt inactivates GSK3 $\alpha$  and GSK3 $\beta$  by phosphorylating them on Ser 21 and Ser9, respectively [30]. Thus, insulin activates Akt, which inactivates GSK3, and derepresses glycogen synthase, leading to a stimulation of glycogen synthesis. Mutation of the Akt phosphorylation sites of GSK3—i.e., mutation of serine 21 to alanine in GSK3 $\alpha$  (S21A) and mutation of serine 9 to alanine in GSK3 $\beta$  (S9A)—renders it insensitive to insulin. Studies of mice with knockin of the S21A mutation in GSK3 $\alpha$  or the S9A mutation in GSK3 $\beta$  show that GSK3 $\beta$  is more important in the regulation of muscle glycogen synthase by insulin [31]. Consistent with this, mice with muscle-specific knockout of GSK3 $\beta$  show improved glucose tolerance, due to enhanced stimulation of glycogen synthase by insulin [32]. GSK3 $\alpha$  may have a more important role in the liver, as mice with whole body knockout of GSK3 $\alpha$  have improved whole-body glucose tolerance and hepatic insulin sensitivity, but mice with liver-specific knockout of GSK3 $\beta$  show no change in glucose or insulin tolerance or glycogen content [32, 33].

**AS160.** Akt substrate of 160 kDa (AS160) is phosphorylated by Akt. AS160 contains an intrinsic rab GTPase activating domain. In its GDP bound form, AS160 is inactive; phosphorylation by Akt inhibits its GTPase activity, allowing a switch to the GTP bound form, which promotes translocation of GLUT 4-containing vesicles to the cell surface, thereby increasing glucose uptake [34].



**FoxO1.** Insulin exerts many of its effects at the transcriptional level. Of the many transcription factors and coactivators involved, FoxO1 and SREBP-1c are among the most well studied. Akt inactivates FoxO1 by phosphorylating it on residues Thr-24, Ser-256 and Ser-319 though other kinases have been implicated [35]. Phosphorylated FoxO1 is excluded from the nucleus and targeted for degradation. Insulin may also regulate FoxO1 by acetylation [35] and modulation of its transcriptional co-activator, peroxisome proliferator-activated receptor gamma, coactivator 1 alpha (PGC)-1 $\alpha$ .<sup>36</sup> In the absence of insulin, FoxO1 activates transcription both directly, by binding to insulin response elements (IREs) in the promoters of its target genes [37], and indirectly by co-activating other transcription factors [38]. FoxO1 induces the gluconeogenic enzymes, glucose-6-phosphatase (*G6pc*) and phosphoenolpyruvate carboxykinase (*Pck1*). Therefore, increased expression of FoxO1 leads to increased fasting glucose and impaired glucose tolerance [39] whereas knockdown of FoxO1 decreases gluconeogenic gene expression and decreases serum glucose levels[40].

Although the effects of FoxO1 on glucose metabolism are its most prominent effects in insulin signaling, FoxO1 has many other important effects in the cell. For example, FoxO1 regulates triglyceride metabolism by inducing microsomal triglyceride transfer protein (*Mttp*), which promotes the lipidation of apolipoprotein B (ApoB), a rate-determining step in VLDL secretion; [41] FoxO1 induces ApoCIII, an apolipoprotein which inhibits lipoprotein lipase activity and promotes hypertriglyceridemia; [42] FoxO1 inhibits lipogenic gene expression [39]. In addition, FoxO1 promotes the expression of the cholesterol efflux transporters, *Abcg5* and *Abcg8* [43], which stimulates cholesterol efflux into the bile, [44] and plays an important role in the regulation of bile acid metabolism, [45] cell growth and differentiation, protection from reactive oxygen species and may even modulate insulin sensitivity (reviewed in [46]).

Insulin also regulates the related protein FoxA2. Akt phosphorylates Foxa2 on Thr156, preventing its nuclear localization and thereby inactivating FoxA2. FoxA2 promotes the transcription of the enzymes of fatty acid oxidation, *Mttp*, and gluconeogenic genes [47, 48]. FoxA2 also regulates the bile acid transporters, and the knockout of FoxA2 in the liver leads to intrahepatic cholestasis [49].

**SREBP-1c.** The sterol regulatory element-binding proteins (SREBPs) are a family of three nuclear transcription factors encoded by two genes [50]. SREBP-1a and SREBP-1c are derived from the same gene, and both appear to regulate lipogenic gene transcription. SREBP-1c, however, is the dominant isoform in liver and adipose. SREBP-1c is capable of activating the entire program of monounsaturated fatty acid synthesis. Mice expressing a constitutively active isoform of SREBP-1c have an increase in lipogenic gene expression and hepatic triglyceride content [51]. In addition, SREBP-1c inhibits transcription of IRS-2 [52] and the gluconeogenic genes [53], potentially contributing to changes in glucose metabolism as well.

The SREBPs are subject to complex regulation at the transcriptional and post-translational levels [50]. Their transcripts encode membrane bound precursors, which are retained in the endoplasmic reticulum by Insig proteins. Sterol depletion causes dissociation of the Insig proteins, allowing the SREBPs to proceed to the

golgi, where the Site 1 and Site 2 proteases reside. These proteases release a soluble fragment of SREBP that can translocate into the nucleus to activate transcription.

Several lines of evidence suggest that SREBP-1c is regulated by insulin. First, SREBP-1c transcript and nuclear protein are increased by insulin treatment in hepatocytes [54]. Second, streptozotocin treatment, which renders mice insulin deficient, results in a decrease in SREBP-1c [54]. Similarly, fasting, which also lowers insulin levels, decreases SREBP-1c [55]. Conversely, refeeding induces an exaggerated insulin response which is accompanied by an increase in SREBP-1c [55]. Knockout of SREBP-1c impairs the lipogenic response to insulin in the context of re-feeding [56].

The mechanisms by which insulin induces SREBP-1c are not clear. Insulin appears to induce transcription of SREBP-1c via the nuclear hormone receptor, Liver X Receptor (LXR), as knockout of LXR prevents insulin from inducing SREBP-1c and its targets [57]. Insulin has been reported to increase the stability of LXR mRNA, [58] and has been suggested to induce the oxysterol ligand of LXR. Insulin also acts post-transcriptionally to induce the processing of SREBP-1c to its active nuclear form by suppressing expression of Insig2a [59]. As Insig levels fall, SREBP-1c is no longer retained in the endoplasmic reticulum. In addition, SREBP-1c may undergo other modifications which regulate its activity, including phosphorylation and ubiquitination. The insulin signaling components which mediate insulin's effects on SREBP-1c are not clear, and PI 3-kinase, PKC- $\lambda$ , GSK-3 $\beta$  and MAPK have been implicated.

It should also be noted that SREBP-1c is under complex control, and insulin is not the only, or even the dominant, regulator of SREBP-1c. Thus, dietary factors, such as carbohydrates and polyunsaturated fatty acids and hormonal factors, such as leptin, also regulate SREBP-1c [60]. It is therefore possible that these other pathways activate SREBP-1c independent of insulin signaling in the metabolic syndrome.

## **Metabolic Effects of Insulin Resistance: Lessons from Knockout Mice**

The existence of so many redundant components and branches of the insulin signaling cascade suggests that, (a) a lesion in a given node in the insulin signaling cascade will only have phenotypic consequences if the other isoforms in that node are unable to compensate for it, and (b) the phenotype produced by a given lesion in the insulin signaling cascade will depend on the location of the lesion. These concepts have been validated by studies using mice with targeted mutations of different components of the insulin signaling cascade (reviewed in [16]). In particular, mice with liver-specific mutations in the insulin receptor, IRS-1 and -2, PI 3-kinase and PKC- $\lambda$  have been generated. Here, we will discuss how defects in the different insulin signaling components, even within the same tissue, vary in their contribution to the metabolic syndrome.



***Insulin Receptor Knockout (LIRKO) Mice.*** LIRKO mice show greater than 95% deletion of the insulin receptor in liver. This results in complete insulin resistance, as the insulin receptor is unable to activate any of its downstream targets. Consistent with the role of insulin in suppressing hepatic gluconeogenesis, LIRKO mice are hyperglycemic, with increased expression of the gluconeogenic genes, increased hepatic glucose output, marked glucose intolerance and hyperglycemia [61]. LIRKO mice are also markedly hyperinsulinemic, and this is due both to  $\beta$  cell compensation, because the  $\beta$  cells secrete excess insulin in response to the hyperglycemic stimulus, and to decreased insulin clearance, because insulin receptors in the liver play an important role in the clearance of insulin from the serum. However, unlike diet-induced obese mice and humans with the metabolic syndrome, LIRKO mouse livers do not respond at all to hyperinsulinemia.

LIRKO mice also show a decrease in SREBP-1c and lipogenic gene expression, particularly in the re-fed state [62]. Although the triglyceride content of the liver is similar to wild type controls, VLDL secretion is markedly abnormal in LIRKO mice. VLDL contains triglycerides, cholesterol and phospholipids in complex with ApoB, the principal apolipoprotein component of VLDL. As expected from the decrease in SREBP-1c and its targets, VLDL-triglyceride secretion is decreased in LIRKO mice. However, ApoB secretion is increased. This discrepancy could be due to the fact that insulin inhibits ApoB lipidation, by inhibiting transcription of *Mttp* transcription by FoxO1,[41] and that insulin targets ApoB protein for degradation [63]. Consequently, LIRKO livers secrete VLDL particles that are relatively poor in triglycerides and rich in cholesterol.

In addition to abnormal VLDL particles, LIRKO mice show reduced levels of HDL-cholesterol [62]. When stressed with an atherogenic diet, LIRKO mice develop marked hypercholesterolemia, which is associated with decreased expression of the low density lipoprotein (LDL) receptor, and decreased LDL clearance. Consequently, all of the LIRKO mice but none of the controls develop atherosclerosis after being fed the atherogenic diet for less than 4 months [62].

In addition, the cholesterol transporters *Abcg5* and *Abcg8* are increased threefold at the mRNA levels in LIRKO livers. These transporters reside on the canalicular membrane of the hepatocyte and regulate the efflux of cholesterol into bile. Consequently, biliary cholesterol secretion is increased threefold in LIRKO mice [43]. This finding is important because increased biliary cholesterol secretion contributes to gallstone formation in obese humans [64]. Not surprisingly, when fed a lithogenic diet, 36% of LIRKO mice, but none of the control mice, develop cholesterol gallstones within 1 week [43].

***Knockout of the Insulin Receptor Substrates (IRS).*** Unlike LIRKO mice, liver-specific knockout of either IRS-1 or IRS-2 showed very subtle phenotypes [8]. Consistent with the fact that IRS-1 is expressed at higher levels in the fed state, mice with liver-specific knockout of IRS-1 alone showed increased gluconeogenic gene expression and hepatic glucose production, glucose intolerance and decreased lipogenic gene expression in the fed state [12]. However, these abnormalities were absent in the fasted state. In contrast, mice with liver-specific knockout of IRS-2 alone showed increased gluconeogenic gene expression, increased hepatic glucose

production, glucose intolerance and decreased lipogenic gene expression in the fasted, but not fed state [12]. Mice with hepatic knockout of either IRS-1 or IRS-2 did not show changes in serum triglyceride or cholesterol levels [12].

On the other hand, mice with knockout of both IRS-1 and IRS-2 in the liver show marked metabolic changes including increased hepatic glucose production and glucose intolerance in both the fed and fasted states, decreased serum triglyceride secretion, decreased serum triglycerides and decreased HDL cholesterol [12, 65]. In addition, genetic ablation of FoxO1 in the livers of these mice restores gluconeogenic gene expression, fasting glucose, insulin levels, serum triglyceride and HDL levels towards normal. This underscores the importance of FoxO1 in this phenotype [12, 65].

**Knockout of PI 3-Kinase.** The two major PI 3-kinase regulatory subunits expressed in the liver are p85 $\alpha$  and p85 $\beta$ . By mating mice with whole-body knockout of the p85 $\beta$  ( $\beta$ KO) to mice with liver-specific knockout of the *Pik3r1* gene, which encodes p85 $\alpha$ , as well as the less abundant isoforms, p55 $\alpha$  and p50 $\alpha$  ( $\alpha$ LKO), mice harboring both these deletions (p85 $\alpha/\beta$ -DKO) were generated [66]. Mice with knockout of either p85 $\alpha$  in the liver or p85 $\beta$  in the whole body show no changes in the activation of the downstream targets of PI 3-kinase, glucose or triglyceride metabolism. However, p85 $\alpha/\beta$ -DKO mice fail to activate PI 3-kinase [66]. Consequently, p85 $\alpha/\beta$ -DKO mice show blunted responses of Akt, PKC $\lambda$ , FoxO1, GSK3 $\beta$ , TSC2 and p70S6 kinase to insulin. They show increased gluconeogenic gene expression, hyperglycemia and hyperinsulinemia. In addition, SREBP-1c, its downstream target, fatty acid synthase, and serum triglycerides are decreased. Mice with acute knockdown of PI 3-kinase in the liver show a similar phenotype [67]. These mice were generated by injecting wild type mice with adenovirus encoding a dominant negative mutant of p85 $\alpha$ , which abolishes basal and insulin stimulated PI 3-kinase. Acute knockdown of PI 3-kinase also produces hyperglycemia, hyperinsulinemia and a marked reduction in serum triglyceride and cholesterol levels.

**PKC- $\lambda$  knockout.** Matsumoto and colleagues [68] have characterized mice with knockout of PKC- $\lambda$  in the liver (L- $\lambda$ KO mice). Although knockout of PKC- $\lambda$  induced hyperinsulinemia, it did not impair the ability of insulin to suppress gluconeogenic gene expression in vitro or in vivo. Consequently, L- $\lambda$ KO mice showed normal serum glucose levels and liver glycogen content after being challenged with a glucose load. However, they showed reduced levels of SREBP-1c mRNA and protein, decreased expression of the SREBP-1c target gene, fatty acid synthase and reduced hepatic triglyceride levels. Serum triglyceride levels were normal. In addition, treatment with an LXR agonist was able to fully restore SREBP-1c levels in L- $\lambda$ KO mice.

Similarly, knockout of liver PKC- $\lambda$  impairs the ability of constitutively active PI 3-kinase, delivered by adenovirus, to increase expression of SREBP-1c but not its ability to decrease serum glucose levels. Interestingly, L- $\lambda$ KO mice show normal levels of serum and hepatic cholesterol. Taken together, these data indicate that PKC- $\lambda$  is an important driver of lipogenesis, but not glucose metabolism. Consistent with this, restoration of PKC- $\lambda$  in L- $\lambda$ KO livers was able to increase expression of SREBP-1c and liver triglyceride content, but did not alter serum glucose levels.

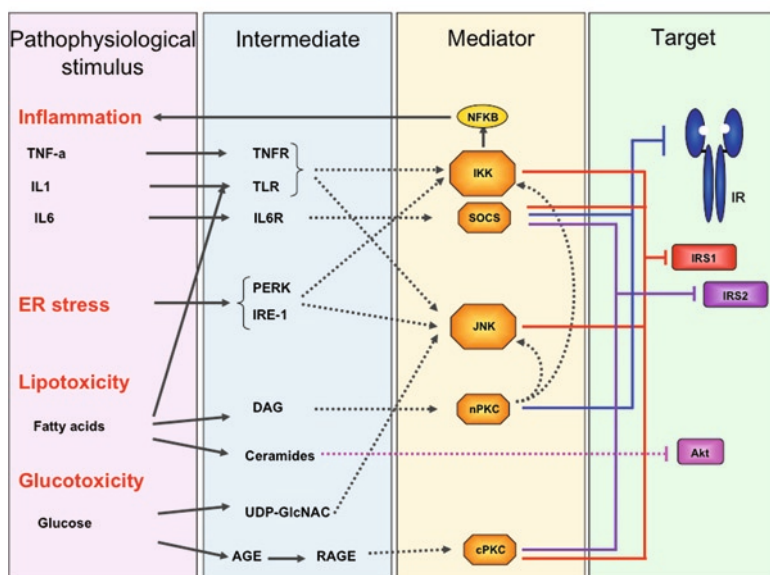
Taken together, these data illustrate several points about insulin resistance. First, studies in LIRKO mice show that insulin resistance can produce several components of the metabolic syndrome: hyperglycemia, low HDL cholesterol and increased susceptibility to atherosclerosis and cholesterol gallstones. This is a very important finding because it indicates that, at least in mice, insulin can play a causative role in the metabolic syndrome phenotype. Whether defects in the IRS proteins or other downstream insulin signaling molecules also produce this phenotype is yet to be determined. Second, the insulin resistance phenotype varies with the particular node involved. For example, LIRKO mice and p85 $\alpha/\beta$ -DKO show hyperglycemia and decreased levels of SREBP-1c. In contrast, mice with knockout of PKC- $\lambda$  only show decreased levels of SREBP-1c. L- $\lambda$ KO mice also show that molecular insulin resistance can exist even in the absence of hyperglycemia. Third, studies in mice with knockout of the IRS proteins or the PI 3-kinase regulatory subunits show that the severity of the phenotype produced by an insulin signaling defect depends on the extent to which the node itself is compromised. Thus, knockout of IRS-1, IRS-2, p85 $\alpha$  or p85 $\beta$  alone produces rather subtle phenotypes. However, when both IRS isoforms or both p85 isoforms are knocked out in the liver, a phenotype very similar to the LIRKO results.

## Molecular Mechanisms of Insulin Resistance

The metabolic syndrome is caused by a combination of genetic and environmental factors. One of the most important environmental factors is overnutrition. Here, we will review some of the mechanisms which have been suggested to underlie the associations between obesity and insulin resistance, including inflammation, lipotoxicity, ER stress and hyperglycemia (Figs. 3, 4).

**Inflammation.** Overnutrition and obesity appear to trigger an inflammatory response. Hence, macrophage activation and infiltration are commonly observed in the adipose tissue of obese humans and mice [22]. This is associated with increased secretion of chemokines and pro-inflammatory cytokines such as TNF- $\alpha$ , interleukin (IL)-1 and IL-6, resulting in a generalized state of inflammation [69]. Inflammation can produce insulin resistance through several mechanisms. Inflammation increases phosphorylation of IRS-1 on Ser307. TNF- $\alpha$ , for example, initiates the formation of a multiprotein signaling complex that triggers the activation of a kinase cascade which activates JNK [22]. JNK phosphorylates IRS-1 on Ser 307. Though IRS-1 Ser-307 phosphorylation has been extensively described as a marker of insulin resistance in mice and humans, it is not clear how this modification impairs insulin signaling. Ser-307 phosphorylation may interfere with IRS-1 function by disrupting its interaction with the insulin receptor or promoting the interaction of IRS-1 with 14-3-3 proteins, impairing its ability to activate its downstream targets [8]. Serine phosphorylation has also been shown to alter the intracellular localization of IRS-1 and induce its degradation [8].

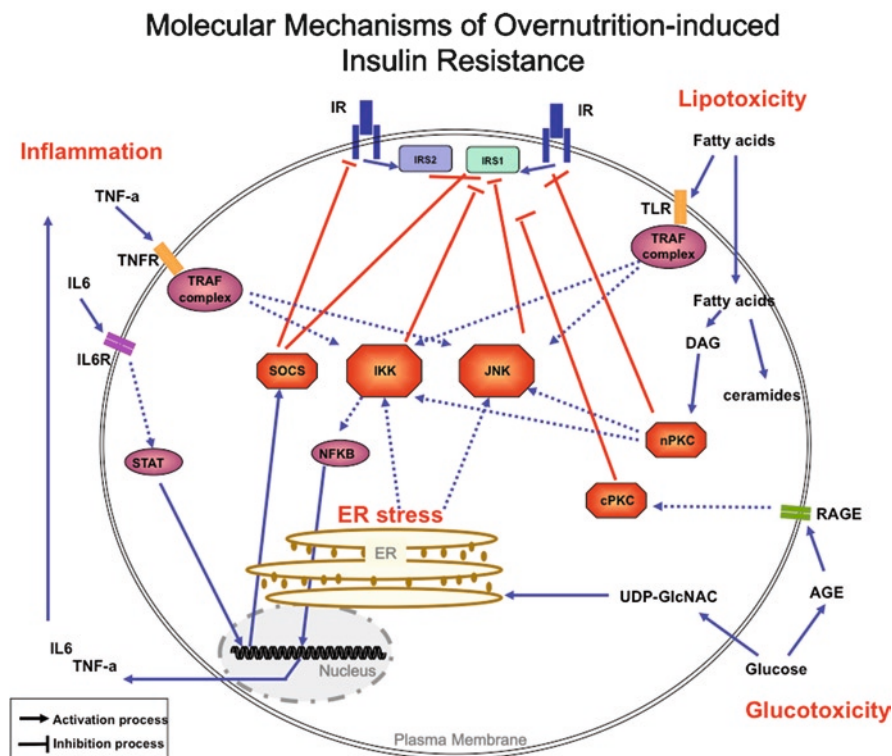
### Molecular Mechanisms of Overnutrition-induced Insulin Resistance



**Fig. 3** Molecular mechanisms of overnutrition-induced insulin resistance. Overnutrition appears to induce insulin resistance through many pathways, including inflammation, ER stress, lipotoxicity and glucotoxicity. Together, these processes induce the SOCS proteins and activate JNK, IKK, the conventional PKCs and novel PKCs. This ultimately inhibits signaling through the insulin receptor, IRS-1, IRS-2 and Akt. However, it is not clear which of these pathways, if any, plays the major role in causing the insulin resistance associated with obesity

Inflammation also increases expression of the suppressors of cytokine signaling (SOCS) proteins. For example, IL-6 induces transcription of the SOCS proteins by activating the STAT family of transcription factors [70]. SOCS -1 and SOCS-3 in particular decrease insulin signaling by (a) direct interaction with the insulin receptor, which could prevent binding of the IRS proteins or decrease the kinase activity of the insulin receptor, and (b) by promoting degradation of the IRS proteins [8]. In addition, SOCS3 promotes leptin resistance in the hypothalamus [70] and may regulate  $\beta$  cell mass and proliferation [71]. Consistent with this, mice heterozygous for a deletion of SOCS3 are resistant to diet-induced obesity and insulin resistance [70].

Inflammation activates the NF $\kappa$ B pathway. Cytokines, such as IL-1, stimulate the formation of signaling platforms involving TNF-receptor associated factor (TRAF) proteins and transforming growth factor- $\beta$  activated kinase (TAK) 1 (reviewed in [72]), which activate the inhibitor  $\kappa$ B kinase (IKK). IKK is a central mediator of the inflammation response, as it activates the transcription factor, nuclear factor kappa B (NF $\kappa$ B). NF $\kappa$ B drives the production of the pro-inflammatory cytokines TNF- $\alpha$ , IL-1 $\beta$  and IL-6, thereby further promoting inflammation and insulin resistance [73]. Forced expression of IKK is sufficient to induce insulin



**Fig. 4** Negative effects of glucotoxicity, lipotoxicity and inflammation on insulin signaling

resistance [22] whereas deletion of the gene protects against high fat diet-induced insulin resistance [22]. In addition, obesity increases IKK activity and salicylates, IKK- $\beta$  inhibitors, have proven to be efficient insulin sensitizers in mice and humans [22].

**Lipotoxicity.** Free fatty acids themselves promote inflammation by activation of the toll-like receptor (TLR)-2 and -4 pathways [22]. TLRs signal via the formation of TRAF protein-containing complexes, and activate the JNK and the IKK/NF $\kappa$ B pathways [72]. Excess fatty acids can also become deposited into tissues such as the liver and muscle, where they drive the formation of diacylglycerol (DAG) and other potentially toxic lipid metabolites, such as GM3 ganglioside or ceramides [74]. DAG activates JNK and the novel PKCs, PKC $\theta$  and PKC $\epsilon$ . PKC $\theta$  is present in muscle and stimulates activation of IKK- $\beta$  and JNK [75]. PKC $\epsilon$  is present in liver and has been shown to directly interact with the insulin receptor and decrease its activity [76]. GM3 ganglioside and ceramides, on the other hand, have been reported to induce insulin resistance by interfering with the activation of the insulin receptor and Akt, respectively (as reviewed in [74]).

**Endoplasmic Reticulum (ER) Stress.** The ER is an organelle dedicated to the synthesis, folding and maturation of all secreted and membrane proteins. When the

ER becomes overloaded with misfolded proteins, it triggers the unfolded protein response (UPR), a coordinate response involving inositol-requiring enzyme 1 (IRE-1), double stranded RNA-activated protein kinase-like endoplasmic reticulum kinase (PERK) and activating transcription factor 6 (ATF6). Under normal physiological conditions, the protein chaperone, BiP, interacts with IRE-1, PERK and ATF6, and maintains them in an inactive conformation. Under conditions of stress, the misfolded proteins bind and sequester BiP. As a result of BiP being removed from IRE-1, PERK and ATF-6, these proteins become activated, producing the UPR [77].

The UPR leads to a general attenuation of translation, which decreases the influx of more unfolded proteins, and increases expression of the chaperone proteins necessary to bind the unfolded proteins present. The UPR therefore represents a homeostatic mechanism. However, it also leads to insulin resistance. IRE-1 directly binds to TRAF-2 and triggers the activation of JNK [22] and IKK [22]. Furthermore, PERK phosphorylates the alpha subunit of the translation initiation factor 2 (eIF2a), and this results in decreased expression of I $\kappa$ B- $\alpha$ , a negative regulator of NF $\kappa$ B [22]. Consequently, genetic manipulations or chemical treatment that reduce the UPR have been shown to improve insulin sensitivity [22].

**Glucose Toxicity.** Insulin resistance eventually leads to hyperglycemia. In a vicious cycle, hyperglycemia leads to glucose toxicity, which further impairs insulin signaling, as well as insulin secretion. The effects of hyperglycemia on insulin signaling are thought to be mediated by the hexosamine pathway. This starts with the production of glucosamine 6-phosphate from fructose 6-phosphate by the rate-limiting enzyme glutamine:fructose-6-phosphate amidotransferase (GFAT). The major end product is UDP-*N*-acetylglucosamine (UDP-GlcNAc), which provides a substrate for the glycosylation of proteins and lipids [78]. Although the mechanism by which this pathway impairs insulin action is unclear, possible mechanisms include ER stress and JNK activation [79]. Alternatively, the fact that overexpression of *O*-GlcNAc transferase (OGT) decreases Akt Thr308 phosphorylation and increases IRS-1 Ser307, 632/635 phosphorylation suggests that glycosylation of key signaling molecules may also occur [79]. In addition, hyperglycemia leads to the formation of advanced glycation end (AGE) products through non-enzymatic modification of proteins by reducing sugars. AGEs bind and activate the receptor for AGE (RAGE). The RAGE receptor activates PKC $\alpha$ , which in turn induces serine phosphorylation of IRS-1 and IRS-2 [80].

**Other Mechanisms of Insulin Resistance.** Studies to understand how environmental factors interfere with insulin signaling have focused on IRS-1 Ser 307 phosphorylation by JNK. However, there are several important points to note. First, there are over 70 potential serine phosphorylation sites on IRS-1, and other IRS proteins may also undergo serine phosphorylation. The importance of IRS-1 Ser 307 relative to these other sites has not been adequately studied. Second, although many studies have demonstrated negative effects of serine phosphorylation, there is some evidence that serine phosphorylation of certain sites may serve a positive role. Finally, multiple other mechanisms could contribute to insulin resistance in the metabolic syndrome [8].



Insulin resistance could result from increases in the activity or amount of the enzymes which normally terminate the insulin signal, including the phosphotyrosine phosphatases, such as PTP1b, which de-phosphorylate and de-activate the insulin receptor. Increased tyrosine phosphatase activity has been described in insulin resistant states, and knockout of PTP1b improves insulin sensitivity in different models of insulin resistance [8]. Similarly, increased levels of the PIP<sub>3</sub> phosphatases, e.g., PTEN and SHIP, which dephosphorylate the PIP<sub>3</sub> produced by PI 3-kinase, would be expected to impair insulin signaling. Consistent with this, reduction of PTEN and SHIP2 expression improves insulin sensitivity in various models of insulin resistance through upregulation of PI3-K and Akt signaling [8]. In addition, the pseudo-kinase TRB3, a mammalian homolog of drosophila tribbles, has been proposed to contribute to obesity-induced insulin resistance by interacting with Akt and downregulating its activity, [8] and endogenous Akt inhibitors such as CTMP and PHLPP may also play a role [8].

Thus, it is likely that many mechanisms, independent of JNK and IRS-1 Ser 307 phosphorylation, play a role in mediating the effects of overnutrition on insulin signaling. In particular, it is likely that different genetic and environmental insults will produce their own signature of molecular insulin resistance, with a defined cluster of defects in the insulin signaling pathway.

## Insulin Resistance in Humans

Identifying insulin signaling defects associated with the metabolic syndrome in humans is complicated by significant methodological challenges (reviewed in [81]). Two approaches have been used. First, biopsies have been taken from insulin-resistant subjects and their controls both before and after being subject to a hyperinsulinemic euglycemic clamp, and insulin signaling has been examined. Alternatively, biopsies have been taken from individuals, and cultured in vitro in the presence or absence of insulin. Comparing the results of these different studies is difficult because of the numerous variables present, such as the patient population, the dose and duration of insulin, the site of biopsy and how the biopsy is processed for signaling studies. Therefore it is not surprising that some studies have identified defects in the insulin signaling pathway whereas others have not (reviewed in [16]). Nonetheless, defects in insulin binding, insulin receptor tyrosine phosphorylation, IRS-1 tyrosine phosphorylation have been documented. Similarly defects in PI 3-kinase and its downstream targets, Akt and aPKC have been shown. The most consistent insulin signaling defect to be observed in muscle and adipose is in the aPKCs, and this occurs in the presence or absence of defects in Akt. In contrast, activities of ERK1/2 and JNK appear to be relatively intact.

In a complementary approach, humans with defined lesions in the insulin signaling cascade have been studied [82]. Patients with mutations in the insulin receptor are hyperglycemic and hyperinsulinemic. Consistent with the studies in LIRKO mice, they show low serum triglycerides, their VLDL is relatively poor in triglycerides

and they show no increase in either lipogenesis or hepatic triglyceride content. In contrast, humans with mutations in Akt2 develop hyperglycemia, hyperinsulinemia, hypertriglyceridemia, and increased lipogenesis and hepatic triglyceride content. Taken together, these studies indicate that multiple defects in the insulin signaling pathway are possible in humans, but that not all pathways are equally affected. Furthermore, the phenotype of the individual will depend on the specific complement of lesions he or she harbors.

## Conclusions

The role of insulin resistance in the metabolic syndrome is yet to be resolved. However, recent advances in our understanding of the insulin signaling cascade show that many of the ideas put forth by Dr. Reaven, over 20 years ago, are likely correct. First, studies, particularly in LIRKO mice, indicate that many features of the metabolic syndrome, in addition to hyperglycemia, can be produced by insulin resistance, namely, low HDL cholesterol, susceptibility to atherosclerosis and susceptibility to cholesterol gallstones. Further characterization of mice and humans with defined lesions in the insulin signaling pathway may even show other features to be related. Second, the existence of multiple branches and nodes in the insulin signaling pathway explain how some pathways of the metabolic syndrome can remain sensitive to insulin, while others become resistant. For example, a specific defect in Akt could allow continued signaling through the insulin receptor/IRS/PI 3-kinase/PKC- $\lambda$  pathway leading to the activation of lipogenesis. Since Akt regulates FoxO1 and gluconeogenesis, defects in Akt could produce both hyperglycemia and increased serum and hepatic triglycerides, as observed in humans with mutations in Akt. Although the precise lesions that cause insulin resistance in humans, particularly in tissues such as the liver, are yet to be determined, studies to date suggest that defects do occur.

Finally, the unexpected finding of so many redundant components in the insulin signaling cascade suggests that the metabolic syndrome may be even more complex than we had initially thought. In other words, the number of defects possible in the insulin signaling cascade is staggering, and each defect is likely to produce a slightly different phenotype. The exact complement of lesions harbored by any individual will depend on both the genetic and environmental insults to which he or she is subjected. Ultimately, we hope to be able to diagnose and treat individuals with the metabolic syndrome based on their specific insulin signaling defects, leading to more effective therapy with fewer adverse effects.

**Acknowledgements** This work was funded in part by National Institutes of Health grants DK063696 and DK083697 (SBB). Because of space limitations, we were unable to include all of the references we would have liked. We apologize to our many colleagues whose work is not directly cited.



## References

1. Reaven, G. M. (1988). Banting lecture 1988. Role of insulin resistance in human disease. *Diabetes*, 37(12), 1595–1607.
2. Cornier, M. A., Dabelea, D., Hernandez, T. L., et al. (2008). The metabolic syndrome. *Endocrine Reviews*, 29(7), 777–822.
3. Ford, E. S., Giles, W. H., & Mokdad, A. H. (2004). Increasing prevalence of the metabolic syndrome among U.S. adults. *Diabetes Care*, 27(10), 2444–2449.
4. Kahn, R., Buse, J., Ferrannini, E., & Stern, M. (2005). The metabolic syndrome: time for a critical appraisal: joint statement from the American Diabetes Association and the European Association for the Study of Diabetes. *Diabetes Care*, 28(9), 2289–2304.
5. Reaven, G. (2004). The metabolic syndrome or the insulin resistance syndrome? Different names, different concepts, and different goals. *Endocrinology and Metabolism Clinics of North America*, 33(2), 283–303.
6. Yamaguchi, Y., Flier, J. S., Benecke, H., Ransil, B. J., & Moller, D. E. (1993). Ligand-binding properties of the two isoforms of the human insulin receptor. *Endocrinology*, 132(3), 1132–1138.
7. Leibiger, B., Leibiger, I. B., Moede, T., et al. (2001). Selective insulin signaling through A and B insulin receptors regulates transcription of insulin and glucokinase genes in pancreatic beta cells. *Molecular cell*, 7(3), 559–570.
8. Taniguchi, C. M., Emanuelli, B., & Kahn, C. R. (2006). Critical nodes in signalling pathways: insights into insulin action. *Nature Reviews. Molecular Cell Biology*, 7(2), 85–96.
9. Fasshauer, M., Klein, J., Ueki, K., et al. (2000). Essential role of insulin receptor substrate-2 in insulin stimulation of Glut4 translocation and glucose uptake in brown adipocytes. *The Journal of Biological Chemistry*, 275(33), 25494–25501.
10. Wu, J., Tseng, Y. D., Xu, C. F., Neubert, T. A., White, M. F., & Hubbard, S. R. (2008). Structural and biochemical characterization of the KRLB region in insulin receptor substrate-2. *Nature Structural & Molecular Biology*, 15(3), 251–258.
11. Hirashima, Y., Tsuruzoe, K., Kodama, S., et al. (2003). Insulin down-regulates insulin receptor substrate-2 expression through the phosphatidylinositol 3-kinase/Akt pathway. *The Journal of Endocrinology*, 179(2), 253–266.
12. Kubota, N., Kubota, T., Itoh, S., et al. (2008). Dynamic functional relay between insulin receptor substrate 1 and 2 in hepatic insulin signaling during fasting and feeding. *Cell Metabolism*, 8(1), 49–64.
13. Oriente, F., Andreozzi, F., Romano, C., et al. (2005). Protein kinase C- $\alpha$  regulates insulin action and degradation by interacting with insulin receptor substrate-1 and 14-3-3 epsilon. *The Journal of Biological Chemistry*, 280(49), 40642–40649.
14. Luan, B., Zhao, J., Wu, H., et al. (2009). Deficiency of a beta-arrestin-2 signal complex contributes to insulin resistance. *Nature*, 457(7233), 1146–1149.
15. Bard-Chapeau, E. A., Hevener, A. L., Long, S., Zhang, E. E., Olefsky, J. M., & Feng, G. S. (2005). Deletion of Gab1 in the liver leads to enhanced glucose tolerance and improved hepatic insulin action. *Nature Medicine*, 11(5), 567–571.
16. Biddinger, S. B., & Kahn, C. R. (2006). From mice to men: insights into the insulin resistance syndromes. *Annual Review of Physiology*, 68, 123–158.
17. Brachmann, S. M., Ueki, K., Engelman, J. A., Kahn, R. C., & Cantley, L. C. (2005). Phosphoinositide 3-kinase catalytic subunit deletion and regulatory subunit deletion have opposite effects on insulin sensitivity in mice. *Molecular and Cellular Biology*, 25(5), 1596–1607.
18. Terauchi, Y., Tsuji, Y., Satoh, S., et al. (1999). Increased insulin sensitivity and hypoglycaemia in mice lacking the p85 alpha subunit of phosphoinositide 3-kinase. *Nature Genetics*, 21(2), 230–235.
19. Cheatham, B., Vlahos, C. J., Cheatham, L., Wang, L., Blenis, J., & Kahn, C. R. (1994). Phosphatidylinositol 3-kinase activation is required for insulin stimulation of pp70 S6 kinase,

- DNA synthesis, and glucose transporter translocation. *Molecular and Cellular Biology*, 14(7), 4902–4911.
20. Shepherd, P. R., Withers, D. J., & Siddle, K. (1998). Phosphoinositide 3-kinase: the key switch mechanism in insulin signalling. *The Biochemical Journal*, 333, (Pt 3):471–490.
  21. Bishop, A. L., & Hall, A. (2000). Rho gtpases and their effector proteins. *The Biochemical Journal*, 348, Pt 2:241–255.
  22. Hotamisligil, G. S. (2006). Inflammation and metabolic disorders. *Nature*, 444(7121), 860–867.
  23. Vanhaesebroeck, B., & Alessi, D. R. (2000). The PI3K-PDK1 connection: more than just a road to PKB. *The Biochemical Journal*, 346, Pt 3:561–576.
  24. Sarbassov, D. D., Guertin, D. A., Ali, S. M., & Sabatini, D. M. (2005). Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. *Science*, 307(5712), 1098–1101.
  25. Bandyopadhyay, G., Standaert, M. L., Zhao, L., et al. (1997). Activation of protein kinase C (alpha, beta, and zeta) by insulin in 3T3/L1 cells. Transfection studies suggest a role for PKC-zeta in glucose transport. *The Journal of Biological Chemistry*, 272(4), 2551–2558.
  26. Tremblay, F., Lavigne, C., Jacques, H., & Marette, A. (2001). Defective insulin-induced GLUT4 translocation in skeletal muscle of high fat-fed rats is associated with alterations in both Akt/protein kinase B and atypical protein kinase C (zeta/lambda) activities. *Diabetes*, 50(8), 1901–1910.
  27. Kotani, K., Ogawa, W., Matsumoto, M., et al. (1998). Requirement of atypical protein kinase clambda for insulin stimulation of glucose uptake but not for Akt activation in 3T3-L1 adipocytes. *Molecular and Cellular Biology*, 18(12), 6971–6982.
  28. Inoki, K., Li, Y., Zhu, T., Wu, J., Guan, K. L. (2002). TSC2 is phosphorylated and inhibited by Akt and suppresses mTOR signalling. *Nature Cell Biology*, 4(9), 648–657.
  29. Inoki, K., Li, Y., Xu, T., & Guan, K. L. (2003). Rheb GTPase is a direct target of TSC2 GAP activity and regulates mTOR signaling. *Genes & Development*, 17(15), 1829–1834.
  30. Cross, D. A., Alessi, D. R., Cohen, P., Andjelkovich, M., & Hemmings, B. A. (1995). Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase B. *Nature*, 378(6559), 785–789.
  31. McManus, E. J., Sakamoto, K., Armit, L. J., et al. (2005). Role that phosphorylation of GSK3 plays in insulin and Wnt signalling defined by knockin analysis. *The EMBO journal*, 24(8), 1571–1583.
  32. Patel, S., Doble, B. W., MacAulay, K., Sinclair, E. M., Drucker, D. J., & Woodgett, J. R. (2008). Tissue-specific role of glycogen synthase kinase 3beta in glucose homeostasis and insulin action. *Molecular and Cellular Biology*, 28(20), 6314–6328.
  33. MacAulay, K., Doble, B. W., Patel, S., et al. (2007). Glycogen synthase kinase 3alpha-specific regulation of murine hepatic glycogen metabolism. *Cell Metabolism*, 6(4), 329–337.
  34. Ishikura, S., Bilan, P. J., & Klip, A. (2007). Rabs 8A and 14 are targets of the insulin-regulated Rab-GAP AS160 regulating GLUT4 traffic in muscle cells. *Biochemical and Biophysical Research Communications*, 353(4), 1074–1079.
  35. Accili, D., & Arden, K. C. (2004). Foxos at the crossroads of cellular metabolism, differentiation, and transformation. *Cell*, 117(4), 421–426.
  36. Puigserver, P., Rhee, J., Donovan, J., et al. (2003). Insulin-regulated hepatic gluconeogenesis through FOXO1-PGC-1alpha interaction. *Nature*, 423(6939), 550–555.
  37. Foufelle, F., & Ferre, P. (2002). New perspectives in the regulation of hepatic glycolytic and lipogenic genes by insulin and glucose: a role for the transcription factor sterol regulatory element binding protein-1c. *The Biochemical Journal*, 366(Pt 2), 377–391.
  38. Kodama, S., Koike, C., Negishi, M., & Yamamoto, Y. (2004). Nuclear receptors CAR and PXR cross talk with FOXO1 to regulate genes that encode drug-metabolizing and gluconeogenic enzymes. *Molecular and cellular biology*, 24(18), 7931–7940.
  39. Zhang, W., Patil, S., Chauhan, B., et al. (2006). Foxo1 regulates multiple metabolic pathways in the liver: effects on gluconeogenic, glycolytic, and lipogenic gene expression. *The Journal of biological chemistry*, 281(15), 10105–10117.
  40. Samuel, V. T., Choi, C. S., Phillips, T. G., et al. (2006). Targeting foxo1 in mice using antisense oligonucleotide improves hepatic and peripheral insulin action. *Diabetes*, 55(7), 2042–2050.

41. Kamagate, A., Qu, S., Perdomo, G., et al. (2008). Foxo1 mediates insulin-dependent regulation of hepatic VLDL production in mice. *The Journal of Clinical Investigation*, 118(6), 2347–2364.
42. Altomonte, J., Cong, L., Harbaran, S., et al. (2004). Foxo1 mediates insulin action on apoc-III and triglyceride metabolism. *The Journal of Clinical Investigation*, 114(10), 1493–1503.
43. Biddinger, S. B., Haas, J. T., Yu, B. B., et al. (2008). Hepatic insulin resistance directly promotes formation of cholesterol gallstones. *Nature Medicine*, 14(7), 778–782.
44. Graf, G. A., Yu, L., Li, W. P., et al. (2003). ABCG5 and ABCG8 are obligate heterodimers for protein trafficking and biliary cholesterol excretion. *The Journal of Biological Chemistry*, 278(48), 48275–48282.
45. Li, T., Kong, X., Owsley, E., Ellis, E., Strom, S., & Chiang, J. Y. (2006). Insulin regulation of cholesterol 7 $\alpha$ -hydroxylase expression in human hepatocytes: roles of forkhead box O1 and sterol regulatory element-binding protein 1c. *The Journal of Biological Chemistry*, 281(39), 28745–28754.
46. Gross, D. N., van den Heuvel, A. P., & Birnbaum, M. J. (2008). The role of foxo in the regulation of metabolism. *Oncogene*, 27(16), 2320–2336.
47. Wolfrum, C., Asilmaz, E., Luca, E., Friedman, J. M., & Stoffel, M. (2004). Foxa2 regulates lipid metabolism and ketogenesis in the liver during fasting and in diabetes. *Nature*, 432(7020), 1027–1032.
48. Zhang, L., Rubins, N. E., Ahima, R. S., Greenbaum, L. E., & Kaestner, K. H. (2005). Foxa2 integrates the transcriptional response of the hepatocyte to fasting. *Cell Metabolism*, 2(2), 141–148.
49. Bochkis, I. M., Rubins, N. E., White, P., Furth, E. E., Friedman, J. R., & Kaestner, K. H. (2008). Hepatocyte-specific ablation of Foxa2 alters bile acid homeostasis and results in endoplasmic reticulum stress. *Nature Medicine*, 14(8), 828–836.
50. Horton, J. D., Goldstein, J. L., Brown, M. S. (2002). Srebps: activators of the complete program of cholesterol and fatty acid synthesis in the liver. *The Journal of Clinical Investigation*, 109(9), 1125–1131.
51. Shimano, H., Horton, J. D., Shimomura, I., Hammer, R. E., Brown, M. S., & Goldstein, J. L. (1997). Isoform 1c of sterol regulatory element binding protein is less active than isoform 1a in livers of transgenic mice and in cultured cells. *The Journal of Clinical Investigation*, 99(5), 846–854.
52. Ide, T., Shimano, H., Yahagi, N., et al. (2004). Srebps suppress IRS-2-mediated insulin signalling in the liver. *Nature Cell Biology*, 6(4), 351–357.
53. Yamamoto, T., Shimano, H., Nakagawa, Y., et al. (2004). SREBP-1 interacts with hepatocyte nuclear factor-4  $\alpha$  and interferes with PGC-1 recruitment to suppress hepatic gluconeogenic genes. *The Journal of Biological Chemistry*, 279(13), 12027–12035.
54. Shimomura, I., Bashmakov, Y., & Horton, J. D. (1999). Increased levels of nuclear SREBP-1c associated with fatty livers in two mouse models of diabetes mellitus. *The Journal of Biological Chemistry*, 274(42), 30028–30032.
55. Horton, J. D., Bashmakov, Y., Shimomura, I., & Shimano, H. (1998). Regulation of sterol regulatory element binding proteins in livers of fasted and refed mice. *Proceedings of the National Academy of Sciences of the United States of America*, 95(11), 5987–5992.
56. Liang, G., Yang, J., Horton, J. D., Hammer, R. E., Goldstein, J. L., & Brown, M. S. (2002). Diminished hepatic response to fasting/refeeding and liver X receptor agonists in mice with selective deficiency of sterol regulatory element-binding protein-1c. *The Journal of Biological Chemistry*, 277(11), 9520–9528.
57. Repa, J. J., Liang, G., Ou, J., et al. (2000). Regulation of mouse sterol regulatory element-binding protein-1c gene (SREBP-1c) by oxysterol receptors, 1x $\alpha$  and 1x $\beta$ . *Genes & Development*, 14(22), 2819–2830.
58. Tobin, K. A., Ulven, S. M., Schuster, G. U., et al. (2002). Liver X receptors as insulin-mediating factors in fatty acid and cholesterol biosynthesis. *The Journal of Biological Chemistry*, 277(12), 10691–10697.

59. Yabe, D., Komuro, R., Liang, G., Goldstein, J. L., & Brown, M. S. (2003). Liver-specific mrna for Insig-2 down-regulated by insulin: implications for fatty acid synthesis. *Proceedings of the National Academy of Sciences of the United States of America*, 100(6), 3155–3160.
60. Kakuma, T., Lee, Y., Higa, M., et al. (2000). Leptin, troglitazone, and the expression of sterol regulatory element binding proteins in liver and pancreatic islets. *Proceedings of the National Academy of Sciences of the United States of America*, 97(15), 8536–8541.
61. Michael, M. D., Kulkarni, R. N., Postic, C., et al. (2000). Loss of insulin signaling in hepatocytes leads to severe insulin resistance and progressive hepatic dysfunction. *Molecular Cell*, 6(1), 87–97.
62. Biddinger, S. B., Hernandez-Ono, A., Rask-Madsen, C., et al. (2008). Hepatic insulin resistance is sufficient to produce dyslipidemia and susceptibility to atherosclerosis. *Cell Metabolism*, 7(2), 125–134.
63. Sparks, J. D., & Sparks, C. E. (1994). Insulin regulation of triacylglycerol-rich lipoprotein synthesis and secretion. *Biochimica et Biophysica Acta*, 1215(1–2), 9–32.
64. Shaffer, E. A., & Small, D. M. (1977). Biliary lipid secretion in cholesterol gallstone disease. The effect of cholecystectomy and obesity. *The Journal of Clinical Investigation*, 59(5), 828–840.
65. Dong, X. C., Copps, K. D., Guo, S., et al. (2008). Inactivation of hepatic Foxo1 by insulin signaling is required for adaptive nutrient homeostasis and endocrine growth regulation. *Cell Metabolism*, 8(1), 65–76.
66. Taniguchi, C. M., Kondo, T., Sajan, M., et al. (2006). Divergent regulation of hepatic glucose and lipid metabolism by phosphoinositide 3-kinase via Akt and pklambda/zeta. *Cell Metabolism*, 3(5), 343–353.
67. Miyake, K., Ogawa, W., Matsumoto, M., Nakamura, T., Sakaue, H., & Kasuga, M. (2002). Hyperinsulinemia, glucose intolerance, and dyslipidemia induced by acute inhibition of phosphoinositide 3-kinase signaling in the liver. *The Journal of Clinical Investigation*, 110(10), 1483–1491.
68. Matsumoto, M., Ogawa, W., Akimoto, K., et al. (2003). Pklambda in liver mediates insulin-induced SREBP-1c expression and determines both hepatic lipid content and overall insulin sensitivity. *The Journal of Clinical Investigation*, 112(6), 935–944.
69. Tilg, H., & Moschen, A. R. (2008). Inflammatory mechanisms in the regulation of insulin resistance. *Molecular Medicine (Cambridge, Mass. )*, 14(3–4), 222–231.
70. Howard, J. K., Cave, B. J., Oksanen, L. J., Tzamelis, I., Bjorbaek, C., & Flier, J. S. (2004). Enhanced leptin sensitivity and attenuation of diet-induced obesity in mice with haploinsufficiency of Socs3. *Nature Medicine*, 10(7), 734–738.
71. Nielsen, J. H., Galsgaard, E. D., Moldrup, A., et al. (2001). Regulation of beta-cell mass by hormones and growth factors. *Diabetes*, 50, Suppl 1:S25–29.
72. Verstrepen, L., Bekaert, T., Chau, T. L., Tavernier, J., Chariot, A., & Beyaert, R. (2008). TLR-4, IL-1R and TNF-R signaling to NF-kappaB: variations on a common theme. *Cellular and Molecular Life Sciences: CMLS*, 65(19), 2964–2978.
73. Shoelson, S. E., Lee, J., & Yuan, M. (2003). Inflammation and the IKK beta/I kappa B/ NF-kappa B axis in obesity- and diet-induced insulin resistance. *International Journal of Obesity and Related Metabolic Disorders: Journal of the International Association for the Study of Obesity*, 27, Suppl 3:S49–52.
74. Holland, W. L., Knotts, T. A., Chavez, J. A., Wang, L. P., Hoehn, K. L., & Summers, S. A. (2007). Lipid mediators of insulin resistance. *Nutrition reviews*, 65(6 Pt 2), S39–46.
75. Gao, Z., Zhang, X., Zuberi, A., et al. (2004). Inhibition of insulin sensitivity by free fatty acids requires activation of multiple serine kinases in 3T3-L1 adipocytes. *Molecular Endocrinology (Baltimore, Md. )*, 18(8), 2024–2034.
76. Savage, D. B., Petersen, K. F., & Shulman, G. I. (2007). Disordered lipid metabolism and the pathogenesis of insulin resistance. *Physiological Reviews*, 87(2), 507–520.
77. Malhotra, J. D., & Kaufman, R. J. (2007). The endoplasmic reticulum and the unfolded protein response. *Seminars in Cell & Developmental Biology*, 18(6), 716–731.

78. Buse, M. G. (2006). Hexosamines, insulin resistance, and the complications of diabetes: current status. *American Journal of Physiology. Endocrinology and Metabolism*, 290(1), E1-E8.
79. Srinivasan, V., Tatu, U., Mohan, V., & Balasubramanyam, M. (2009). Molecular convergence of hexosamine biosynthetic pathway and ER stress leading to insulin resistance in L6 skeletal muscle cells. *Molecular and Cellular Biochemistry*, 328(1–2), 217–224.
80. Miele, C., Riboulet, A., Maitan, M. A., et al. (2003). Human glycated albumin affects glucose metabolism in L6 skeletal muscle cells by impairing insulin-induced insulin receptor substrate (IRS) signaling through a protein kinase C alpha-mediated mechanism. *The Journal of Biological Chemistry*, 278(48), 47376–47387.
81. Frojdo, S., Vidal, H., & Pirola, L. (2009). Alterations of insulin signaling in type 2 diabetes: a review of the current evidence from humans. *Biochimica et Biophysica Acta*, 1792(2), 83–92.
82. Semple, R. K., Sleight, A., Murgatroyd, P. R., et al. (2009). Postreceptor insulin resistance contributes to human dyslipidemia and hepatic steatosis. *The Journal of Clinical Investigation*, 119(2), 315–322.

# Chapter 11

## Pancreatic Islet $\beta$ -Cell Failure in Obesity

Tomoaki Morioka and Rohit N. Kulkarni

### Introduction

It is well recognized that obesity is associated with insulin resistance and a high risk for the development of type 2 diabetes (T2D). Numerous studies show that insulin-resistance precedes the development of hyperglycemia and the onset of diabetes. Most obese, insulin resistant individuals do not develop hyperglycemia because the pancreatic islet  $\beta$ -cells compensate appropriately by increasing insulin release and/or their mass to overcome impaired peripheral insulin action. T2D develops only in those who are unable to compensate fully for the reduced insulin sensitivity. Although evidence supporting this concept has emerged during recent years [1–5], the factors or mechanisms that underlie the  $\beta$ -cell compensation for insulin resistance, and/or those that trigger  $\beta$ -cell failure leading to T2D in obese individuals are not fully understood.

Recent evidence supports a central role for the brain in regulating both body fat content and glucose metabolism. Indeed, studies suggest that the brain is able to sense and integrate information from neural, hormonal, and nutrient cues that are generated in response to body energy status, such as food ingestion, body adiposity, and energy balance. The hypothalamus is also known to directly regulate glucose metabolism by controlling hepatic glucose output, glucose uptake by fat and muscle, and insulin secretion by the endocrine pancreas. Nutrient sensing by the brain is impaired by overeating and obesity, and sustained impairment of hypothalamic nutrient sensing can also lead to obesity. Thus, the hypothalamus plays a crucial role in energy and glucose metabolism and is involved in mechanisms linking obesity to T2D [6–8].

Among the signals that originate in the central nervous system (CNS), the autonomic nerves mediate important signals to the pancreatic islets to influence physiological and pathophysiological functions in the islet [9]. Indeed, the islets are

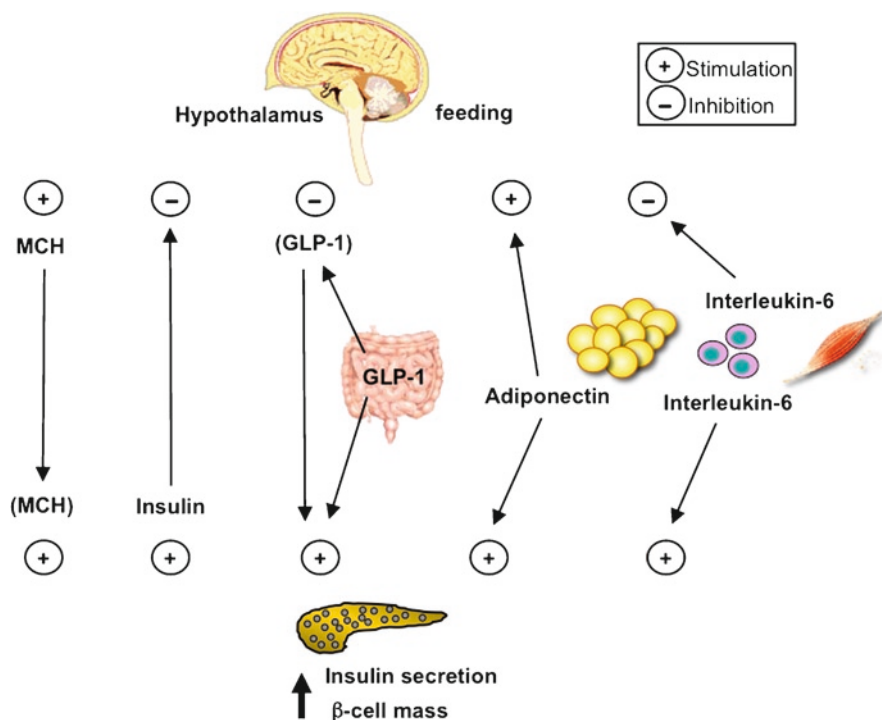
---

R.N. Kulkarni (✉)

Division of Islet Cell Biology and Regenerative Medicine, Joslin Diabetes Center,  
and Department of Medicine, Brigham and Women's Hospital, Harvard Medical School,  
One Joslin Place, Boston MA 02215, USA  
e-mail: Rohit.Kulkarni@joslin.harvard.edu

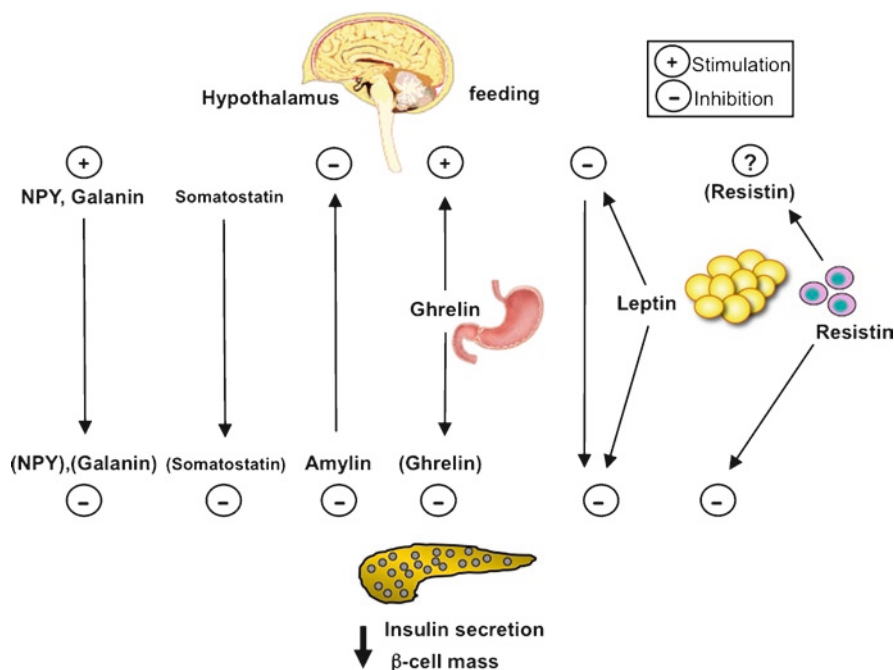
richly innervated by autonomic nerves and have been suggested to be involved in the islet adaptation to insulin resistance with possible implications for  $\beta$ -cell failure leading to the development of T2D[9]. Interestingly, several neurotransmitters or neuropeptides that act on the CNS to regulate appetite and energy homeostasis have also been reported to regulate pancreatic islet function [9]. In this chapter, we will discuss, recent evidence regarding the roles of neuropeptides, including melanin-concentrating hormone (MCH), neuropeptide Y (NPY), orexins, galanin, and somatostatin, that could be implicated in regulating  $\beta$ -cell function and its adaptation to insulin resistance in obesity as components of a putative “islet–hypothalamic axis” [10] (Figs. 1 and 2).

It is notable that obesity and insulin resistance are associated with changes in the abundance of bioactive factors released from the adipose tissue, termed adipokines, that also regulate  $\beta$ -cell growth and function and may be linked to the pathogenesis of obesity-related diabetes[11]. Several of these adipokines affect islet function and/or growth directly and/or indirectly by acting via the central/autonomic nervous system, contributing to an “adipo-insular axis” or “islet–hypothalamic axis”[11, 12]. In the latter part of this chapter, we will consider adipokines and other factors originating from the gut and the endocrine pancreas, including leptin, adiponectin, resistin, interleukin-6, ghrelin, glucagon-like peptide (GLP)-1, and



**Fig. 1** Stimulation of pancreatic islet  $\beta$ -cells by neuropeptides, gut-derived peptides, and adipokines





**Fig. 2** Inhibition of pancreatic islet  $\beta$ -cells by neuropeptides, gut-derived peptides, and adipokines

amylin, that have been recently reported to play important roles in regulating both hypothalamic and islet function and that could potentially contribute to  $\beta$ -cell adaptation and/or its failure in obesity-related diabetes (Figs. 1 and 2).

## Neuropeptides Regulating the Islet–Hypothalamic Axis

**MCH:** MCH is expressed in the lateral hypothalamus and known to play a critical role in feeding and energy balance in rodents [13]. MCH expression in the hypothalamus is upregulated by fasting and exogenous administration of MCH stimulates feeding behavior in mice [14]. Mice overexpressing MCH have mild obesity, hyperinsulinemia, and islet hyperplasia that is independent of their degree of obesity [15]. Conversely, mice lacking the MCH gene are lean, hypophagic, and resistant to aging-associated obesity and insulin resistance [13, 16]. A stimulatory effect of MCH on insulin release and expression of MCH and its receptors has been observed in mouse and human islets and rodent  $\beta$ -cell lines [17, 18]. The compensatory islet expansion in response to a HFD, which was observed in control mice, was attenuated in mice lacking MCH, suggesting MCH signaling is a necessary link in the islet adaptation to insulin resistance [17]. Although  $\beta$ -cell function and mass could be modulated by central action of MCH on feeding, adiposity, and



whole body insulin sensitivity, the rodent models discussed above indicate a potential local and autocrine role for MCH in the regulation of islet secretory function and  $\beta$ -cell mass in obese states.

**NPY:** NPY is a neurotransmitter that is widely distributed in mammalian central and peripheral nervous systems and is a potent orexigenic peptide that has been suggested to play an important role in the regulation of body weight and feeding [19]. Chronic exposure to elevated NPY in the hypothalamus of mice produced hyperphagia, obesity, and insulin resistance [20], and an increase in activity of NPY and its receptors has been found in the brain in many forms of experimental obesity [19, 21]. A recent study revealed a direct action of NPY on fat tissue that may contribute to stress-induced obesity and metabolic syndrome [22]. NPY is also expressed in pancreatic islets and implicated in the regulation of islet function. NPY decreases glucose-stimulated insulin secretion from rodent and human islets [23, 24]. Conversely, islets from NPY-deficient mice have higher basal and glucose-stimulated insulin secretion, and greater islet mass compared with wild-type mice [25]. Furthermore, the islet adaptation to obesity, that is, enhanced basal/glucose-stimulated insulin secretion, in C57BL/6J mice on a HFD was associated with decreased NPY and Y1 receptor mRNA levels in islets [25]. These findings imply that local action of NPY in pancreatic  $\beta$ -cells is involved in insulin hypersecretion and islet growth in response to obesity and insulin resistance, although its direct role in islet  $\beta$ -cell failure in obesity and diabetes remains to be determined.

**Orexins:** Orexins, also called hypocretins, are peptides originally found to be produced in the lateral hypothalamic area and are implicated in the regulation of feeding, energy balance, and wakefulness [26]. Orexin mRNA is upregulated by fasting and central administration of orexins has been shown to increase food intake in rodents [27]. Recent evidence indicates that orexins and functional orexin receptors are expressed in the periphery including the gastrointestinal tract and the endocrine pancreas. Orexin A release is increased in response to hypoglycemia and inhibited when glucose levels are high, suggesting these peptides participate in the control of pancreatic hormone secretion and glucose homeostasis [28]. Recent in vitro and in vivo studies suggest that orexins influence both islet insulin and glucagon secretion. However, no clear role for orexins has been established in the regulation of islet function and glucose metabolism [28].

**Galanin:** Galanin is widely distributed in the central and peripheral nervous systems and regulates a variety of physiological and pathological processes, including feeding, seizure, cognitive performance, mood, and pain threshold, by acting on three G-protein-coupled receptors, GalR1, GalR2, and GalR3 [29]. The evidence that galanin receptor is expressed in insulin producing cells and localization of the peptide in sympathetic nerve terminals that surround islet cells suggests that galanin is involved in the sympathetic regulation of islet function. Although general conclusions are complicated by species differences, galanin is reported to be a sympathetic neurotransmitter in islets inhibiting insulin secretion in many studies using mice, rats, and dogs [30, 31]. The pancreatic content of galanin is decreased in obese-diabetic *ob/ob* and *db/db* mice exhibiting hyperinsulinemia [32], suggesting that the peptide is involved in obesity-related hyperinsulinemia and  $\beta$ -cell failure.

**Somatostatin:** Somatostatin is a peptide hormone produced and released in neuroendocrine neurons in the hypothalamus and acts to suppress growth hormone (GH) secretion. It is also secreted in the gastro-intestinal system including stomach, intestine, and  $\delta$ -cells of the pancreas, and regulates gastro-intestinal motility, gastric acid flow, and secretions from the exocrine and endocrine pancreas [33]. Somatostatin is a potent inhibitor of insulin and glucagon secretion, and therefore indirectly regulates glucose homeostasis. Among the five somatostatin receptor subtypes ( $sst_1$  through  $sst_5$ ),  $sst_5$  is reported to be implicated in regulating  $\beta$ -cell insulin secretion [33]. A recent study reported that islets from  $sst_5$  knockout (KO) mice exhibit enhanced glucose-stimulated insulin secretion. The  $sst_5$  KO mice also exhibited improved insulin sensitivity compared to controls, resulting in attenuated obesity, insulin resistance, and improved glucose tolerance after high-fat feeding, suggesting a potential role for  $sst_5$  antagonists in improving metabolic abnormalities associated with obesity and insulin resistance [33]. Octreotide, a synthetic somatostatin analog, that is used for treatment of pituitary tumors and neuroendocrine tumors in the gastrointestinal tract, has also been shown to be effective in reducing insulin secretion and body weight in children with hypothalamic obesity [34]. Recent human studies suggest the analog is also effective in controlling adult obesity with hyperinsulinemia and retarding the progression of diabetic microvascular complications [35–37].

**Other Neuropeptides:** Arginine vasopressin (AVP) is a neurohypophyseal hormone that exerts a number of physiological roles in mammals in regulating body fluid volume, osmolality, and blood pressure. Although AVP does not act on the hypothalamus or affect appetite, it regulates glucose metabolism by its direct glycogenolytic and gluconeogenic effects on the liver and by modulating insulin and glucagon release from the endocrine pancreas, with the effects dependent on ambient glucose concentrations [38]. AVP has also been shown to potentiate insulin release synergistically with the hypothalamic neuropeptide corticotrophin-releasing hormone (CRH) by raising intracellular  $Ca^{2+}$  influx in mouse islet cells [39]. However, it is unclear whether these peptides are directly involved in obesity-related  $\beta$ -cell pathophysiology.

## Gut Peptides Regulating the Islet–Hypothalamic Axis

**Insulin:** It is well-known that insulin, which is secreted by the pancreatic  $\beta$ -cells to promote anabolic metabolism in peripheral tissues such as skeletal muscle and fat, plays important roles in the maintenance of islet  $\beta$ -cell function and mass. Mice lacking insulin receptors (IRs) in  $\beta$ -cells ( $\beta$ IRKO) exhibited a loss of glucose-stimulated insulin secretion and a progressive impairment of glucose tolerance with reduced  $\beta$ -cell mass [40]. Mice lacking functional receptors for both insulin and IGF-1 only in  $\beta$ -cells [41], and mice with  $\beta$ -cells deficient in PDK1, a common downstream mediator of both insulin and IGF-1 signaling [42], both develop early-onset overt diabetes due to loss of  $\beta$ -cell mass, indicating that  $\beta$ -cell failure leading

to T2D might result from a defect in insulin and IGF-1 signaling in pancreatic  $\beta$ -cells. Studies using BIRKO mice further demonstrated that functional IR signaling in  $\beta$ -cells is crucial for islet compensatory growth response to insulin resistance in mice fed with high-fat diet or mice with liver-specific IR knockout (LIRKO) [43].

Insulin is also known to enter the brain via the circulation and act as a hormonal signal in the regulation of body weight by the CNS [44]. As a consequence of increased positive energy balance and adiposity that is seen in obesity, circulating insulin is increased in proportion to body fat content, providing a feedback adiposity signal to the CNS to reduce energy intake [44, 45]. IRs are expressed in the hypothalamus, particularly in the arcuate nucleus (ARC), which is important in the regulation of food intake and energy homeostasis, and i.c.v. insulin infusion inhibits food intake [45]. Experimental evidence supports an important role of central insulin action in the regulation of energy and glucose homeostasis. Mice with a neuron-specific deletion of the IR gene throughout the brain (NIRKO) showed increased food intake and developed diet-sensitive obesity [46]. A selective decrease of the IR protein in the ARC neurons containing NPY and AgRP by an antisense oligodeoxynucleotide was accompanied by rapid onset of hyperphagia and increase in fat mass in rats [47]. Other studies have shown that the insulin effect in the hypothalamus is mediated by insulin receptor substrate 2 (IRS-2)-phosphatidylinositol 3-kinase (PI3K) pathway with a plausible neuronal cross-talk between insulin and leptin signaling [48–51].

Considering the evidence that reduced hypothalamic neuronal insulin signaling also causes hepatic insulin resistance [47] and subsequent increase in insulin demand in  $\beta$ -cells, alterations in hypothalamic insulin action may potentially be linked to  $\beta$ -cell failure in obese and insulin-resistant subjects. The studies on neural or  $\beta$ -cell insulin action discussed above, demonstrate that, besides insulin resistance in classical insulin target tissues, nonclassical insulin target tissues such as the brain and pancreatic  $\beta$ -cell likely act synergistically in the induction of obesity, insulin resistance, and glucose intolerance associated with T2D.

*Amylin:* Amylin, also known as islet amyloid polypeptide (IAPP), is a pancreatic  $\beta$ -cell hormone co-released with insulin in response to nutrient stimuli. The actions of the peptide, including reducing food intake, slowing gastric emptying, and reducing postprandial glucagon secretion, mainly through actions in the hypothalamus, are the basis for the use of a nonamyloidogenic analog, pramlintide, in the treatment of obesity and T2D [52]. Human, but not rodent, IAPP is capable of forming amyloid fibrils in pancreatic islet cells. The presence of islet amyloid deposition in a vast majority of individuals with T2D has led to the speculation that human IAPP (hIAPP) plays a role in the pathogenesis of  $\beta$ -cell failure observed in T2D [53, 54]. Both in vitro and in vivo studies have revealed that amyloid formation in islets causes islet  $\beta$ -cell death in obesity and insulin resistance. Animal studies using a transgenic mice expressing hIAPP in islet  $\beta$ -cells, a model of islet amyloid formation as it occurs in T2D, reported the transgenic mice fed a diet rich in fat led to an increase in islet amyloid formation, reduced islet  $\beta$ -cell mass, decrease in glucose-stimulated insulin secretion, and glucose intolerance; whereas nontransgenic mice, without islet amyloid deposition, adapted to the diet-induced obesity by increasing their islet mass and function [55–57].

Recent studies have suggested the IAPP-induced  $\beta$ -cell death is caused by apoptosis and/or reduced  $\beta$ -cell replication that could be associated with oxidative stress or endoplasmic reticulum (ER) stress induced by the HFD [54, 58, 59]. The rodent studies indicate that the amyloid, formed in islets of humans with obesity, is cytotoxic to  $\beta$ -cells and contributes to  $\beta$ -cell failure, leading them to diabetes. It remains to be understood how exactly hIAPP forms, aggregates, and causes cytotoxicity in islet  $\beta$ -cells [60, 61]. The 20–29 amino acid region of hIAPP, which is a highly amyloidogenic segment, has been suggested to be a target for the development of islet amyloid inhibitors [54].

**Ghrelin:** Ghrelin, a peptide predominantly produced in the stomach, is a natural ligand of the GH secretagogue receptor (GHS-R). It potently stimulates GH secretion and feeding by acting on the pituitary and hypothalamic arcuate nucleus, respectively [62, 63]. Ghrelin and GHS-Rs are also expressed in the pancreatic islet cells, as well as CNS, stomach, and intestine. Plasma ghrelin levels are negatively correlated with body weight, fasting insulin levels, and insulin resistance [62]. In humans and rodents, systemic administration of ghrelin has been shown to inhibit plasma insulin levels, followed by a persistent increase in plasma glucose levels. Ghrelin treatment inhibits, whereas GHS-R antagonists enhances glucose-induced insulin secretion (GSIS) and cytosolic  $\text{Ca}^{2+}$  concentration in isolated islets, indicating an inhibitory effect of the peptide on insulin release that contributes to restrain its secretory activity in the fasting state [62, 64]. Mice with targeted deletion of ghrelin (Ghr-KO) displayed essentially normal metabolic phenotypes when fed a regular diet; however, the Ghr-KO mice exhibited markedly enhanced insulin responses with reduced glycemia during GTT. Isolated islets from the Ghr-KO mice also showed greater insulin response to glucose than that of wild-type mice and the two groups showed no significant difference in islet size, number, or insulin content [62, 64].

The significance of islet ghrelin-GHS-R system in  $\beta$ -cell pathophysiology in obesity has been examined using the Ghr-KO mice. The HFD-induced glucose intolerance that was seen in wild-type mice, was largely prevented in Ghr-KO mice due to a markedly enhanced secretory insulin response to glucose [64, 65]. Deletion of ghrelin augmented GSIS and markedly improved hyperglycemia in genetically obese *ob/ob* mice, the effect of which was associated with reduction of UCP2 expression in the pancreatic islets [66]. Conversely, ghrelin transgenic (Tg) mice, whose ghrelin expression and production are increased in the stomach and brain, exhibited an increased circulating bioactive ghrelin, resulting in hyperphagia, glucose intolerance, and an attenuated GSIS [67]. Thus, ghrelin plays a role in regulating  $\beta$ -cell function and antagonism of ghrelin function may be a potential therapeutic approach for treating T2D by enhancing insulin secretion.

**GLP-1:** GLP-1 is secreted from intestinal endocrine L-cells in a nutrient-dependent manner, and directly regulates many aspects of pancreatic  $\beta$ -cell growth and function. GLP-1 stimulates glucose-dependent insulin secretion, promotes gene transcription, mRNA stability, and biosynthesis of insulin, improves  $\beta$ -cell glucose sensitivity, stimulates  $\beta$ -cell proliferation and neogenesis, and inhibits  $\beta$ -cell apoptosis, thereby improving  $\beta$ -cell function and increasing  $\beta$ -cell mass (reviewed in [68, 69]). GLP-1 has also been reported to inhibit glucagon and stimulate

somatostatin secretion (reviewed in [68, 70]. Increased intestinal GLP-1 production and enhanced sensitivity of islets to the incretin effect of GLP-1 was observed in animals with HFD-induced insulin resistance [71, 72].

Enhanced insulin production in response to insulin resistance has been reported to cause ER stress, perturbation of which leads to impairment of insulin biosynthesis  $\beta$ -cell survival, and glucose homeostasis [73]. GLP-1R activation has been shown to reduce ER stress-associated  $\beta$ -cell death [74], and this effect may contribute to the ability of GLP-1 to expand  $\beta$ -cell mass and enhance  $\beta$ -cell function and survival [2]. Besides its direct effects on the endocrine pancreas, GLP-1 also acts as a neurotransmitter to control gastric emptying, food intake, and body weight via mechanism involving the autonomic and the central nerves that express GLP-1 receptors (GLP-1R) or produce GLP-1 [68–70].

Several studies suggest that peripheral, gut-derived GLP-1 stimulates insulin secretion indirectly, in part, through an autonomic pathway, based on the observation of a rapid degradation of GLP-1 by DPP-IV in the peripheral circulation. Moreover, hypothalamic neurons expressing GLP-1 and GLP-1R regulate glucose metabolism by modulating autonomic output [70]. Interacerebroventricular (i.c.v.) administration or hypothalamic injection of GLP-1 or its agonist, exendin-4 has been shown to increase glucose-stimulated insulin secretion and reduce hepatic glucose production [70, 75, 76]. Based on these observations, the current understanding is that both brain and peripheral GLP-1 action play a role in the control of both feeding and glucose metabolism [70, 75, 76]. Therefore, GLP-1 could be among the important components of the “islet–hypothalamic axis” necessary for promoting  $\beta$ -cell adaptation and survival in insulin-resistant states in obesity.

## **Adipokines and Cytokines Regulating the Islet–Hypothalamic Axis**

*Leptin:* Leptin is an adipocyte-derived hormone that represents the endocrine function of adipose tissue, and mediates a wide range of physiological actions on energy and glucose homeostasis. Leptin is secreted by white adipose tissue in proportion to fat content and signals the status of body energy stores to the hypothalamus to regulate appetite and energy expenditure [77–79]. In addition to its well-characterized effects on body weight, leptin regulates glucose homeostasis both directly and indirectly through its actions on food intake and body weight [78, 80]. Furthermore, several studies suggest that leptin can directly impact glucose metabolism that is independent of its central effect on body weight reduction. Thus, the brain is a key target for the glucose-lowering actions of leptin, since i.c.v., but not peripheral, administration of leptin, and increased CNS expression of leptin in insulin resistant, obese/diabetic animals have all been found to improve peripheral insulin sensitivity and decrease insulin secretion [6, 78, 80]. Interestingly, although reduced insulin secretion by leptin administration appears to be secondary to the improvement of peripheral insulin sensitivity via its action on the CNS, growing evidence indicates that leptin can directly regulate

insulin release and  $\beta$ -cell growth by acting via its receptors (ObRb) expressed in the pancreatic  $\beta$ -cells [11, 12]. These data provide important evidence that leptin can act in the periphery independent of its actions on the CNS.

In vitro studies have shown an inhibitory effect of leptin on insulin gene expression and insulin release in clonal  $\beta$ -cell lines and in isolated murine and human islets [12, 81, 82]. The acute inhibitory effect of leptin on insulin release in leptin-deficient *ob/ob* mice has been demonstrated in several studies [81, 82]. Furthermore, in obese/diabetic rodent models lacking systemic leptin action, such as *ob/ob*, *db/db* mice, and Zucker fatty rats, hyperinsulinemia and/or increased  $\beta$ -cell mass were observed to precede the development of obesity and insulin resistance [12, 83]. These in vivo findings indicate that hyperinsulinemia and islet hyperplasia in the mutant animals is due, in part, to a lack of leptin signaling in  $\beta$ -cells. A direct role for leptin signaling on  $\beta$ -cell function and growth has been recently investigated in genetically engineered mice that lack leptin receptors (ObRs) either specifically in  $\beta$ -cells [84] or the whole pancreas [85]. Both models exhibited fasting hyperinsulinemia and an increase in islet mass, possibly due primarily to the absence of leptin action in  $\beta$ -cells. The mice lacking ObRs in pancreas also exhibited enhanced glucose-stimulated insulin secretion both in vivo and in vitro, leading to improved glucose tolerance, supporting an inhibitory role for leptin on insulin secretion in agreement with previous studies [85]. Intriguingly, the pancreas-ObR KO mice were susceptible to HFD-induced glucose intolerance compared to the control mice, due to a failure of compensatory  $\beta$ -cell secretory function and islet expansion, consistent with the concept that leptin protects islets from lipid overload and subsequent “lipoapoptosis,” as suggested by previous reports [86, 87].

Based on these experimental data, it is conceivable that leptin resistance at the level of the  $\beta$ -cell plays a role in regulating  $\beta$ -cell function. For example, obese people are resistant to hypothalamic effects of leptin on satiety and energy expenditure despite higher leptin levels in circulation and are “leptin resistant” [78]. Thus, in obese individuals, increased adiposity and prolonged elevated plasma leptin levels potentially promote  $\beta$ -cells to become unresponsive to leptin action, leading to chronic hyperinsulinemia – a characteristic feature of obesity and T2D. This would also lead to insulin resistance in  $\beta$ -cells and consequent defects in secretory function [43]. Finally, leptin-resistant  $\beta$ -cells may also become sensitive to lipid-induced  $\beta$ -cell dysfunction, leading to  $\beta$ -cell failure and overt diabetes.

**Adiponectin:** Adiponectin is an adipokine secreted exclusively by the white adipose tissue and is present at relatively high concentrations in circulation [88]. It increases peripheral insulin sensitivity and a decrease in circulating adiponectin in obesity has been suggested to contribute to insulin resistance, metabolic syndrome, glucose intolerance, and atherosclerosis [88, 89]. Administration of a fragment of adiponectin that contains the globular domain to rodents increases lipid oxidation and glucose uptake in muscle, reduces hepatic glucose production, and improves whole body insulin sensitivity through the activation of AMP-activated protein kinase (AMPK) in the peripheral tissues [90, 91]. In addition to its peripheral actions, this adipokine has also been shown to mediate regulation of energy homeostasis via its central actions. Indeed, adiponectin receptors, AdipoR1 and



AdipoR2 [92], have been shown to be abundantly expressed in the hypothalamus, and adiponectin is known to cross the blood–brain barrier from the systemic circulation [93, 94]. A recent study reported that adiponectin enhances AMPK activity in the arcuate hypothalamus (ARH) and stimulates food intake under fasting conditions, and that it also decreases energy consumption in mice [94].

The findings that serum and CSF adiponectin levels and AdipoR1 expression in the ARH are increased during fasting and decreased after refeeding suggest that adiponectin acts mainly during fasting conditions to serve as a starvation hormone to promote fat storage [88, 94]. Adiponectin has also been shown to play a role in pancreatic  $\beta$ -cell function and survival via its receptors AdipoR1 and AdipoR2 which are both expressed in islet  $\beta$ -cells. Other studies have reported that adiponectin inhibits fatty acid-, or glucotoxicity-induced  $\beta$ -cell apoptosis and prevents fatty acid-induced secretory dysfunction in INS-1 rat insulinoma cell lines [95, 96]. In vitro studies have also demonstrated that adiponectin augments insulin secretion from isolated rodent islets at both low [97] and high [98] glucose concentrations. Studies in humans have indicated that a low-circulating adiponectin is associated with  $\beta$ -cell dysfunction and insulin resistance [99–103]. Thus, low plasma adiponectin levels observed in obese subjects could be one of many factors contributing to  $\beta$ -cell failure and consequent development of T2D in obese humans.

**Resistin:** Resistin, a cysteine-rich protein secreted by adipocytes in rodents and macrophages in humans, is one of the adipocyte-derived hormones implicated in insulin resistance, inflammation, and impaired glucose homeostasis in obesity [104]. Following its discovery [105], studies indicate that increased circulating resistin in genetic- or diet-induced obesity is a primary cause of hepatic insulin resistance, which is mediated by resistin-induced SOCS-3 or inhibition of AMP-kinase activity in the liver [106–108]. Resistin mRNA and protein are expressed in rodent hypothalamus [104] and recent studies demonstrated that resistin delivered to the cerebral ventricle (i.c.v) stimulated hepatic glucose production by impairing insulin action and inducing proinflammatory cytokines (TNF- $\alpha$ , IL-6, and SOCS-3) in the liver [109, 110]. Central resistin was shown to activate hypothalamic neurons and cause impaired hepatic insulin sensitivity and hyperglycemia by increasing orexigenic NPY expression in hypothalamus [110].

Resistin is also expressed in pancreatic islets at relatively high levels in humans and rodents [111]. An adenovirus-mediated transient increase in circulating resistin in mice resulted in impaired glucose tolerance due to both insulin resistance and impaired insulin secretory response to glucose [112]. In vitro studies, albeit from only a few available so far, have demonstrated that a long-term exposure (~24 h) of isolated mouse islets, or  $\beta$ -cell lines, to resistin, at high concentrations, caused insulin secretory dysfunction by inducing insulin resistance in  $\beta$ -cells [112, 113], and that it also caused a significant increase in  $\beta$ -cell viability at lower concentrations [113]. These reports indicate that resistin delivered from adipose tissue is involved in insulin resistance partly by acting in the CNS and also by regulating  $\beta$ -cell function/viability and is one potential mechanism by which increased adiposity causes  $\beta$ -cell dysfunction in obese states.

**Interleukin-6:** Interleukin-6 (IL-6) is a multifunctional cytokine that is produced by the immune system and other tissues such as the adipose and contracting skeletal

muscle. Increased circulating IL-6 is related to obesity, insulin resistance, and cardiovascular morbidity, and many studies indicate its pro-inflammatory effects contribute to insulin resistance in liver and adipose tissue [114]. IL-6 appears to have central actions. For example, mice deficient in IL-6 were shown to develop maturity-onset obesity due to lack of central action of IL-6 to increase energy expenditure [115]. Since chronic i.c.v. administration of IL-6 decreased body fat mass and food intake in rats [116], and IL-6 concentrations in CSF are negatively correlated with body fat mass in humans [117], IL-6 appears to exert an antiobesity effect through its central actions [114]. IL-6 also plays direct roles in pancreatic islets. IL-6 has been shown to stimulate insulin secretion and increase the viability of  $\beta$ -cells in rodent pancreatic islets and some clonal  $\beta$ -cell lines [11].

These effects of IL-6 indicate that it has protective effects on  $\beta$ -cells and that the elevation of IL-6 production in obese and T2D individuals may be involved in the  $\beta$ -cell compensation for insulin resistance. Supporting this possibility, a recent clinical study indicated that IL-6, but not TNF- $\alpha$ , independently contributed to the hyperinsulinemia in subjects with excess visceral adiposity [118]. Another recent study has shown that the  $\alpha$ -cell is a primary target of IL-6 action in pancreatic islets and that IL-6 regulates pro-glucagon production and glucagon secretion, stimulates  $\alpha$ -cell proliferation, and inhibits  $\alpha$ -cell apoptosis induced by high glucose and free-fatty acid in vitro [119]. This study further showed the expansion of  $\alpha$ -cell mass in response to HFD-feeding, which was evident before changes in  $\beta$ -cell mass, is dependent on IL-6 in vivo and suggested that it's required for functional  $\beta$ -cell compensation for increased metabolic demand in insulin resistance [119].

## Potential Therapeutic Approaches to Pancreatic $\beta$ -Cell Failure in Obesity

Several neuropeptides that control appetite also expressed and act at the level of pancreatic islets to regulate  $\beta$ -cell function. Those peptides are possibly involved in modulating islet function and mass to compensate for insulin resistance in obesity. Among them, according to the recent animal studies discussed above, reduced local action of NPY, galanin, and ghrelin in islets, all of which stimulate appetite, appears to contribute to islet compensation for insulin resistance in obesity. The local presence of the orexigenic peptide, MCH, also appears to be required for islet adaptation to insulin resistance. Upregulating local MCH or downregulating local NPY action in islets, and systemic antagonism of ghrelin could be potential therapeutic approaches to promote  $\beta$ -cell function and mass in obesity-related diabetes. The incretin hormone, GLP-1, which acts on the hypothalamus to suppress appetite, seems to be involved both in promoting islet compensation and preventing islet failure in obesity-related diabetes. The GLP-1 analog and DPP-IV inhibitors, which are currently available for diabetes treatment, could be protective for  $\beta$ -cells and prevent diabetes progression in patients with obesity.

A number of adipokines and cytokines could modulate the islet-hypothalamic axis, to regulate both appetite and islet pathophysiology in individuals manifesting



obesity and insulin resistance. Among these adipokines, reduced leptin action or “leptin resistance,” in both hypothalamus and pancreatic islets, could be associated with both hyperinsulinemia and islet expansion in response to insulin resistance on the one hand, and with islet failure possibly secondary to  $\beta$ -cell lipotoxicity in obesity-related diabetes on the other. Increased levels of IL-6 and mild elevation of resistin in obesity appear to contribute to islet adaptation, whereas low adiponectin and high resistin levels in obesity seem to be associated with  $\beta$ -cell failure in insulin resistance, leading to diabetes. The antidiabetic class of drugs, thiazolidinediones (TZDs), have been reported to increase body weight in most clinical studies and to positively regulate food consumption and energy balance in animals [120, 121]. However, many studies have shown that TZDs improve  $\beta$ -cell function in T2D patients [122, 123] and slow the rate of loss of  $\beta$ -cell function to a greater extent than did metformin or sulphonylureas in recently diagnosed T2D patients [124]. Recent studies have shown that TZD treatment modulates circulating adipocytokine levels including adiponectin, FFA, TNF- $\alpha$ , and leptin [125], reduces islet amyloid deposition [126, 127], decreases islet triglyceride content [128, 129], and improves ER stress in  $\beta$ -cells [130], each of which directly or indirectly leads to improvement of  $\beta$ -cell function and survival in individuals with obesity and insulin resistance.

## Conclusions

Teleologically, the concept of an islet–hypothalamus axis plays an important role in the maintenance of glucose homeostasis. Bioactive factors from metabolic tissues including adipose tissue, gut, skeletal muscle, and endocrine pancreas, may all modulate the function of this “axis” to control both islet adaptation and failure in insulin resistance in obesity. The islet–hypothalamic axis should be considered a significant ‘node’, and actions of these regulatory peptides both at the hypothalamus and the islet should be considered when developing therapeutic approaches to treat obesity-related diabetes.

**Acknowledgments** The authors thank Lindsay Huse and Kellianne Parlee for excellent assistance with the manuscript. R.N. Kulkarni is supported by grant RO1 DK 67536 from the National Institutes of Health and the American Diabetes Association. Tomoaki Morioka was supported by a grant from the Noguchi Medical Research Institute.

## References

1. Muoio, D. M. & Newgard, C. B. (2008). Mechanisms of disease: Molecular and metabolic mechanisms of insulin resistance and beta-cell failure in type 2 diabetes. *Nature Reviews Molecular Cell Biology* 9(3), 193–205.
2. Prentki, M. & Nolan, C. J. (2006). Islet beta cell failure in type 2 diabetes. *The Journal of Clinical Investigation* 116(7), 1802–1812.
3. Kahn, S. E., Hull, R. L., & Utzschneider, K. M. (2006). Mechanisms linking obesity to insulin resistance and type 2 diabetes. *Nature* 444(7121), 840–846.

4. Kasuga, M. (2006). Insulin resistance and pancreatic beta cell failure. *The Journal of Clinical Investigation* 116(7), 1756–1760.
5. Lingohr, M. K., Buettner, R., & Rhodes, C. J. (2002). Pancreatic beta-cell growth and survival – a role in obesity-linked type 2 diabetes? *Trends in Molecular Medicine* 8(8), 375–384.
6. Sandoval, D. A., Obici, S., & Seeley, R. J. (2009). Targeting the CNS to treat type 2 diabetes. *Nature Reviews Drug Discovery* 8(5), 386–398.
7. Schwartz, M. W. & Porte, D., Jr. (2005). Diabetes, obesity, and the brain. *Science* 307(5708), 375–379.
8. He, W., Lam, T. K., Obici, S., & Rossetti, L. (2006). Molecular disruption of hypothalamic nutrient sensing induces obesity. *Nature Neuroscience* 9(2), 227–233.
9. Ahren, B. (2000). Autonomic regulation of islet hormone secretion – implications for health and disease. *Diabetologia* 43(4), 393–410.
10. Konturek, S. J., Pepera, J., Zabielski, K., et al., (2003). Brain-gut axis in pancreatic secretion and appetite control. *Journal of Physiology and Pharmacology: An Official Journal of the Polish Physiological Society* 54(3), 293–317.
11. Zhao, Y. F., Feng, D. D., & Chen, C. (2006). Contribution of adipocyte-derived factors to beta-cell dysfunction in diabetes. *The International Journal of Biochemistry & Cell Biology* 38(5–6), 804–819.
12. Kieffer, T. J. & Habener, J. F. (2000). The adipoinsular axis: Effects of leptin on pancreatic beta-cells. *American Journal of Physiology Endocrinology and Metabolism* 278(1), E1–E14.
13. Shimada, M., Tritos, N. A., Lowell, B. B., Flier, J. S., & Maratos-Flier, E. (1998). Mice lacking melanin-concentrating hormone are hypophagic and lean. *Nature* 396(6712), 670–674.
14. Pissios, P. & Maratos-Flier, E. (2003). Melanin-concentrating hormone: From fish skin to skinny mammals. *Trends in Endocrinology and Metabolism: TEM* 14(5), 243–248.
15. Ludwig, D. S., Tritos, N. A., Mastaitis, J. W., Kulkarni, R., Kokkotou, E., Elmquist, J., et al., (2001). Melanin-concentrating hormone overexpression in transgenic mice leads to obesity and insulin resistance. *The Journal of Clinical Investigation* 107(3), 379–386.
16. Jeon, J. Y., Bradley, R. L., Kokkotou, E. G., Marino, F. E., Wang, X., Pissios, P., et al., (2006). MCH-/- mice are resistant to aging-associated increases in body weight and insulin resistance. *Diabetes* 55(2), 428–434.
17. Pissios, P., Ozcan, U., Kokkotou, E., Okada, T., Liew, C. W., Liu, S., et al., (2007). Melanin concentrating hormone is a novel regulator of islet function and growth. *Diabetes* 56(2), 311–319.
18. Tadayyon, M., Welters, H. J., Haynes, A. C., Cluderay, J. E., & Hervieu, G. (2000). Expression of melanin-concentrating hormone receptors in insulin-producing cells: MCH stimulates insulin release in RINm5F and CRI-G1 cell-lines. *Biochemical and Biophysical Research Communications* 275(2), 709–712.
19. Beck, B. (2006). Neuropeptide Y in normal eating and in genetic and dietary-induced obesity. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* 361(1471), 1159–1185.
20. Raposinho, P. D., Pierroz, D. D., Broqua, P., White, R. B., Pedrazzini, T., & Aubert, M. L. (2001). Chronic administration of neuropeptide Y into the lateral ventricle of C57BL/6J male mice produces an obesity syndrome including hyperphagia, hyperleptinemia, insulin resistance, and hypogonadism. *Molecular and Cellular Endocrinology* 185(1–2), 195–204.
21. Kalra, S. P. & Kalra, P. S. (2004). NPY and cohorts in regulating appetite, obesity and metabolic syndrome: Beneficial effects of gene therapy. *Neuropeptides* 38(4), 201–211.
22. Kuo, L. E., Kitlinska, J. B., Tilan, J. U., Li, L., Baker, S. B., Johnson, M. D., et al., (2007). Neuropeptide Y acts directly in the periphery on fat tissue and mediates stress-induced obesity and metabolic syndrome. *Nature Medicine* 13(7), 803–811.
23. Myrsen-Axerona, U., Karlsson, S., Sundler, F., & Ahren, B. (1997). Dexamethasone induces neuropeptide Y (NPY) expression and impairs insulin release in the insulin-producing cell line RINm5F. Release of NPY and insulin through different pathways. *The Journal of Biological Chemistry* 272(16), 10790–10796.
24. Morgan, D. G., Kulkarni, R. N., Hurley, J. D., Wang, Z.L., Wang, R.M., Ghatei, M.A., et al., (1998). Inhibition of glucose stimulated insulin secretion by neuropeptide Y is mediated via

- the Y1 receptor and inhibition of adenylyl cyclase in RIN 5AH rat insulinoma cells. *Diabetologia* 41(12), 1482–1491.
25. Imai, Y., Patel, H. R., Hawkins, E. J., Doliba, N. M., Matschinsky, F. M., & Ahima, R. S. (2007). Insulin secretion is increased in pancreatic islets of neuropeptide Y-deficient mice. *Endocrinology* 148(12), 5716–5723.
  26. Kirchgessner, A. L. (2002). Orexins in the brain-gut axis. *Endocrine Reviews* 23(1), 1–15.
  27. Sakurai, T., Amemiya, A., Ishii, M., Matsuzaki, I., Chemelli, R.M., Tanaka, H., et al., (1998). Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. *Cell* 92(4), 573–585.
  28. Heinonen, M. V., Purhonen, A. K., Makela, K. A., & Herzog, K. H. (2008). Functions of orexins in peripheral tissues. *Acta Physiologica (Oxford, England)* 192(4), 471–485.
  29. Mitsukawa, K., Lu, X., & Bartfai, T. (2008). Galanin, galanin receptors and drug targets. *Cellular and Molecular Life Sciences: CMLS* 65(12), 1796–1805.
  30. Manabe, T., Okada, Y., Sawai, H., Funahashi, H., Yamamoto, M., Hayakawa, T., et al., (2003). Effect of galanin on plasma glucose, insulin and pancreatic glucagon in dogs. *The Journal of International Medical Research* 31(2), 126–132.
  31. Ahren, B., Pacini, G., Wynick, D., Wierup, N., & Sundler, F. (2004). Loss-of-function mutation of the galanin gene is associated with perturbed islet function in mice. *Endocrinology* 145(7), 3190–3196.
  32. Dunning, B. E. & Ahren, B. (1992). Reduced pancreatic content of the inhibitory neurotransmitter galanin in genetically obese, hyperinsulinemic mice. *Pancreas* 7(2), 233–239.
  33. Strowski, M. Z., Kohler, M., Chen, H. Y., Trumbauer, M. E., Li, Z., Szalkowski, D., et al., (2003). Somatostatin receptor subtype 5 regulates insulin secretion and glucose homeostasis. *Molecular Endocrinology (Baltimore, Md.)* 17(1), 93–106.
  34. Tzotzas, T., Papazisis, K., Perros, P., & Krassas, G. E. (2008). Use of somatostatin analogues in obesity. *Drugs* 68(14), 1963–1973.
  35. Boehm, B. O. & Lustig, R. H. (2002). Use of somatostatin receptor ligands in obesity and diabetic complications. *Best Practice & Research. Clinical Gastroenterology* 16(3), 493–509.
  36. Boehm, B. O. (2003). The therapeutic potential of somatostatin receptor ligands in the treatment of obesity and diabetes. *Expert Opinion on Investigational Drugs* 12(9), 1501–1509.
  37. Lustig, R. H., Greenway, F., Velasquez-Mieyer, P., Heimbürger, D., Schumacher, D., Smith, D., et al., (2006). A multicenter, randomized, double-blind, placebo-controlled, dose-finding trial of a long-acting formulation of octreotide in promoting weight loss in obese adults with insulin hypersecretion. *International Journal of Obesity (London)* 30(2), 331–341.
  38. Abu-Basha, E. A., Yibchok-Anun, S., & Hsu, W. H. (2002). Glucose dependency of arginine vasopressin-induced insulin and glucagon release from the perfused rat pancreas. *Metabolism: Clinical and Experimental* 51(9), 1184–1190.
  39. O'Carroll, A. M., Howell, G. M., Roberts, E. M., & Lolait, S. J. (2008). Vasopressin potentiates corticotropin-releasing hormone-induced insulin release from mouse pancreatic beta-cells. *The Journal of Endocrinology* 197(2), 231–239.
  40. Kulkarni, R. N., Bruning, J. C., Winnay, J. N., Postic, C., Magnuson, M. A., & Kahn, C. R. (1999). Tissue-specific knockout of the insulin receptor in pancreatic beta cells creates an insulin secretory defect similar to that in type 2 diabetes. *Cell* 96(3), 329–339.
  41. Ueki, K., Okada, T., Hu, J., Liew, C.W., Assmann, A., Dahlgren, G. M., et al., (2006). Total insulin and IGF-I resistance in pancreatic beta cells causes overt diabetes. *Nature Genetics* 38(5), 583–588.
  42. Hashimoto, N., Kido, Y., Uchida, T., Asahara, S., Shiqeyama, Y., Matsuda, T., et al., (2006). Ablation of PDK1 in pancreatic beta cells induces diabetes as a result of loss of beta cell mass. *Nature Genetics* 38(5), 589–593.
  43. Okada, T., Liew, C. W., Hu, J., Hinault, C., Michael, M. D., Krtzfeldt, J., et al., (2007). Insulin receptors in beta-cells are critical for islet compensatory growth response to insulin resistance. *Proceedings of the National Academy of Sciences of the United States of America* 104(21), 8977–8982.

44. Schwartz, M. W., Woods, S. C., Porte, D., Jr., Seeley, R. J., & Baskin, D. G. (2000). Central nervous system control of food intake. *Nature* 404(6778), 661–671.
45. Baskin, D. G., Figlewicz Lattemann, D., Seeley, R. J., Woods, S. C., Porte, D., Jr., & Schwartz, M. W. (1999). Insulin and leptin: Dual adiposity signals to the brain for the regulation of food intake and body weight. *Brain Research* 848(1–2), 114–123.
46. Bruning, J. C., Gautam, D., Burks, D. J., Gillette, J., Schubert, M., Orban, P. C., et al., (2000). Role of brain insulin receptor in control of body weight and reproduction. *Science* 289(5487), 2122–2125.
47. Obici, S., Feng, Z., Karkanias, G., Baskin, D. G., & Rossetti, L. (2002). Decreasing hypothalamic insulin receptors causes hyperphagia and insulin resistance in rats. *Nature Neuroscience* 5(6), 566–572.
48. Kubota, N., Terauchi, Y., Tobe, K., Yano, W., Suuki, R., Ueki, K., et al., (2004). Insulin receptor substrate 2 plays a crucial role in beta cells and the hypothalamus. *The Journal of Clinical Investigation* 114(7), 917–927.
49. Lin, X., Taguchi, A., Park, S., Kushner, J. A., Li, F., Li, Y., et al., (2004). Dysregulation of insulin receptor substrate 2 in beta cells and brain causes obesity and diabetes. *The Journal of Clinical Investigation* 114(7), 908–916.
50. Niswender, K. D., Morrison, C. D., Clegg, D. J., Olson, R., Baskin, D. G., Myers, M. G. Jr., et al., (2003). Insulin activation of phosphatidylinositol 3-kinase in the hypothalamic arcuate nucleus: A key mediator of insulin-induced anorexia. *Diabetes* 52(2), 227–231.
51. Porte, D., Jr., Baskin, D. G., & Schwartz, M. W. (2005). Insulin signaling in the central nervous system: a critical role in metabolic homeostasis and disease from *C. elegans* to humans. *Diabetes* 54(5), 1264–1276.
52. Lutz, T. A. (2009). Control of food intake and energy expenditure by amylin-therapeutic implications. *International Journal of Obesity (London)* 33(Suppl 1), S24–S27.
53. Hoppener, J. W. & Lips, C. J. (2006). Role of islet amyloid in type 2 diabetes mellitus. *The International Journal of Biochemistry & Cell Biology* 38(5–6), 726–736.
54. Hull, R. L., Westermark, G. T., Westermark, P., & Kahn, S. E. (2004). Islet amyloid: A critical entity in the pathogenesis of type 2 diabetes. *The Journal of Clinical Endocrinology and Metabolism* 89(8), 3629–3643.
55. Hull, R. L., Andrikopoulos, S., Verchere, C. B., Vidal, J., Wang, F., Cnop, M., et al., (2003). Increased dietary fat promotes islet amyloid formation and beta-cell secretory dysfunction in a transgenic mouse model of islet amyloid. *Diabetes* 52(2), 372–379.
56. Butler, A. E., Janson, J., Soeller, W. C., & Butler, P. C. (2003). Increased beta-cell apoptosis prevents adaptive increase in beta-cell mass in mouse model of type 2 diabetes: Evidence for role of islet amyloid formation rather than direct action of amyloid. *Diabetes* 52(9), 2304–2314.
57. Matveyenko, A. V., Gurlo, T., Daval, M., Butler, A. E., & Butler, P. C. (2009). Successful versus failed adaptation to high-fat diet-induced insulin resistance: The role of IAPP-induced beta-cell endoplasmic reticulum stress. *Diabetes* 58(4), 906–916.
58. Matveyenko, A. V. & Butler, P. C. (2006). Beta-cell deficit due to increased apoptosis in the human islet amyloid polypeptide transgenic (HIP) rat recapitulates the metabolic defects present in type 2 diabetes. *Diabetes* 55(7), 2106–2114.
59. Zraika, S., Hull, R. L., Udayasankar, J., Aston-Mourmey, K., Subramanian, S. L., Kisilevsky, R., et al., (2009). Oxidative stress is induced by islet amyloid formation and time-dependently mediates amyloid-induced beta cell apoptosis. *Diabetologia* 52(4), 626–635.
60. Brender, J. R., Durr, U. H., Heyl, D., Budarapu, M. B., & Ramamoorthy, A. (2007). Membrane fragmentation by an amyloidogenic fragment of human Islet Amyloid Polypeptide detected by solid-state NMR spectroscopy of membrane nanotubes. *Biochimica et biophysica acta* 1768(9), 2026–2029.
61. Jiang, P., Xu, W., & Mu, Y. (2009). Amyloidogenesis abolished by proline substitutions but enhanced by lipid binding. *PLoS Computational Biology* 5(4), e1000357.
62. Broglio, F., Gottero, C., Benso, A., Prodam, F., Volante, M., Destefanis, S., et al., (2003). Ghrelin and the endocrine pancreas. *Endocrine* 22(1), 19–24.

63. Kojima, M. & Kangawa, K. (2006). Drug insight: The functions of ghrelin and its potential as a multitherapeutic hormone. *Nature Clinical Practice. Endocrinology & Metabolism* 2(2), 80–88.
64. Yada, T., Dezaki, K., Sone, H., Koizumi, M., Damdindorj, B., Nakata, M., et al., (2008). Ghrelin regulates insulin release and glycemia: Physiological role and therapeutic potential. *Current Diabetes Reviews*, 4(1), 18–23.
65. Dezaki, K., Sone, H., Koizumi, M., Nakata, M., Kakei, M., Nagai, H., et al., (2006). Blockade of pancreatic islet-derived ghrelin enhances insulin secretion to prevent high-fat diet-induced glucose intolerance. *Diabetes* 55(12), 3486–3493.
66. Sun, Y., Asnicar, M., Saha, P. K., Chan, L., & Smith, R. G. (2006). Ablation of ghrelin improves the diabetic but not obese phenotype of ob/ob mice. *Cell Metabolism* 3(5), 379–386.
67. Bewick, G. A., Kent, A., Campbell, D., Patterson, M., Ghatei, M. A., Bloom, S. R., et al., (2009). Mice with hyperghrelinemia are hyperphagic and glucose intolerant and have reduced leptin sensitivity. *Diabetes* 58(4), 840–846.
68. Baggio, L. L. & Drucker, D. J. (2007). Biology of incretins: GLP-1 and GIP. *Gastroenterology* 132(6), 2131–2157.
69. Drucker, D. J. (2006). The biology of incretin hormones. *Cell Metabolism* 3(3), 153–165.
70. Williams, D. L. (2009). Finding the sweet spot: Peripheral vs. central GLP-1 action in feeding and glucose homeostasis. *Endocrinology*, doi:10.1210/en.2009-0220.
71. van Citters, G. W., Kabir, M., Kim, S. P., Mittelman, S. D., Dea, M. K., Brubaker, P. L., et al., (2002). Elevated glucagon-like peptide-1-(7–36)-amide, but not glucose, associated with hyperinsulinemic compensation for fat feeding. *The Journal of Clinical Endocrinology and Metabolism* 87(11), 5191–5198.
72. Nolan, C. J., Leahy, J. L., Delghingaro-Augusto, V., Moibi, J., Soni, K., Peyot, M. L., et al., (2006). Beta cell compensation for insulin resistance in Zucker fatty rats: Increased lipolysis and fatty acid signalling. *Diabetologia* 49(9), 2120–2130.
73. Eizirik, D. L., Cardozo, A. K., & Cnop, M. (2008). The role for endoplasmic reticulum stress in diabetes mellitus. *Endocrine Reviews* 29(1), 42–61.
74. Yusta, B., Baggio, L. L., Estall, J. L., Koehler, J. A., Holland, D. P., Li, H., et al., (2006). GLP-1 receptor activation improves beta cell function and survival following induction of endoplasmic reticulum stress. *Cell Metabolism* 4(5), 391–406.
75. Sandoval, D. A., Bagnol, D., Woods, S. C., D'Alessio, D. A., & Seeley, R. J. (2008). Arcuate glucagon-like peptide 1 receptors regulate glucose homeostasis but not food intake. *Diabetes* 57(8), 2046–2054.
76. Knauf, C., Cani, P. D., Perrin, C., Iglesias, M. A., Maury, J. F., Bernard, E., et al., (2005). Brain glucagon-like peptide-1 increases insulin secretion and muscle insulin resistance to favor hepatic glycogen storage. *The Journal of Clinical Investigation* 115(12), 3554–3563.
77. Trayhurn, P. & Bing, C. (2006). Appetite and energy balance signals from adipocytes. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* 361(1471), 1237–1249.
78. Ahima, R. S., Qi, Y., & Singhal, N. S. (2006). Adipokines that link obesity and diabetes to the hypothalamus. *Progress in Brain Research* 153, 155–174.
79. Villanueva, E. C. & Myers, M. G., Jr. (2008). Leptin receptor signaling and the regulation of mammalian physiology. *International Journal of Obesity (London)* 32(Suppl 7), S8–S12.
80. Morton, G. J. (2007). Hypothalamic leptin regulation of energy homeostasis and glucose metabolism. *The Journal of Physiology* 583(Pt 2), 437–443.
81. Kulkarni, R. N., Wang, Z. L., Wang, R. M., Hurley, J. D., Smith, D. M., Ghatei, M. A., et al., (1997). Leptin rapidly suppresses insulin release from insulinoma cells, rat and human islets and, in vivo, in mice. *The Journal of Clinical Investigation* 100(11), 2729–2736.
82. Seufert, J., Kieffer, T. J., & Habener, J. F. (1999). Leptin inhibits insulin gene transcription and reverses hyperinsulinemia in leptin-deficient ob/ob mice. *Proceedings of the National Academy of Sciences of the United States of America* 96(2), 674–679.
83. Jetton, T. L., Lausier, J., LaRock, K., Trotman, W. E., Larmie, B., Habibovic, A., et al., (2005). Mechanisms of compensatory beta-cell growth in insulin-resistant rats: Roles of Akt kinase. *Diabetes* 54(8), 2294–2304.

84. Covey, S. D., Wideman, R. D., McDonald, C., Unniappan, S., Huynh, F., Asadi, A., et al., (2006). The pancreatic beta cell is a key site for mediating the effects of leptin on glucose homeostasis. *Cell Metabolism* 4(4), 291–302.
85. Morioka, T., Asilmaz, E., Hu, J., Dishinger, J. F., Rurpad, A. J., Elias, C. F., et al., (2007). Disruption of leptin receptor expression in the pancreas directly affects beta cell growth and function in mice. *The Journal of Clinical Investigation* 117(10), 2860–2868.
86. Poirout, V. & Robertson, R. P. (2002). Minireview: Secondary beta-cell failure in type 2 diabetes – a convergence of glucotoxicity and lipotoxicity. *Endocrinology* 143(2), 339–342.
87. Unger, R. H. & Zhou, Y. T. (2001). Lipotoxicity of beta-cells in obesity and in other causes of fatty acid spillover. *Diabetes* 50(Suppl 1), S118–S121.
88. Kadowaki, T., Yamauchi, T., Kubota, N., Hara, K., Ueki, K., & Tobe, K. (2006). Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. *The Journal of Clinical Investigation* 116(7), 1784–1792.
89. Meier, U. & Gressner, A. M. (2004). Endocrine regulation of energy metabolism: review of pathobiochemical and clinical chemical aspects of leptin, ghrelin, adiponectin, and resistin. *Clinical Chemistry* 50(9), 1511–1525.
90. Yamauchi, T., Kamon, J., Minokoshi, Y., Ito, Y., Waki, H., Uchida, S., et al., (2002). Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. *Nature Medicine* 8(11), 1288–1295.
91. Yamauchi, T., Kamon, J., Waki, H., Terauchi, Y., Kubota, N., Hara, K., et al., (2001). The fat-derived hormone adiponectin reverses insulin resistance associated with both lipodystrophy and obesity. *Nature Medicine* 7(8), 941–946.
92. Yamauchi, T., Kamon, J., Ito, Y., Tsuchida, A., Yokomizo, T., Kita, S., et al., (2003). Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. *Nature* 423(6941), 762–769.
93. Qi, Y., Takahashi, N., Hileman, S. M., Patel, H. R., Berg, A. H., Pajvani, U. B., et al., (2004). Adiponectin acts in the brain to decrease body weight. *Nature Medicine* 10(5), 524–529.
94. Kubota, N., Yano, W., Kubota, T., Yamauchi, T., Itoh, S., Kumagai, H., et al., (2007). Adiponectin stimulates AMP-activated protein kinase in the hypothalamus and increases food intake. *Cell Metabolism* 6(1), 55–68.
95. Rakatzi, I., Mueller, H., Ritzeler, O., Tennagels, N., & Eckel, J. (2004). Adiponectin counteracts cytokine- and fatty acid-induced apoptosis in the pancreatic beta-cell line INS-1. *Diabetologia* 47(2), 249–258.
96. Lin, P., Chen, L., Li, D., Liu, J., Yang, N., Sun, Y., et al., (2009). Adiponectin reduces glucotoxicity-induced apoptosis of INS-1 rat insulin-secreting cells on a microfluidic chip. *The Tohoku Journal of Experimental Medicine* 217(1), 59–65.
97. Okamoto, M., Ohara-Imaizumi, M., Kubota, N., Hashimoto, S., Eto, K., Kanno, T., et al., (2008). Adiponectin induces insulin secretion in vitro and in vivo at a low glucose concentration. *Diabetologia* 51(5), 827–835.
98. Gu, W., Li, X., Liu, C., Yang, J., Ye, L., Tang, J., et al., (2006). Globular adiponectin augments insulin secretion from pancreatic islet beta cells at high glucose concentrations. *Endocrine* 30(2), 217–221.
99. Bacha, F., Saad, R., Gungor, N., & Arslanian, S. A. (2004). Adiponectin in youth: Relationship to visceral adiposity, insulin sensitivity, and beta-cell function. *Diabetes care* 27(2), 547–552.
100. Osei, K., Gaillard, T., & Schuster, D. (2005). Plasma adiponectin levels in high risk African-Americans with normal glucose tolerance, impaired glucose tolerance, and type 2 diabetes. *Obesity Research* 13(1), 179–185.
101. Retnakaran, R., Hanley, A. J., Raif, N., Hirning, C. R., Connelly, P. W., Sermer, M., et al., (2005). Adiponectin and beta cell dysfunction in gestational diabetes: pathophysiological implications. *Diabetologia* 48(5), 993–1001.
102. Chailurkit, L. O., Chanprasertyotin, S., Jongjaroenprasert, W., & Ongphiphadhanakul, B. (2008). Differences in insulin sensitivity, pancreatic beta cell function and circulating adiponectin across glucose tolerance status in Thai obese and non-obese women. *Endocrine* 33(1), 84–89.



103. Thamer, C., Haap, M., Heller, E., Joel, L., Brain, S., Tschritter, O., et al., (2006). Beta cell function, insulin resistance and plasma adiponectin concentrations are predictors for the change of postprandial glucose in non-diabetic subjects at risk for type 2 diabetes. *Hormone and Metabolic Research. Hormon- und Stoffwechselforschung. Hormones et metabolisme* 38(3), 178–182.
104. Steppan, C. M. & Lazar, M. A. (2004). The current biology of resistin. *Journal of Internal Medicine* 255(4), 439–447.
105. Steppan, C. M., Bailey, S. T., Bhat, S., Brown, E. J., Bangerjee, R. R., Wright, C. M., et al., (2001). The hormone resistin links obesity to diabetes. *Nature* 409(6818), 307–312.
106. Banerjee, R. R., Rangwala, S. M., Shapiro, J. S., Rich, A. S., Rhoades, B., Qi, Y., et al., (2004). Regulation of fasted blood glucose by resistin. *Science* 303(5661), 1195–1198.
107. Qi, Y., Nie, Z., Lee, Y. S., Singhal, N. S., Scherer, P. E., Lazar, M. A., et al., (2006). Loss of resistin improves glucose homeostasis in leptin deficiency. *Diabetes* 55(11), 3083–3090.
108. Muse, E. D., Obici, S., Bhanot, S., Monica, B. P., McKay, R. A., Rajala, M. W., et al., (2004). Role of resistin in diet-induced hepatic insulin resistance. *The Journal of Clinical Investigation* 114(2), 232–239.
109. Muse, E. D., Lam, T. K., Scherer, P. E., & Rossetti, L. (2007). Hypothalamic resistin induces hepatic insulin resistance. *The Journal of Clinical Investigation* 117(6), 1670–1678.
110. Singhal, N. S., Lazar, M. A., & Ahima, R. S. (2007). Central resistin induces hepatic insulin resistance via neuropeptide Y. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 27(47), 12924–12932.
111. Minn, A. H., Patterson, N. B., Pack, S., Hoffmann, S. C., Gavrilova, O., Vinson, C., et al., (2003). Resistin is expressed in pancreatic islets. *Biochemical and Biophysical Research Communications* 310(2), 641–645.
112. Nakata, M., Okada, T., Ozawa, K., & Yada, T. (2007). Resistin induces insulin resistance in pancreatic islets to impair glucose-induced insulin release. *Biochemical and Biophysical Research Communications* 353(4), 1046–1051.
113. Brown, J. E., Onyango, D. J., & Dunmore, S. J. (2007). Resistin down-regulates insulin receptor expression, and modulates cell viability in rodent pancreatic beta-cells. *FEBS Letters* 581(17), 3273–3276.
114. Hoene, M. & Weigert, C. (2008). The role of interleukin-6 in insulin resistance, body fat distribution and energy balance. *Obesity Reviews: An Official Journal of the International Association for the Study of Obesity* 9(1), 20–29.
115. Wallenius, V., Wallenius, K., Ahren, B., Rudling, M., Carlsten, H., Dickson, S. L., et al., (2002). Interleukin-6-deficient mice develop mature-onset obesity. *Nature Medicine* 8(1), 75–79.
116. Wallenius, K., Wallenius, V., Sunter, D., Dickson, S. L., & Jansson, J. O. (2002). Intracerebroventricular interleukin-6 treatment decreases body fat in rats. *Biochemical and Biophysical Research Communications* 293(1), 560–565.
117. Stenlof, K., Wernstedt, I., Fjallman, T., Wallenius, V., Wallenius, K., & Jansson, J. O. (2003). Interleukin-6 levels in the central nervous system are negatively correlated with fat mass in overweight/obese subjects. *The Journal of Clinical Endocrinology and Metabolism* 88(9), 4379–4383.
118. Cartier, A., Lemieux, I., Almeras, N., Tremblay, A., Bergeron, J., & Despres, J. P. (2008). Visceral obesity and plasma glucose-insulin homeostasis: contributions of interleukin-6 and tumor necrosis factor-alpha in men. *The Journal of Clinical Endocrinology and Metabolism* 93(5), 1931–1938.
119. Ellingsgaard, H., Ehses, J. A., Hammar, E. B., Van Lommel, L., Quintes, R., Martens, G., et al., (2008). Interleukin-6 regulates pancreatic alpha-cell mass expansion. *Proceedings of the National Academy of Sciences of the United States of America* 105(35), 13163–13168.
120. Larsen, P. J., Wulff, E. M., Gotfredsen, C. F., Brand, C. I., Sturis, J., Vrang, N., et al., (2008). Combination of the insulin sensitizer, pioglitazone, and the long-acting GLP-1 human analog, liraglutide, exerts potent synergistic glucose-lowering efficacy in severely diabetic ZDF rats. *Diabetes, Obesity & Metabolism* 10(4), 301–311.

121. Festuccia, W. T., Oztezcan, S., Laplante, M., Berthiaume, M., Michel, C., Dohgu, S., et al., (2008). Peroxisome proliferator-activated receptor-gamma-mediated positive energy balance in the rat is associated with reduced sympathetic drive to adipose tissues and thyroid status. *Endocrinology* 149(5), 2121–2130.
122. Dorkhan, M., Magnusson, M., Frid, A., Grubb, A., Groop, L., & Jovinge, S. (2006). Glycaemic and nonglycaemic effects of pioglitazone in triple oral therapy of patients with type 2 diabetes. *Journal of Internal Medicine* 260(2), 125–133.
123. Gastaldelli, A., Ferrannini, E., Miyazaki, Y., Matsuda, M., Mari, A., & DeFronzo, R. A. (2007). Thiazolidinediones improve beta-cell function in type 2 diabetic patients. *American Journal of Physiology. Endocrinology and Metabolism* 292(3), E871–E883.
124. Kahn, S. E., Haffner, S. M., Heise, M. A., Herman, W.H., Holman, R. R., Jones, N. P., et al., (2006). Glycemic durability of rosiglitazone, metformin, or glyburide monotherapy. *The New England Journal of Medicine* 355(23), 2427–2443.
125. Miyazaki, Y., Mahankali, A., Wajsborg, E., Bajaj, M., Mandarino, L. J., & DeFronzo, R. A. (2004). Effect of pioglitazone on circulating adipocytokine levels and insulin sensitivity in type 2 diabetic patients. *The Journal of Clinical Endocrinology and Metabolism* 89(9), 4312–4319.
126. Lin, C. Y., Gurlo, T., Haataja, L., Hsueh, W. A., & Butler, P. C. (2005). Activation of peroxisome proliferator-activated receptor-gamma by rosiglitazone protects human islet cells against human islet amyloid polypeptide toxicity by a phosphatidylinositol 3'-kinase-dependent pathway. *The Journal of Clinical Endocrinology and Metabolism* 90(12), 6678–6686.
127. Hull, R. L., Shen, Z. P., Watts, M. R., Kodama, K., Carr, D. B., Utzschneider, K. M., et al., (2005). Long-term treatment with rosiglitazone and metformin reduces the extent of, but does not prevent, islet amyloid deposition in mice expressing the gene for human islet amyloid polypeptide. *Diabetes* 54(7), 2235–2244.
128. Shimabukuro, M., Zhou, Y. T., Lee, Y., & Unger, R. H. (1998). Troglitazone lowers islet fat and restores beta cell function of Zucker diabetic fatty rats. *The Journal of Biological Chemistry* 273(6), 3547–3550.
129. Matsui, J., Terauchi, Y., Kubota, N., Takamoto, I., Eto, K., Yamashita, T., et al., (2004). Pioglitazone reduces islet triglyceride content and restores impaired glucose-stimulated insulin secretion in heterozygous peroxisome proliferator-activated receptor-gamma-deficient mice on a high-fat diet. *Diabetes* 53(11), 2844–2854.
130. Evans-Molina, C., Robbins, R. D., Kono, T., Tersey, S. A., Vestermarck, G. L., Nunemaker, C. S., et al., (2009). Peroxisome proliferator-activated receptor gamma activation restores islet function in diabetic mice through reduction of endoplasmic reticulum stress and maintenance of euchromatin structure. *Molecular and Cellular Biology* 29(8), 2053–2067.





# Chapter 12

## Non-Alcoholic Fatty Liver Disease and the Metabolic Syndrome

Sonia M. Najjar

### Introduction

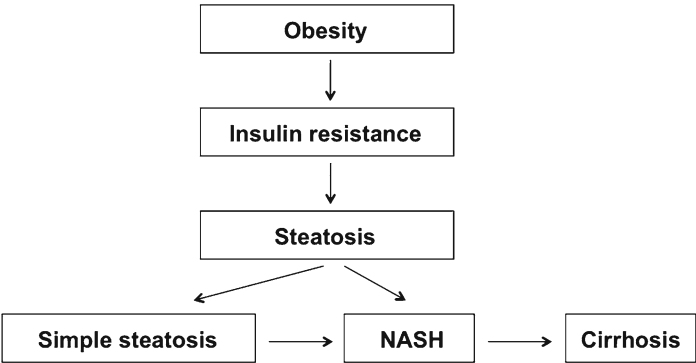
Non-alcoholic fatty liver disease (NAFLD) is a constellation of diseases ranging from benign hepatic steatosis to fibrosing nonalcoholic steatohepatitis (NASH) [1, 2]. The incidence of NAFLD is growing worldwide in parallel to the epidemic rise of obesity and metabolic syndrome [3]. It is currently estimated to be at ~20–25% in the general population, and at ~75–90% in the morbidly obese population; ~3–5% of patients with NAFLD will develop the more progressive form, NASH [4, 5] (Fig. 1). With NASH progressing to cirrhosis and/or hepatocellular carcinoma [6], the disease is projected to become the leading liver disease and cause of liver transplantation due to cirrhosis in western countries [7]. In 1980, Ludwig et al. [8] first described NASH in a small pool of patients with no history of alcoholism. Histologically, these patients exhibited liver macrosteatosis with inflammatory infiltrates, Mallory bodies, fibrosis, and cirrhosis.

The pathogenesis of NASH is not fully elucidated, but a “multiple-hit” hypothesis, previously referred to as the “two-hit hypothesis” has gained traction in recent years [9]. According to this hypothesis, hepatic steatosis develops initially (first hit) (Fig. 2), and predisposes to lipid peroxidation and inflammation, leading to hepatitis, hepatocyte loss by apoptosis, fibrosis and ultimately, cirrhosis. With liver steatosis being mechanistically linked to insulin action, it is natural to associate NAFLD with insulin resistance (Fig. 3). However, this notion remains somehow controversial, largely because of the limited availability of animal models that replicate the human disease. This review will discuss novel mechanisms linking NASH pathogenesis to the Carcino-Embryonic Antigen-related Cell Adhesion Molecule 1 (CEACAM1), a protein that regulates insulin sensitivity by mediating hepatic insulin clearance [10, 11] (Fig. 4), and a negative acute effect of insulin on fatty acid synthase (FAS) activity [12]. CEACAM1 also exerts an anti-inflammatory effect [13].

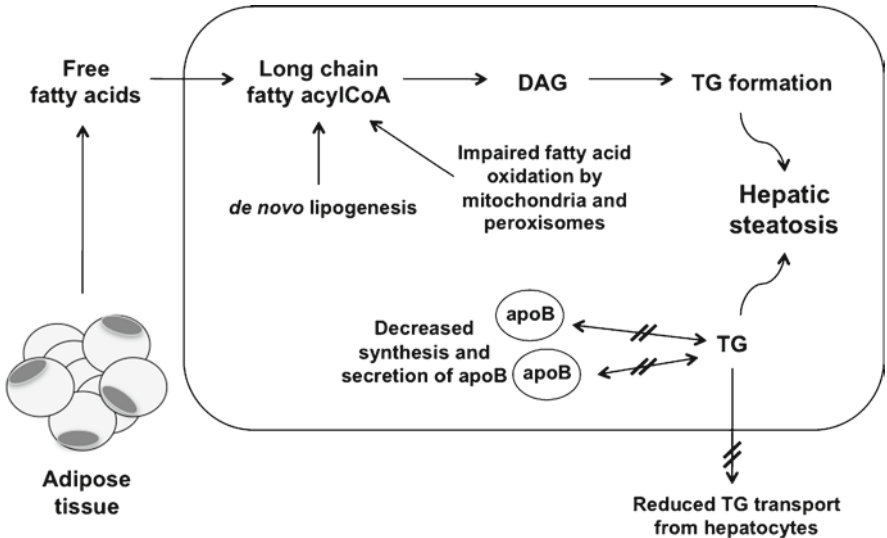
---

S.M. Najjar (✉)

Department of Physiology and Pharmacology, Center for Diabetes and Endocrine Research,  
University of Toledo College of Medicine, Health Science Campus, 3000 Arlington Avenue,  
Mail Stop 1008, Block Health Science Building, CeDER, Toledo, OH, USA, 43614-2598  
e-mail: Sonia.Najjar@utoledo.edu

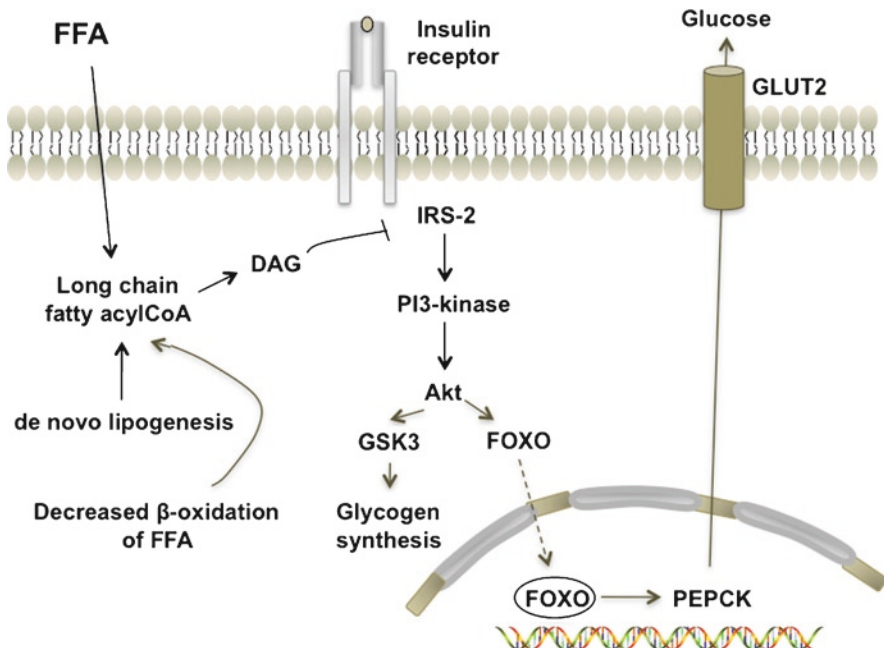


**Fig. 1** Natural history of Non-alcoholic fatty liver disease (NAFLD). Obesity is associated with insulin resistance and hepatic steatosis. A subset of patients (~5%) develop nonalcoholic steatohepatitis (NASH), which may progress to cirrhosis

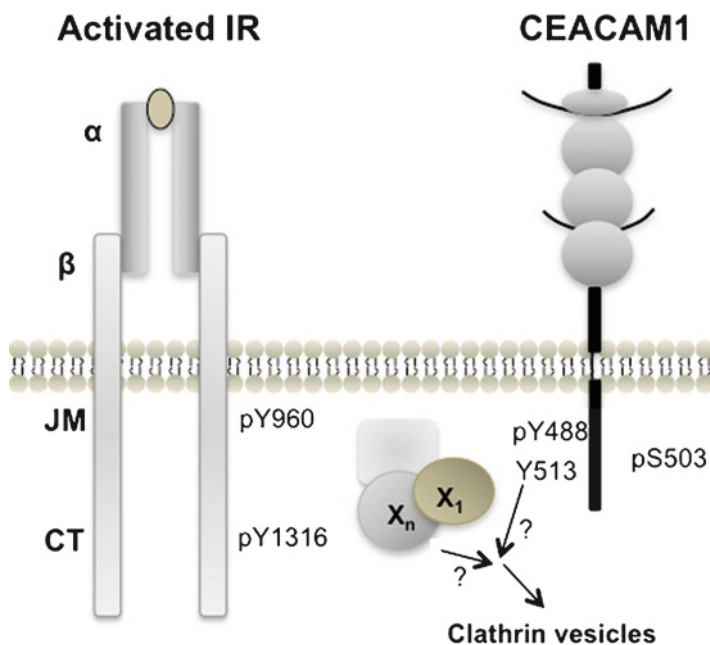


**Fig. 2** Pathogenesis of hepatic steatosis. In obese individuals, insulin resistance in adipose tissue increases lipolysis, leading to enhanced hepatic fatty acid influx, generation of LCFACoAs, and triglyceride formation. De novo hepatic lipogenesis is also increased in obesity. On the other hand, hepatic fatty acid oxidation is attenuated in obesity. The result is a net accumulation of hepatic triglycerides, i.e., steatosis

**Fig. 4** (continued) binding phosphorylates the receptor at many sites, including Y960 and Y1316 of the  $\beta$ -subunit of the IR. Phosphorylation of Y1316 regulates phosphorylation of CEACAM1 on Y488, causing CEACAM1 binding to an intracellular molecule (X1), which then interacts with phosphorylated Y960 in IR. CEACAM1, through Y513 and/or other proteins targets insulin for degradation



**Fig. 3** Hepatic insulin resistance in NAFLD. Diacylglycerol (DAG) content is increased as a result of free fatty acids (FFA) influx from circulation or increased de novo lipogenesis. DAGs activate protein kinase C (PKC), which inhibits the insulin signaling cascade



**Fig. 4** Model of insulin endocytosis mediated through Carcino-Embryonic Antigen-related Cell Adhesion Molecule (CEACAM). Activation of tyrosine kinase of the insulin receptor (IR) by insulin

## **NAFLD and the Metabolic Syndrome**

As pointed out by Marra et al. [14], NASH can be viewed as the liver manifestation of the metabolic syndrome. Abundant epidemiological evidence indicates that the two conditions are often associated, and that treating the metabolic syndrome improves liver function. The metabolic syndrome is also known as insulin resistance and is characterized clinically by increased body fat content, dyslipidemia, increased blood pressure, and by biochemical features of reduced insulin action, such as fasting hyperinsulinemia, impaired glucose tolerance, and reduced glucose disposal during glucose clamps. It is therefore all the more surprising that no clear-cut molecular mechanism linking hepatic insulin resistance to NAFLD has been identified [15]. This could be due to the fact that insulin affects virtually all the pathways implicated in the pathogenesis of NAFLD, including lipid and glucose metabolism, cellular turnover and survival, production of inflammatory cytokines, and fibrogenesis. The main stumbling block appears to be that insulin affects these processes in different ways: for example, hepatocyte survival is decreased, consistent with resistance to the pro-survival actions of insulin, but lipogenesis is increased, consistent with sensitivity to insulin's lipogenic actions [16–19]. Reconciling these disparate findings is challenging and this could cast doubt about a positive relationship between insulin resistance and NAFLD.

## **The Intertwined Paths of Insulin and Lipid Metabolism in the Pathogenesis of Hepatic Steatosis**

Insulin action is tightly regulated by insulin and fat metabolism in liver. By promoting insulin clearance [10, 11] and mediating a decrease in FAS activity [12], CEACAM1 is well positioned to act as a unifying mechanism for the regulation of insulin action and lipid metabolism in liver.

Insulin resistance is a key factor in the etiology of metabolic diseases, and is commonly associated with hyperinsulinemia. Considerable evidence in humans supports the view that impaired hepatic insulin extraction causes chronic hyperinsulinemia in obesity [20, 21]. Hyperinsulinemia, caused by impaired insulin clearance, worsens insulin resistance by downregulating insulin receptors and escalating *de novo* lipogenesis, by virtue of activating the nuclear sterol regulatory element-binding protein 1c (SREBP-1c), a master transcriptional regulator of lipogenic enzymes, including FAS [22].

Studies on the role of CEACAM1 in insulin clearance provide more convincing evidence that hyperinsulinemia causes insulin resistance [10]. CEACAM1, a trans-membrane glycoprotein in liver, but not muscle or adipose tissue [23], undergoes phosphorylation on tyrosine (Tyr<sup>488</sup>) by the insulin receptor tyrosine kinase [24]. This requires an intact serine (Ser<sup>503</sup>) residue. Whereas other substrates of the insulin receptor

mediate insulin action by taking part in the insulin signaling pathways, CEACAM1 regulates insulin action by promoting insulin extraction via endocytotic vesicular insulin uptake and degradation. This finding is buttressed by impaired hepatic insulin clearance and resulting hyperinsulinemia in mice expressing a liver-specific dominant-negative, phosphorylation-defective S503A CEACAM1 mutant (L-SACC1), null mutant mice (*Cc1<sup>-/-</sup>*) [10, 11], and mice with activated SH-containing phosphatase-1 (SHP-1), which dephosphorylates CEACAM1 [25]. Hyperinsulinemic clamp studies reveal that hyperinsulinemia causes secondary insulin resistance in these mice [11, 25, 26]. Ceacam1 mutant mice also develop liver steatosis, resulting from the lipogenic effect of chronic hyperinsulinemia. This increases triglyceride output and redistribution to white adipose tissue, as reflected by increased visceral obesity.

The phenotype of Ceacam1 mutant mice demonstrates a connection between insulin clearance and insulin action in lipid metabolism [27]. It reveals that impaired insulin clearance causes hyperinsulinemia and subsequently, hepatic insulin resistance and increased hepatic de novo fatty acid synthesis. Based on the normal physiology of insulin action, one would predict that insulin resistance would not be associated with increased hepatic triglyceride content [18, 19], as demonstrated by unaltered triglyceride synthesis in the liver-specific insulin receptor knockout mouse (LIRKO) [28]. However, unlike LIRKO, Ceacam1 mutant mice maintain a certain level of insulin receptor signaling. This may explain the peculiar admixture of insulin sensitivity (increased lipogenesis) and resistance (altered glucose homeostasis), observed in these mice. This mixed insulin sensitivity-insulin resistance is the defining feature of NASH [17], and for this reason the Ceacam1 mutant model could be used as a useful tool to investigate the relationship between insulin resistance and hepatic steatosis.

FAS, a key enzyme in the de novo synthesis of fatty acids, is highly expressed in liver and to a lower extent, in white visceral adipose tissue [29]. In contrast to the long-term positive effect of insulin on FAS transcription, we have presented evidence that insulin acutely decreases FAS activity in liver, but not in adipose tissue [12]. The decrease in hepatic FAS activity depends on the ability of insulin to induce CEACAM1 phosphorylation, internalization as part of the insulin endocytosis complex and binding to FAS. We propose that insulin acutely decreases FAS activity to limit lipogenesis and protect the liver against higher levels of insulin in the portal circulation [30]. The negative effect of insulin on FAS activity is abolished in chronic hyperinsulinemia, in light of reduced insulin signaling and CEACAM1 phosphorylation. Together with increase FAS levels as a consequence of activating SREBP-1c by elevated insulin levels, which may result from altered insulin removal in liver, this leads to increased de novo lipogenesis and accumulation of fatty acids in microsomal compartments.

In addition to fatty acid synthesis, CEACAM1 exerts a downregulatory effect on the de novo synthesis of cholesterol [31] and the accumulation of free cholesterol in mitochondria, as suggested by reduced Niemann Pick type C1 (NPC-1) in the liver of L-SACC1 mice [32]. With the latter being an important determinant of glutathione (GSH) level and progression to steatohepatitis [33], this suggests that loss-of-CEACAM1 is at the crossroads of altered insulin metabolism and action and hepatic steatosis, and progression to steatohepatitis.

## Pathogenesis of NASH

NAFLD is a multi-faceted disease. The mechanisms underlying the pathogenesis of the progressive form-NASH are not well delineated. The genetic and environmental factors underlying the disease and the progression of fibrosing steatohepatitis have not been fully identified. The “multiple-hit” hypothesis has only slightly promoted our understanding of the pathogenesis of the disease and its progression. Several factors contribute to this limitation. In addition to the mixed insulin sensitivity-insulin resistance in metabolic diseases, the paucity of animal models that replicate adequately all features of the human disease [34, 35] has not helped resolve the issue concerning the relationship between insulin resistance and hepatic steatosis. Few models develop some of the clinical manifestation of the disease, in particular its progressive NASH form [36, 37]. The most common method to induce fibrosing steatohepatitis is the use of a methionine-choline deficient diet in animals. However, this diet does not cause insulin resistance, and NASH patients do not develop methionine or choline deficiency. Mice with liver-specific null deletion of the inositol-phosphatase Pten exhibit severe steatosis while maintaining insulin sensitivity [38]. Transgenic mice with adipose tissue-specific expression of SREBP-1c display marked steatosis and histological changes similar to NASH [39], but they also develop severe insulin resistance. With much dissimilarity with human NASH, these models fail to adequately probe the role of insulin resistance in the disease process.

In contrast, Ceacam1 mutant mice which manifest insulin resistance resulting from hyperinsulinemia, display features of benign NAFLD when fed a regular chow diet, and of the most progressive form -NASH when fed a high-fat diet [32]. Mechanistically, this progression implicates a rise in TNF $\alpha$  secretion from activated resident macrophages and presentation to the increased pool of intrahepatic CD4+ T cells [40]. In addition to infiltrated adipokines from the white adipose tissue, excessive lipid accumulation in the hepatocyte activates liver macrophages [40, 41]. Progression to steatohepatitis involves a Th1 cytokine response, which is characterized by increased release of cytokines from intrahepatic CD4+ T cells [42–44]. With CEACAM1 mediating an anti-inflammatory effect in T cell [13], which depends on SHP-1 activation and on the phosphorylation of immunoreceptor tyrosine-based inhibition motifs (ITIM) within the cytoplasmic domain [45], inactivating Ceacam1 in hepatocytes would limit the CEACAM1-dependent inhibitory responses in T lymphocytes and lead to a robust inflammatory response to cytokines. Thus, progression to steatohepatitis in Ceacam1 mutant mice results from increased accumulation of free cholesterol and hence, reduction in the GSH-defense system against elevated levels of cytotoxic TNF $\alpha$ , which by activating T cells, can lead to a robust inflammatory response especially in light of the loss of the anti-inflammatory function of CEACAM1.

The Ceacam1 mutant mice provide evidence that CEACAM1 protects against NASH in a cell-autonomous fashion through its actions in the hepatocyte, which include prevention of metabolic (insulin resistance and increased triglyceride synthesis) and inflammatory abnormalities (Th1 cytokine response). With its



shared role in all these processes, loss-of-CEACAM1 function provides a unifying underlying mechanism of progressive NAFLD, which results from the culmination of multiple interconnected abnormalities in insulin action, lipid metabolism, and inflammatory response.

## Conclusions

NAFLD is becoming an epidemic in parallel to obesity and metabolic syndrome. It is predicted that the incidence of the disease would be higher if better diagnostic means are developed. Identifying the mechanisms linking insulin action with hepatocyte response to oxidative stress, regulation of lipid synthesis, and inflammatory function, can be exploited for diagnosis and treatment of NASH. It can also lead to the development of strategies to prevent the progression from steatosis to steatohepatitis and cirrhosis.

## References

1. Marrero, J. A., Fontana, R. J., Su, G. L., Conjeevaram, H. S., Emick, D. M., & Lok, A. S. (2002). NAFLD may be a common underlying liver disease in patients with hepatocellular carcinoma in the United States. *Hepatology*, 36(6), 1349–1354.
2. Bullock, R. E., Zaitoun, A. M., Aithal, G. P., Ryder, S. D., Beekingham, I. J., & Lobo, D. N. (2004). Association of non-alcoholic steatohepatitis without significant fibrosis with hepatocellular carcinoma. *Journal of Hepatology*, 41(4), 685–686.
3. Erickson, S. K. (2009). Nonalcoholic fatty liver disease. *Journal of Lipid Research*, 50(Suppl), S412–S416.
4. Lazo, M., & Clark, J. M. (2008). The epidemiology of nonalcoholic fatty liver disease: A global perspective. *Seminars in Liver Disease*, 28(4), 339–350.
5. Ong, J. P., & Younossi, Z. M. (2007). Epidemiology and natural history of NAFLD and NASH. *Clinics in Liver Disease*, 11, 1–16.
6. Hui, J. M., Kench, J. G., Chitturi, S., et al. (2003). Long-term outcomes of cirrhosis in nonalcoholic steatohepatitis compared with hepatitis C. *Hepatology*, 38, 420–427.
7. Clark, J. M. (2006). The epidemiology of nonalcoholic fatty liver disease in adults. *Journal of Clinical Gastroenterology*, 40(3 Suppl 1), S5–S10.
8. Ludwig, J., Viggiano, T. R., McGill, D. B., & Oh, B. J. (1980). Nonalcoholic steatohepatitis: Mayo Clinic experiences with a hitherto unnamed disease. *Mayo Clinic Proceedings. Mayo Clinic*, 55, 434–438.
9. Day, C. P., & James, O. F. (1998). Steatohepatitis: A tale of two “hits”? *Gastroenterology*, 114, 842–845.
10. Poy, M. N., Yang, Y., Rezaei, K., et al. (2002). CEACAM1 regulates insulin clearance in liver. *Nature Genetics*, 30(3), 270–276.
11. DeAngelis, A. M., Heinrich, G., Dai, T., et al. (2008). Carcinoembryonic antigen-related cell adhesion molecule 1: A link between insulin and lipid metabolism. *Diabetes*, 57(9), 2296–2303.
12. Najjar, S. M., Yang, Y., Fernstrom, M. A., et al. (2005). Insulin acutely decreases hepatic fatty acid synthase activity. *Cell Metabolism*, 2, 43–53.

13. Gray-Owen, S. D., & Blumberg, R. S. (2006). CEACAM1: Contact-dependent control of immunity. *Nature Reviews. Immunology*, 6, 433–446.
14. Marra, F., Gastaldelli, A., Svegliati Baroni, G., Tell, G., & Tiribelli, C. (2008). Molecular basis and mechanisms of progression of non-alcoholic steatohepatitis. *Trends in Molecular Medicine*, 14, 72–81.
15. Farrell, G. C. (2009). The liver and the waistline: Fifty years of growth. *Journal of Gastroenterology and Hepatology*, 24(Suppl 3), S105–S118.
16. Choi, S. S., & Diehl, A. M. (2008). Hepatic triglyceride synthesis and nonalcoholic fatty liver disease. *Current Opinion in Lipidology*, 19(3), 295–300.
17. Ginsberg, H. N. (2006). Is the slippery slope from steatosis to steatohepatitis paved with triglyceride or cholesterol? *Cell Metabolism*, 4, 179–181.
18. Haeusler, R. A., & Accili, D. (2008). The double life of Irs. *Cell Metabolism*, 8, 7–9.
19. Brown, M. S., & Goldstein, J. L. (2008). Selective versus total insulin resistance: A pathogenic paradox. *Cell Metabolism*, 7, 95–96.
20. Polonsky, K. S., Given, B. D., Hirsch, L., et al. (1988). Quantitative study of insulin secretion and clearance in normal and obese subjects. *Journal of Clinical Investigation*, 81, 435–441.
21. Escobar, O., Mizuma, H., Sothorn, M. S., et al. (1999). Hepatic insulin clearance increases after weight loss in obese children and adolescents. *American Journal of the Medical Sciences*, 317, 282–286.
22. Horton, J. D., Goldstein, J. L., & Brown, M. S. (2002). SREBPs: Activators of the complete program of cholesterol and fatty acid synthesis in the liver. *Journal of Clinical Investigation*, 109, 1125–1131.
23. Accili, D., Perrotti, N., Rees, J. R., & Taylor, S. I. (1986). Tissue distribution and subcellular localization of an endogenous substrate (pp120) for the insulin receptor-associated tyrosine kinase. *Endocrinology*, 119, 1274–1280.
24. Najjar, S. M., Philippe, N., Suzuki, Y., et al. (1995). Insulin-stimulated phosphorylation of recombinant pp120/HA4, an endogenous substrate of the insulin receptor tyrosine kinase. *Biochemistry*, 34, 9341–9349.
25. Dubois, M. J., Bergeron, S., Kim, H. J., et al. (2006). The SHP-1 protein tyrosine phosphatase negatively modulates glucose homeostasis. *Nature Medicine*, 12, 549–556.
26. Park, S. Y., Cho, Y. R., Kim, H. J., et al. (2006). Mechanism of glucose intolerance in mice with dominant negative mutation of CEACAM1. *American Journal of Physiology. Endocrinology and Metabolism*, 291, E517–E524.
27. Bergman, R. N. (2000). Non-esterified fatty acids and the liver: Why is insulin secreted into the portal vein? *Diabetologia*, 43, 946–952.
28. Biddinger, S. B., Hernandez-Ono, A., Rask-Madsen, C., et al. (2008). Hepatic insulin resistance is sufficient to produce dyslipidemia and susceptibility to atherosclerosis. *Cell Metabolism*, 7, 125–134.
29. Cantley, L. C. (2002). The phosphoinositide 3-kinase pathway. *Science*, 296, 1655–1657. Review.
30. Ward, G. M., Walters, J. M., Aitken, P. M., Best, J. D., & Alford, F. P. (1990). Effects of prolonged pulsatile hyperinsulinemia in humans. Enhancement of insulin sensitivity. *Diabetes*, 39(4), 501–507.
31. Lee, S. J., Heinrich, G., Fedorova, L., et al. (2008). Development of non-alcoholic steatohepatitis in insulin resistant L-SACC1 mice. *Gastroenterology*, 135, 2084–2095.
32. Lee, S. J., Heinrich, G., Fedorova, L., et al. (2008). Development of nonalcoholic steatohepatitis in insulin-resistant liver-specific S503A carcinoembryonic antigen-related cell adhesion molecule 1 mutant mice. *Gastroenterology*, 135(6), 2084–2095.
33. Mari, M., Caballero, F., Colell, A., et al. (2006). Mitochondrial free cholesterol loading sensitizes to TNF- and Fas-mediated steatohepatitis. *Cell Metabolism*, 4, 185–198.
34. Ariz, U., Mato, J. M., Lu, S. C., & Martinez Chantar, M. L. (2010). Nonalcoholic steatohepatitis, animal models, and biomarkers: What is new? *Methods in Molecular Biology (Clifton, N.J.)*, 593, 109–136.
35. Anstee, Q. M., & Goldin, R. D. (2006). Mouse models in non-alcoholic fatty liver disease and steatohepatitis research. *International Journal of Experimental Pathology*, 87(1), 1–16.

36. Green, R. M. (2003). NASH – hepatic metabolism and not simply the metabolic syndrome. *Hepatology*, 38(1), 14–17.
37. Otagawa, K., Kinoshita, K., Fujii, H., et al. (2007). Erythrophagocytosis by liver macrophages (Kupffer cells) promotes oxidative stress, inflammation, and fibrosis in a rabbit model of steatohepatitis: Implications for the pathogenesis of human nonalcoholic steatohepatitis. *American Journal of Pathology*, 170, 967–980.
38. Kurlawalla-Martinez, C., Stiles, B., Wang, Y., Devaskar, S. U., Kahn, B. B., & Wu, H. (2005). Insulin hypersensitivity and resistance to streptozotocin-induced diabetes in mice lacking PTEN in adipose tissue. *Molecular and Cellular Biology*, 25, 2498–2510.
39. Nakayama, H., Otabe, S., Ueno, T., et al. (2007). Transgenic mice expressing nuclear sterol regulatory element-binding protein 1c in adipose tissue exhibit liver histology similar to nonalcoholic steatohepatitis. *Metabolism*, 56, 470–475.
40. Bigorgne, A. E., Bouchet-Delbos, L., Naveau, S., et al. (2008). Obesity-induced lymphocyte hyperresponsiveness to chemokines: A new mechanism of fatty liver inflammation in obese mice. *Gastroenterology*, 134, 1459–1469.
41. Sheth, S. G., Gordon, F. D., & Chopra, S. (1997). Nonalcoholic steatohepatitis. *Annals of Internal Medicine*, 126, 137–145.
42. Li, Z., Soloski, M. J., & Diehl, A. M. (2005). Dietary factors alter hepatic innate immune system in mice with nonalcoholic fatty liver disease. *Hepatology*, 42, 880–885.
43. Kremer, M., Hines, I. N., Milton, R. J., & Wheeler, M. D. (2006). Favored T helper 1 response in a mouse model of hepatosteatosis is associated with enhanced T cell-mediated hepatitis. *Hepatology*, 44, 216–227.
44. Tiegs, G. (2007). Cellular and cytokine-mediated mechanisms of inflammation and its modulation in immune-mediated liver injury. *Zeitschrift für Gastroenterologie*, 45, 63–70.
45. Nagaishi, T., Pao, L., Lin, S. H., et al. (2006). SHP1 phosphatase-dependent T cell inhibition by CEACAM1 adhesion molecule isoforms. *Immunity*, 25, 769–781.



# Chapter 13

## Sleep, Circadian Rhythms and Metabolism

Eleonore Maury, Kathryn Moynihan Ramsey, and Joseph Bass

### Introduction

Obesity and cardiometabolic disease are closely linked disorders that have recently accelerated throughout the industrialized world, coincident with more sedentary lifestyle and poor nutrition; however a complete understanding of the environmental precipitants underlying metabolic disease remains obscure. Mounting evidence from epidemiological studies has pointed towards a novel yet less appreciated factor that correlates with the recent expansion of these epidemics, namely, the introduction of artificial light and work at night-time, in addition to the rise in sleep curtailment. At the physiological level, it has been well-documented that many processes, including glucose and lipid metabolism, body temperature, and corticosterone production vary in a circadian fashion; moreover, there is an established temporal variation to health catastrophes such as myocardial infarction, cerebrovascular accident, and hypertensive crises. Over the past decade, major advances have emerged in our understanding of the underlying molecular mechanisms linking circadian rhythms, sleep, and metabolism, primarily through studies in experimental genetic models that became available following the landmark discovery of the first mammalian circadian clock gene *Clock* in 1997 [1, 2].

In this chapter, we highlight evidence at the intersection of clinical medicine and experimental genetics that illustrates how perturbations of the internal circadian system, and alterations in clock gene function, participate in the onset and progression of obesity and related disorders. An exciting aspect of the field has been the integration

---

J. Bass (✉)

Department of Medicine, Division of Endocrinology, Metabolism,  
and Molecular Medicine, Northwestern University, Feinberg  
School of Medicine, 2200 Campus Drive, Evanston, Illinois 60208, USA  
and

Department of Neurobiology and Physiology, Northwestern University,  
2200 Campus Drive, Evanston, Illinois 60208, USA  
e-mail: j-bass@northwestern.edu

of behavioral and physiological approaches and the emerging insight into integration of neural and peripheral tissues in disease pathogenesis.

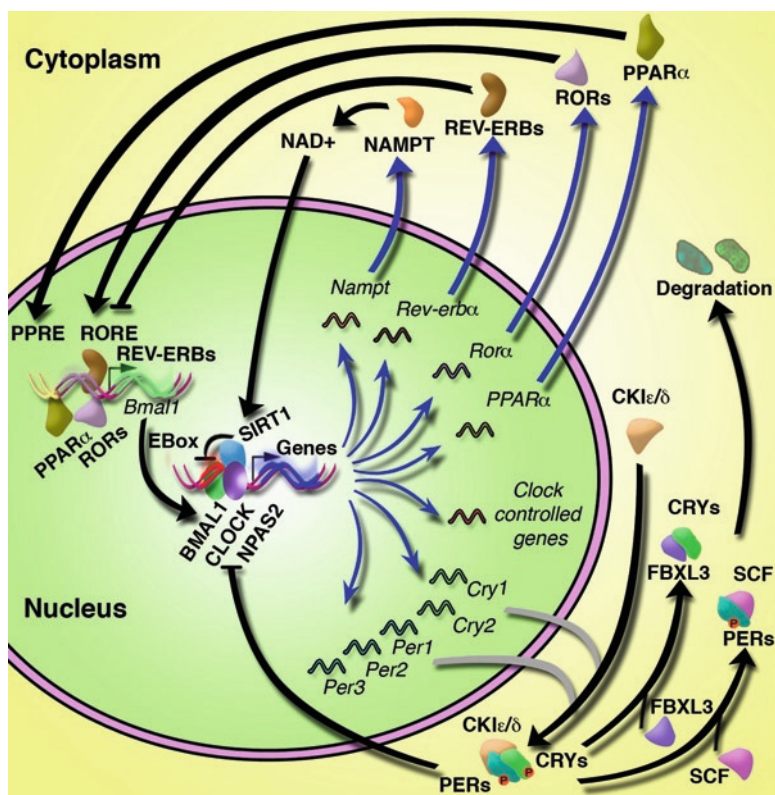
## Molecular Relationships Between Metabolism and Circadian Rhythms

### *The Core Clock Molecular Network: The Basis of Circadian Rhythms*

Circadian rhythms regulate a wide variety of physiological and metabolic functions in most organisms [3, 4]. At the molecular level, a network of autoregulatory coordinated transcription–translation feedback loops regulates the core molecular clock, maintaining approximately 24 h rhythmicity in order to match the Earth's rotation around its axis.

In mammals, the positive elements of these loops include members of basic helix-loop-helix (*bHLH*)-PAS (*Period-Arnt-Single-minded*) transcription-factor family of the transcription factors CLOCK (*Circadian locomotor output cycles kaput*), its paralogue NPAS2 (*Neuronal PAS domain protein 2*), and BMAL1/ARNTL (*Aryl-hydrocarbon receptor nuclear translocator-like*). CLOCK or NPAS2 and BMAL1 heterodimerize to activate the rhythmic transcription of genes containing E-box enhancer sequences, including the *Period* (*Per1*, *Per2*, and *Per3*) and *Cryptochrome* (*Cry1* and *Cry2*) genes (Fig. 1). The PER and CRY proteins comprise the negative limb of the feedback loop; upon translation, PER and CRY proteins multimerize and subsequently translocate to the nucleus and directly inhibit the transcriptional activity of the CLOCK:BMAL1 complex (Fig. 1). Posttranslational modifications, including phosphorylation and ubiquitination, provide further regulation of the clock network. *Casein kinase 1 epsilon* and *delta* (*CK1 $\epsilon$*  and *CK1 $\delta$* ) phosphorylate PER and CRY, tagging them for polyubiquitylation by the E3 ubiquitin ligase complexes  $\beta$ TrCP1 and FBXL3, respectively, ultimately leading to their degradation by the 26S proteasome. In addition to phosphorylation mediated by the casein kinase family, a role for GSK3- $\beta$  signaling has also been established in flies [5]. Subsequent to PER and CRY phosphorylation, CLOCK/BMAL1 is released from repression, activating the forward limb of the 24 h cycle. The biochemical mechanisms involved in generating 24 h periodicity to CLOCK/BMAL1 activity remains an area of active investigation, although recent results suggest that post-translational modification via phosphorylation of these factors may mediate the termination of their occupancy on promoters of the repressors [6, 7].

CLOCK and BMAL1 further drive expression of the orphan nuclear receptors, *Rev-erb $\alpha$*  and *Ror $\alpha$* , which inhibit and activate *Bmal1* expression, respectively, by binding to the retinoic acid-related orphan receptor response element (RORE) within the *Bmal1* promoter, constituting a short-feedback loop [8, 9]. It is important to note that REV-ERB $\alpha$  and ROR $\alpha$  are also key nutrient sensors (heme binds to



**Fig. 1** The core molecular clock components (adapted from [32]). The core molecular clock machinery is encoded by a series of interlocking transcription–translation feedback loops that oscillates with a 24-h periodicity within both pacemaker neurons and within peripheral tissues. The positive limb of the clock is composed of the transcription factors CLOCK/NPAS2 and BMAL1, which heterodimerize and activate transcription of downstream clock target genes, including the period (*Per1*, 2, and 3) and cryptochrome (*Cry1* and 2) genes, *Rev-erba*, *Rora*, and other clock-controlled genes. Upon translation, the PERs and CRYs heterodimerize, translocate back to the nucleus, and inhibit CLOCK/BMAL1 in a negative feedback loop. Multiple additional interlocking loops, including the nuclear receptors ROR $\alpha$  and REV-ERB $\alpha$  which activate and inhibit *Bmal1*, respectively, are shown (please see text for further details)

REV-ERB $\alpha$  [10, 11], while cholesterol and oxysterols bind to ROR $\alpha$  [12]), which provides clues as to a molecular link between metabolism and the circadian clock. This family of nuclear receptors is involved in the regulation of lipid and carbohydrate metabolism, as well as inflammation and thrombosis (reviewed in [13]).

Other nuclear receptors play an important role in the relationship between metabolism and circadian cycles. Indeed, the nuclear receptors known as peroxisome proliferator-activated receptor (PPARs) are lipid-activated transcription factors that have emerged as key regulators of lipid metabolism and inflammation [14]. For example, PPAR $\gamma$  displays circadian oscillation and controls *Bmal1* transcription



(detailed in part “From metabolism to circadian cycles”). Moreover, the PPAR $\gamma$  coactivator 1- $\alpha$  (PGC1 $\alpha$ ) also displays circadian oscillation in liver and skeletal muscle and upregulates the transcription of *Bmal1* and *Rev-erba*. Since PGC1 $\alpha$  levels are elevated in response to starvation, physical activity, and cold exposure, it will be important to learn whether *Pgc1 $\alpha$*  is necessary to maintain circadian homeostasis under varying nutritional conditions.

Two additional molecular autoregulatory feedback loops link nutrient sensing and metabolism with the core circadian clock. The first of these involves the direct regulation of the rate-limiting enzyme in NAD $^{+}$  biosynthesis (nicotinamide phosphoribosyltransferase, *Nampt*) by the positive limb of the clock within peripheral tissues, including liver and white adipose tissue [15, 16]. Direct activation of *Nampt* by CLOCK and BMAL1 leads to elevated NAD $^{+}$  levels, increased activity of the NAD $^{+}$ -dependent deacetylase SIRT1, and subsequently reduced CLOCK/BMAL1 activity, as SIRT1 is an inhibitor of the positive limb of the clock (Fig. 1). SIRT1 is also a key nutrient sensor that plays a critical role in the molecular integration of metabolism and circadian rhythms, as SIRT1 is also involved in a myriad of metabolic functions, including glucose and lipid metabolism, insulin secretion, and adipocyte differentiation (reviewed in [3]). A second mechanism involves circadian regulation via adenosine monophosphate-activated protein kinase (AMPK) signaling, a pathway activated by decreases in ATP production (and increases in AMP). AMPK modulates degradation of the core clock repressor, CRY1 [17]. Interestingly, AMPK has also been shown to modulate NAMPT activity, thus it is tempting to speculate that AMPK may also modulate circadian systems indirectly via activation of NAMPT [18].

Genetic mouse models have provided the opportunity to dissect the function of core clock genes in the generation and maintenance of circadian rhythms. *Bmal1* knockout mice [19] and mice with a dominant-negative *Clock* mutation [2] become arrhythmic in constant darkness. Of note, *Clock* knockout mice have normal locomotor activity rhythms due to developmental compensation by NPAS2 [20, 21]. Furthermore, *Per1/Per2* and *Cry1/Cry2* double knockout mice display a much more pronounced loss of circadian rhythmicity compared to the single mutant counterparts, consistent with either functional redundancy and/or developmental compensation [22–26]. Recent studies have also discovered that mutation of the F-box protein FBXL3 results in a period lengthening in mice [27, 28], and mice lacking PGC1 $\alpha$  have abnormal diurnal locomotor activity rhythms and body temperature, along with altered expression of clock and metabolic genes [29]. While many of these early genetic studies focused on the role of clock genes in the regulation of circadian behavior, more recent studies have expanded the analyses of these mice to include their metabolic phenotypes, as discussed in section “Circadian Genes Involved in Metabolism Regulation”. Further, the recent discovery that many nutrient-responsive factors, including the nuclear hormone receptors and the sirtuins, are key regulators of the clock have provided critical clues as to the molecular mechanisms linking metabolism and nutrient-sensing with the clock and sleep. Indeed, hormones and nutrients might directly modulate the sleep/wake and feeding/fasting cycles (detailed in sections “Neurophysiological Structures: Interconnection Between Circadian,

Sleep and Energy Centers” and “From Metabolism to Circadian Cycles”), and an important question is whether nutrient signaling per se may affect these cycles by modulating the core properties of the suprachiasmatic nucleus (SCN) pacemaker.

### ***Peripheral Clocks: Regulation of Circadian Metabolism***

Molecular analyses have revealed that the clock network is also widely expressed throughout nearly every tissue/cell type in vertebrates [30, 31]. In addition to the master clock in the SCN, independent circadian oscillators have been found in a number of peripheral tissues in mammals and can be maintained and self-sustained ex vivo in appropriate conditions. Gene expression profiling has shown that 3–20% of genes display a 24 h rhythmic expression, and a large proportion of these genes have a role in metabolic processes (for review [3]), including regulation of lipid and cholesterol biosynthesis, carbohydrate metabolism and transport, oxidative phosphorylation, and xenobiotic detoxification pathways (review [32, 33]). While the core clock machinery only directly regulates a small subset of these metabolic genes, oscillation of the nuclear receptors in metabolic tissues appears to indirectly regulate the expression of metabolic genes (for review [3]). Importantly, the period and amplitude of oscillation, as well as the level of expression of each of these metabolic genes, varies among different tissues, suggesting the importance of tissue-specific roles of peripheral clocks for normal cellular function. In this way, circadian patterns of metabolic gene expression may optimize the switch between daily anabolic and catabolic states corresponding with periods of feeding and fasting. For example, the cyclic expression of gastrointestinal tract enzymes may ensure that factors involved in nutrient absorption are expressed in anticipation of daily episodes of food ingestion, while adipose enzymes involved in fatty acid storage peak coincident with feeding. Moreover, components of gluconeogenesis, glycolysis, and fatty acid metabolism in the liver and a large portion of rhythmic genes in the muscle peak during the subjective night (in nocturnal rodents), coinciding with the peak of physical activity.

Thus, peripheral oscillators are cell-autonomous and tissue-specific, but the mechanisms involved in sustaining this synchrony within and between peripheral tissue clocks are still poorly understood. In addition, while the SCN is still considered the master clock, experimental genetic models suggest that the misalignment of local circadian oscillation among peripheral tissues or between peripheral tissue and SCN may contribute to cardiovascular and metabolic pathologies. For example, clock gene disruption targeted to the fat body in flies is sufficient to induce increased food consumption, decreased glycogen levels, and increased sensitivity to starvation [34]. At least in flies, these findings suggest involvement of a peripheral tissue clock in neural energy homeostasis [34]. A recent study reported that mice with a liver-specific deletion of *Bmal1* exhibited hypoglycemia during fasting, indicating a role for the liver clock in maintaining euglycemia during rest [35]. In addition, high-fat feeding (HFD), as well as mouse models of type 2 diabetes, alters

both circadian behavior and sleep [32]. Thus, identifying the signals that impact both central pacemaker neurons and peripheral clock oscillators which remains an area of intensive investigation.

## **From Circadian Disruption to Cardiometabolic Diseases**

### ***Impact of Sleep and Circadian Cycles on Metabolism: Clinical Evidence and Experimental Models***

#### **Impact of Sleep Quantity and Quality**

*Human studies.* Epidemiological evidence has linked obesity and cardiometabolic disease (e.g. cardiovascular disease, type 2 diabetes) with both habitually short and long sleep. Numerous cross-sectional, as well as prospective clinical studies, have demonstrated that short-duration and poor-quality sleep predicts the development of type 2 diabetes and obesity after age, BMI and other variables are taken into account [36–41]. For example, short-term sleep duration (less than 6 h) in a large population of Japanese men has been associated with weight gain and development of obesity [42]. More alarming, such positive associations between short sleep duration and obesity have also been found in children [43, 44].

In addition to obesity, chronic short sleep duration is also strongly associated with cardiovascular disease and hypertension. Possible mechanisms linking sleep deprivation with cardiometabolic disease may include effects on glucose metabolism, appetitive behavior, and energy expenditure (reviewed previously [38, 39]). For example, healthy subjects who underwent six consecutive nights of sleep restricted to 4 h exhibited impaired insulin sensitivity following a glucose challenge. Furthermore, sleep deprivation results in a reduction of leptin and an increase in levels of the orexigenic hormone ghrelin, both of which may lead to increased appetite and altered energy expenditure.

Diseases related to changes in time and/or quality-sleep duration are also associated with metabolic disorders. For example, sleep apnea syndrome, a sleep disorder that is highly prevalent in metabolic syndrome [45], was proposed to cause clock gene dysfunction [46], and effective treatments of sleep apnea have been found to improve glucose metabolism and energy balance [40]. In addition, the circadian oscillation of leptin was found to be disrupted in narcoleptic patients, which may predispose them to weight gain [47]. A challenge for future investigation will be to discern the independent effects of circadian misalignment vs. sleep restriction (and hypoxia) on metabolic functions.

*Animal studies.* Experimental models using chronic sleep deprivation paradigms have strongly corroborated the impact of sleep loss in metabolism (reviewed in [48]). These studies have shown a decrease in blood levels of the satiety hormone leptin, which may contribute both to dysregulation of appetite and hepatic glucose metabo-

lism. Consistent with human studies, these works also demonstrated increased ghrelin levels and alteration in glucose utilization. However, in both human and rodent studies, it will be important to understand the extent to which increased autonomic tone contributes to metabolic dysfunction following sleep-deprivation.

### **Impact of Circadian Misalignment**

*Human studies.* Gradual sleep loss, as well as extension of work during the night, may disrupt synchrony between the periods of sleep/activity with feeding/fasting and corresponding cycles of energy storage/utilization. Indeed, recent evidence has also demonstrated that chronic circadian disruption might also increase susceptibility to such disorders. Behavioral cycles are normally aligned with the light–dark cycle. However, the dysregulation of sleep and activity can result in misalignment of the central and peripheral oscillators and desynchronization of behavior, metabolic gene expression, and hormone release, thereby leading to adverse metabolic physiological consequences. Indeed, these misalignments might lead to obesity and to cardiovascular disease, as often observed in shift workers. For example, shift work is associated with a 1.6 and 3.0-fold increased risk of cardiovascular disease for 45–55 years old men and women, respectively [49]. An altered postprandial lipid excursion has also been reported in shift workers, thereby providing a partial explanation for the increased occurrence of cardiovascular disease [50]. Interestingly, the incidence of acute myocardial infarction is also significantly increased after the transition to daylight saving time, reinforcing the deleterious impact of chronobiologic rhythm disruption [51]. Recently, Sheer et al. demonstrated adverse cardiometabolic endpoints in human subjects who underwent forced misalignment of behavioral and circadian cycles, simulating the conditions of jet lag and shift work within a controlled clinical setting [52]. In this study, the behavioral cycle of the subjects was extended to 28 h, under dim light, with 14 h rest and fasting alternated with 14 h of wakefulness, interspersed with four evenly spaced and isocaloric meals. When subjects ate and slept approximately 12 h out of phase from their habitual times, circadian desynchrony decreased leptin levels and resulted in hyperglycemia and hyperinsulinemia. Thus, this study suggests that synchrony between behavioral and physiological rhythms is advantageous to maintain normal glucose metabolism in otherwise healthy persons.

*Animal studies.* The impact of chronobiology in the pathogenesis of obesity and its related disorders has been mainly substantiated by experimental genetic studies describing the role of clock genes in adipose tissue and other metabolic organs. Please refer to section “animal studies” for details.

### **Impact of Feeding Time**

*Human studies.* Concerning the development of adiposity, there are several clues to suggest that disruption of either behavioral or genetic aspects of circadian synchrony

may contribute to dysmetabolic states. Indeed, several clinical studies have highlighted the importance of feeding time in a society in which people are inclined to eat at irregular times in the sleep-wake cycle (for review [53]). Conversely, regularity of food intake improves postprandial thermogenesis and reduces energy intake [54]. High-energy intake in the evening and/or skipping breakfast has been associated with the development of obesity (for review [53]). Curiously, individuals diagnosed with night eating syndrome appear to have greater propensity towards obesity.

*Animal studies.* Interestingly, mice fed a HFD consumed nearly all of the extra calories during the 12-h light phase, demonstrating a desynchronizing effect of HFD on the normal feeding rhythm [55]. A plausible hypothesis is that consuming calories at the incorrect time in the light-dark cycle (i.e., rest period) exacerbates the obesogenic effects of HF caloric intake due to desynchronization of various behavioral, hormonal, and molecular rhythms involved in maintaining energy balance.

Interestingly, genetically obese animals are resistant to weight gain when feeding is restricted to the active (dark) phase. In agreement with these observations, recent evidence demonstrated that circadian timing of food intake contributes to weight gain [56]. Indeed, mice fed with a HFD only during the 12-h light phase gained significantly more weight compared to isocalorically fed mice which were provided food only during the 12-h dark phase [56]. As expected, food intake during the normal activity phase prevents obesity and circadian desynchrony in a rat model of night work, based on daily 8-h activity schedules during the resting phase [56].

Further studies are necessary to understand how the timing of food intake impacts energy constancy. Interestingly, a study demonstrated that treatment with an antagonist of T-type calcium channel, which is involved in sleep-wake regulation, improved HFD-induced alterations, including a decrease in inactive phase activity, core body temperature, feeding, and adiposity [57]. Taken together, these observations (largely based on animal studies) raise important questions concerning the impact of circadian misalignment and clock gene disruption on obesity and its metabolic complications and suggest avenues for future investigation in human subjects. How does time of feeding affect circadian systems from the physiological to molecular level? Understanding this question has implications for public health, since overnutrition and altered rest-activity behavior are common in modern society, and both factors have been implicated in the pathogenesis of metabolic syndrome and cardiovascular disease.

## ***Clock Genes Regulate Both Sleep and Metabolism: Genetic Evidence in Human and Animal Models***

### **Circadian Genes Involved in Sleep/Wake Phenotypes**

*Human studies.* In addition to environmental sleep disruption (e.g., shift work disorders), genetic polymorphisms in several clock genes have also been linked to sleep phenotypes. For example, an advanced sleep phase has been associated with polymorphism of the human *Per1* gene [58] and with missense mutations of *Per2*

[59] and *CKε/δ* [60, 61]. Mutations in several clock genes have also been identified in the familial (monogenic) sleep disorders in man, but it is not known whether these increase susceptibility to metabolic disorders [60–62].

*Animal studies.* The *Clock*<sup>Δ19</sup> mutant mouse was produced in a deliberate chemical mutagenesis screen designed to identify the genes controlling mammalian circadian locomotor activity [1, 63]. Interestingly, mice homozygous for the *Clock* mutation (on a C57Bl/6J background) sleep approximately 2 h less than wild-type mice in regular 12:12 light:dark conditions and present a smaller amount of rapid eye movement (REM) sleep rebound during 24 h recovery following sleep deprivation [64]. Since that time, there have been major advances in understanding the molecular basis of the clock network (described in section “The Core Clock Molecular Network: The Basis of Circadian Rhythms”), which is now viewed as a centerpiece for the generation of the 24 h rhythms of sleep propensity. Among the other clock genes described above, genetic animal models with *Cry* or *Bmal1* gene deletion also present sleep phenotypes. For example, mice deficient in *Cry1* and *Cry2* showed altered sleep structure, including increases in non-REM time, consolidation, and EEG delta power, in addition to their disrupted circadian period [65, 66]. *Bmal1* is also believed to be involved in the generation of sleep, as *Bmal1* mutant mice display increases in total sleep time, sleep fragmentation and EEG delta power under baseline conditions, and an attenuated compensatory response to acute sleep deprivation [67].

It is important to note that the circadian system is not the only mechanism regulating sleep. The wake-dependent homeostatic sleep process, whereby sleep pressure increases during wake and dissipates during sleep, represents a principal process controlling sleep (for review, see [68, 69]). A better understanding of the neurobiological links between sleep, energetics, and metabolism will likely emerge as the homeostatic process becomes more clearly defined at the molecular level.

## Circadian Genes Involved in Metabolism Regulation

*Human studies.* Polymorphisms in several clock genes have been linked to obesity or to other features of the metabolic syndrome (for review, see [32]). In small sample populations, polymorphisms in the *Clock* gene have been correlated with predisposition to obesity, and two *Bmal1* haplotypes are associated with type 2 diabetes and hypertension. Polymorphisms within other clock core genes (i.e., *Per2* and *Npas2*) have also been associated with hypertension and high fasting blood glucose in studies of similar sample size. Interestingly, a rare variant in *Nampt* (*Visfatin/Pbef1*), which is involved in a negative clock feedback loop, is associated with protection from obesity.

In addition to the clock gene machinery, several genome-wide association studies recently discovered that melatonin, a hormone implicated in seasonal and circadian rhythms, and its receptor melatonin 1B receptor gene (*mntr1b*), may be important in the regulation of mammalian glucose levels [70]. Interestingly, polymorphism in *Cry2* has also recently emerged as a genetic factor involved in fasting glucose regulation in large-scale association studies [71]. Taken together, these studies suggest

that disruption of circadian systems may contribute to human metabolic syndrome and cardiovascular complications, either directly at the level of altered clock gene expression, or indirectly through effects on melatonin.

*Animal studies.* The discovery that *Clock*<sup>Δ19</sup> mutant animals develop hyperglycemia, hyperlipidemia, hepatic steatosis, and increased susceptibility to diet-induced obesity has provided a new entrée into experimental studies to dissect the mechanistic linkages between circadian and metabolic systems [72]. The feeding rhythm in these mice is damped, with increased food intake during the day, and, in addition, these mice have significantly increased food intake overall. HF feeding studies revealed exaggerated weight gain of *Clock*<sup>Δ19</sup> mutant mice, and DEXA scanning and fat pad weight both demonstrated significant increases in fat and lean mass relative to controls following HF feeding. It is likely that the obese phenotype results, at least in part, from altered rhythms of neuropeptides in the hypothalamus, as ghrelin, cocaine- and amphetamine-regulated transcript (CART), and orexin are all expressed at constitutively low-levels in the *Clock*<sup>Δ19</sup> mutant mice. In addition, the anorectic neuropeptide pro-opiomelanocortin (*POMC*) was decreased throughout the entire LD cycle in hypothalami of young *Clock*<sup>Δ19</sup> mutant prior to the onset of weight gain and overt diabetes, consistent with a deficit in the central homeostatic regulation of weight constancy. Disruption of other circadian clock genes also leads to metabolic alterations. For example, gene disruption in *Bmal1* induces an abnormal metabolic phenotype characterized by impaired gluconeogenesis, hyperleptinemia, glucose intolerance, and dyslipidemia [35, 73, 74]. In addition, *Per2* knockout mice developed increased weight gain on HFD [75]. Conversely, mice deficient in the circadian deadenylase nocturin remained lean and resistant to hepatic steatosis when fed a HFD despite equivalent caloric intake, similar metabolic rates, and reduced activity compared with control mice [76].

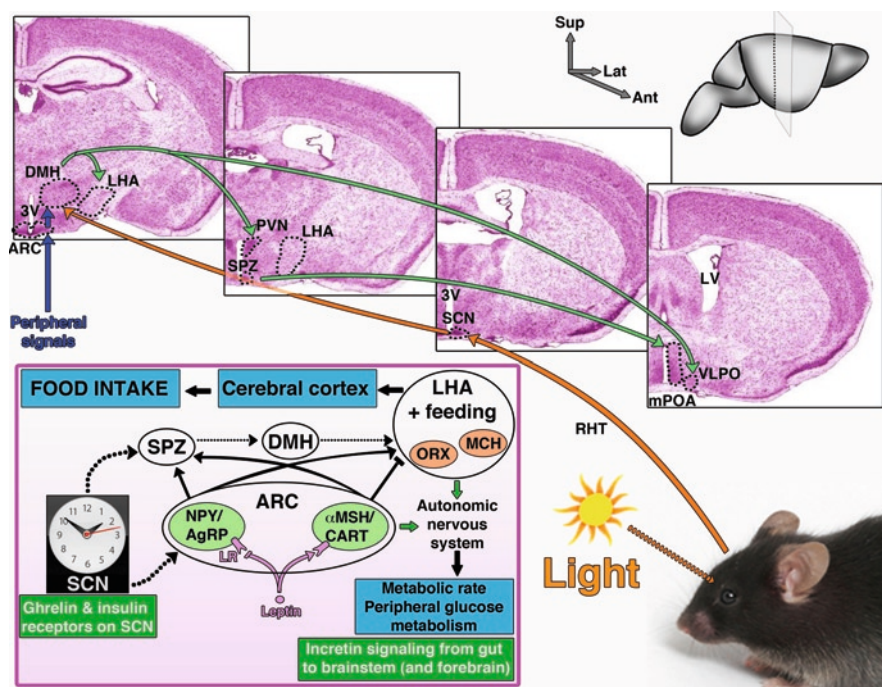
In addition to changes in sleep and circadian cycles affecting metabolism, alterations in metabolism are also able to entrain central and/or peripheral clocks, thereby resulting in changes to the rhythms of sleep/wakefulness, fasting/feeding, and hormonal secretion and energy metabolism. Indeed, nutrient and hormonal cues may also affect the period and phase characteristics of the master clock neurons, although little is known about how metabolic signals are communicated to the SCN. In addition, it is still unclear whether the food-entrainment pathway regulates circadian behavior directly or indirectly through other brain structures.

## **Neurophysiological Structures: Interconnection Between Circadian, Sleep and Energy Centers**

### ***Circadian and Sleep Centers***

The past several decades have witnessed enormous outgrowths of understanding regarding the brain centers important in the regulation of circadian rhythms, sleep, and metabolism (Fig. 2). One of the early seminal experiments to show that





**Fig. 2** Neural pathways linking circadian and metabolic systems (adapted from [3]). Light is the predominant environmental cue that is transmitted from the eyes via the retinohypothalamic tract (RHT) to the SCN. Projections from the SCN extend toward the SPZ and then onward from the SPZ to the dorsomedial hypothalamus (DMH). The SPZ and DMH also project toward the medial preoptic area (mPOA) and the VLPO, two relay regions of the hypothalamus that may be served to integrate circadian and wakefulness signals. The DMH has emerged as an important site in the activity response to food (the food-entrainable oscillator), although this finding remains controversial. The DMH has many outputs to other regions of the brain, including the LHA, which controls circadian regulation of the sleep/wakefulness and fasting/feeding cycles. *Inset:* The LHA also receives neuroendocrine input from the arcuate (ARC) neurons producing anorexigenic and orexigenic neuropeptides. The hormone leptin produced by adipose tissue activates the production of anorexigenic neuropeptides such as  $\alpha$ MSH/CART, which in turn blocks production of the orexigenic peptides orexin (ORX) and MCH in the LHA. In the absence of leptin, orexigenic neurons in the ARC produce the neuropeptides NPY/AgRP that stimulate hunger and decreased energy expenditure via signaling to the LHA. In addition, insulin, ghrelin, and other incretins may also influence circadian behavioral rhythms through direct effects on SCN or indirectly through actions within other regions of midbrain and brainstem. Arrows in inset indicate functional links. 3V third ventricle; LV lateral ventricle

circadian period length was determined by the SCN involved in the restoration of normal circadian locomotor activity in SCN-lesioned hamsters by transplantation of a wild-type SCN from another animal [77]. Of note, these activity rhythms were recovered despite the lack of direct neural connections between the grafted SCN and the host brain, suggesting that a diffusible secreted factor, such as transforming growth factor- $\alpha$ , prokineticin-2, gamma-aminobutyric acid, or vasopressin, might

be important for the generation of at least some circadian rhythms. The SCN has numerous cellular and anatomic projections to hypothalamic centers involved in the regulation of wakefulness, activity, and feeding (reviewed in [68, 78]). In particular, the largest output of projections is directed toward the subparaventricular zone (SPZ); the ventral SPZ regulates circadian rhythms of locomotor activity and sleep/wakefulness, while the dorsal SPZ regulates circadian rhythms of body temperature. Both the SCN and SPZ also project to the dorsomedial nucleus of the hypothalamus (DMH), which has been implicated in circadian rhythms of locomotor activity, sleep/wakefulness, corticosteroid secretion, and feeding. In turn, the DMH projects to other brain centers involved in the regulation of sleep (ventrolateral preoptic nucleus, VLPO), corticosteroid release (the paraventricular nucleus, PVN), and wakefulness/feeding (the lateral hypothalamus, LHA). Of note, the DMH has been implicated in the ability of an organism to be entrained by food, although this finding remains controversial (see section “From Metabolism to Circadian Cycles”). Sleep recording in sham and SCN-lesioned mice under baseline conditions and following sleep deprivation has also established that the SCN plays a central role in the regulation of sleep and wakefulness beyond just the timing of vigilance states [79]. Recent functional magnetic resonance imaging experiments in humans with extreme chronotypes also demonstrate that vigilance state in the evening was associated with higher activity in evening than morning chronotypes in a region of the suprachiasmatic area including the circadian master clock [80]. This activity may be modulated by homeostatic sleep pressure [80].

### ***Link Between Energy Centers with Circadian and Sleep/Wakefulness Centers***

In addition to brain centers regulating circadian locomotor activity and sleep/wakefulness, recent advances have been made toward understanding the overlap between these circadian centers with those involved in energy balance and feeding behavior (Fig. 2). Importantly, a landmark breakthrough in our molecular understanding of the hypothalamic control of appetite regulation and energy balance came in 1994 with the positional cloning of leptin, a secreted adipocyte-derived factor, and subsequent cloning and localization of its receptor within various regions of the hypothalamus, including the arcuate nucleus (ARC), DMH, and VMH, all regions previously implicated in regulation of satiety (reviewed in [68, 81]). Of note, leptin is secreted in proportion to the fat mass, and its levels display circadian rhythmicity in addition to responding to nutrient status. Thus, these findings have provided critical insight into how signals from the periphery may translate the nutritional status of the organism to appetite-regulating regions of the hypothalamus in a circadian-dependent fashion.

Following the initial discovery of leptin and its receptor, came the discovery of the melanocortin system as downstream of leptin signaling (reviewed in [68, 81]). Leptin activates the POMC and CART-expressing neurons within the ARC to release  $\alpha$ -melanocyte-stimulating hormone ( $\alpha$ -MSH), which then activates the

melanocortin receptor subtype 4 (MC4), leading to inhibition of food intake and increased energy expenditure. At the same time, leptin inhibits the neuropeptide Y (NPY) and agouti-related protein (AgRP)-expressing neurons within the ARC, thereby in effect blocking the ability of  $\alpha$ -MSH to act on its MC4 receptor via release of AgRP and inhibiting the POMC/CART-expressing neurons via release of small inhibitor amino acid neurotransmitter  $\gamma$ -aminobutyric acid. Thus, during fasting, when leptin levels are low, the orexigenic neuropeptides (NPY and AgRP) lead to increased appetite and decreased energy expenditure, while during feeding, leptin acts to suppress appetite via activation of POMC/CART.

These orexigenic and anorexigenic neuropeptides further project to additional centers within the brain involved in feeding behavior, including the LHA, which makes the orexigenic melanin-concentrating hormone (MCH) and the orexins A and B (reviewed in [82]). Importantly, orexins A and B display circadian rhythmicity, are induced by fasting, and play a critical link in the regulation of sleep–wake rhythms, as their neurons also project to regions within both the cortex and brainstem which regulate arousal and autonomic function. Disruption of orexin and its receptor result in narcolepsy, which is consistent with its role in the regulation of the sleep–wake axis. Finally, neurons from the ARC also project to the dopaminergic-reward centers of the midbrain, including the ventral tegmental area (VTA), suggesting a direct link between the appetite-regulating regions of the brain with those important in regulating reward in response to food and drugs [83, 84]. The extensive anatomic projections and synaptic relays between the various brain centers involved in circadian rhythmicity, sleep/wakefulness, and feeding indicate that the brain has evolved to be able to rapidly coordinate and allow extensive cross-talk between these centers, thereby allowing the organism to most efficiently adapt to daily changes in its environment.

## From Metabolism to Circadian Cycles

As described above, the master pacemaker has anatomic connections with centers coordinating activity behavior, sleep, appetite, and energetics, suggesting that targeting one of these centers would impact the others. Indeed, feeding behavior in particular plays an essential role in coordinating the circadian rhythms of sleep and activity. Indeed, it is well known that food restriction to the normal rest period in rodents also induces a burst of food anticipatory activity (FAA). While lesioning of the dorsomedial nucleus has been shown to alter FAA [85–87], there remains controversy regarding the involvement of circadian oscillators in FAA since the FAA behavior persists in *Bmal1* nullizygous mice [85, 88]. Recent data also suggests the involvement of the melanocortin signaling pathway in FAA [89]. Further, the FAA may constitute a metabolic oscillator responsive to peripheral neural or circulating signals elicited by food ingestion. Resolution of the precise stimuli and neural pathways involved in FAA, as well as understanding the involvement of nutrient signaling pathways which may affect core properties of the SCN pacemaker, remain important questions for the future.

As mentioned earlier, diet-induced obesity per se alters circadian behavioral and molecular rhythms in C57BL/6J mice [55]. Indeed, HFD in mice leads to increased daytime activity, a lengthened period of the locomotor activity rhythm, and alterations in the expression and cycling of canonical circadian clock genes, nuclear receptors that regulate clock transcription factors, and clock-controlled genes involved in fuel utilization in the hypothalamus, liver, and adipose tissue [55]. Conversely, it has also been demonstrated that caloric restriction induces phase advances in rat behavioral and physiological circadian rhythms (for review [90]). Indeed, prolonged fasting advances the phase of free-running rhythms such as wheel-running and body temperature. Oscillatory expression of clock genes and neuropeptides in the mouse SCN are also altered by hypocaloric feeding, supporting the hypothesis that calorie restriction has effects within the SCN clock (for review [90]).

Changes in dietary nutrient composition, calorie content, or in food availability play an important role in the regulation of circadian behavior and physiology, although the mechanisms of such regulations are still unclear. For instance, availability of food, in addition to other environmental factors such as day length and temperature, might strongly regulate the timing of torpor induction. Torpor is a state of “inactivity” during mammalian hibernation, which is homologous to sleep as demonstrated by numerous EEG studies (for review [91]). Indeed, there is a switch from carbohydrate- to fat-based metabolism during torpor. Most metabolic processes seem to be halted or slowed considerably during deep torpor. Prior to hibernation, an animal dramatically increases its food intake and stores energy in the form of fat. During the low-temperature torpor phases, the burning of fat results in the accumulation of acetyl-CoA, which becomes converted into heat (by uncoupling proteins) and energy upon the transient activation of mitochondrial oxidative phosphorylation during brief interbout arousals. The signals that trigger the periodic arousals from torpor have not yet been elucidated. However, the studies of hibernating species allowed postulating that it is the fluctuating levels of these key metabolites that control the transitions between torpor and interbout arousal. Of note, the SCN keeps a relatively high metabolic activity compared to nearly every other brain structures in torpor, as demonstrated namely by uptake of [ $^{14}\text{C}$ ] 2-deoxyglucose measurement (for review, see [91]).

On the other hand, sleep change affects many aspects of our physiology and behavior, from immunity to hormonal regulation. Indeed, brain metabolism itself changes depending on the sleep state; it is low in NREM sleep but high in REM sleep (for review [92, 93]). Whole-genome transcriptomic studies have revealed a differential expression of many genes between brains of sleeping and awake animals. These changes occur mainly in the cerebral cortex, cerebellum, and hypothalamus. The transcripts that are the most consistently increased during waking and short-term sleep deprivation relative to sleep genes include genes involved in energy metabolism, including those coding for mitochondrial proteins, glucose transporters, and proteins related to glycogen metabolism (for review [92, 93]). Their upregulation has been proposed to be a mechanism by which the brain responds to the high-energy requirements of wakefulness (for review [92, 93]). Conversely, the transcripts with increased expression during sleep appear to be mainly involved in protein synthesis and lipid metabolism (for review [92, 93]).

## ***Fatty Acids/Lipids***

Studies performed more than 20 years ago by Brody and colleagues were the first to identify interactions between fatty acids and circadian oscillator function. Analyses in a fatty acid-requiring strain of *Neurospora* indicated a relationship between the period length of the spore-forming rhythm and unsaturated fatty acid concentration of medium. The period lengthening effects of unsaturated fat were reversed by the addition of saturated fat, indicating a specific correlation between lipid signals and oscillator properties, although the metabolic pathways accounting for these effects at a mechanistic level have not been uncovered [94].

During recent years, studies undertaken in rodents demonstrated that fatty acids can relay the body's nutritional status to the hypothalamic energy center (the arcuate nucleus), thereby controlling feeding behavior (for review [95]). Long chain fatty acids cross the blood brain barrier (BBB) mainly by simple diffusion in the unbound form or via delivery by chylomicrons or other circulating lipoproteins. Cellular accumulation of long-chain fatty acyl-CoA, as well as manipulation of enzymes of the fatty acid synthesis pathway that result in elevated malonyl-CoA, lead to inhibition of food intake (for review [95, 96]). Of note, in addition to hypophagia, mice presenting a deletion of fatty acid synthase in hypothalamus (and islets) showed significantly increased locomotor activity, particularly during the dark phase [97], suggesting that fatty acids might act centrally to control daily behavior.

In addition to the regulation of feeding behavior, lipid metabolism may also play a role in sleep, as HFD fed to female C57BL/6J mice increased their sleep time [98]. Further, QTL analysis identified *Acads*, the short-chain acyl-coenzyme A dehydrogenase involved in fatty acid  $\beta$ -oxidation, as linked to theta frequency, which is prominent during REM sleep; however the mechanism by which its deficiency can slow down the peak theta frequency remains unclear (for review [93]). Finally, forward molecular and reverse genetic approaches have shown that *rar*- $\beta$ , the gene encoding retinoic acid receptor- $\beta$ , is important for determining the contribution of delta activity to the EEG during NREM sleep (for review [93]).

Fatty acids have also been described as interfering in peripheral circadian physiology, especially within the vascular tissue. Conditional deletion of PPAR $\gamma$ , the rhythmically expressed lipid receptor that directly regulates *Bmal1* transcription, within vascular tissue results in abnormalities in blood pressure and heart rate in parallel with a reduction of diurnal variation in the sympathetic nerve activity [99]. Furthermore, vascular PPAR $\gamma$  exhibits a robust cyclic expression, whose rhythmic phase may be reset by changes in feeding time as well as changes in the photoperiod [99]. Thus, the temporal environment may be integrated within the heart by PPAR $\gamma$ . Consistent with this, PPAR $\gamma$  agonists were found to shift the circadian fluctuation of blood pressure in patients with type 2 diabetes, indicating that vasculoprotective actions of thiazolidinediones may in part involve effects on the clock transcription network [100]. Emerging clinical evidence has also uncovered unique actions of the PPAR $\alpha$  agonist fenofibrate in the circadian control of blood pressure and heart rate in diabetic subjects [101–103].

In turn, the circadian clock also controls lipid metabolism. For instance, the clock induces an ultradian rhythm in the expression of genes involved in the unfolded protein response, thereby controlling rhythmic regulation of hepatic lipid metabolism [104].

## ***Amino Acids***

Caloric restriction, amino acid imbalance, and activation of the target in rapamycin pathway *increase* life span in evolutionarily diverse organisms including mammals [105, 106]. While the mechanisms involved are still not completely understood, it has been postulated that the biological clock could be an important mediator of longevity in calorically restricted animals (for review [107]). Interestingly, many amino acids exhibit significant circadian rhythmicity in both mice and humans. For example, glutamine, threonine, proline, valine, phenylalanine, methionine, isoleucine, leucine, and tryptophan peak around midnight, as demonstrated by mass spectrometry analysis of mouse blood samples [108]; however it is unclear how exactly amino acids may be regulated by the clock. Studies show that diets low in proteins increase food intake and, conversely, diets high in protein decrease food intake, potentially implicating the CNS in amino acid sensing (for review [109]). Indeed, central administration of leucine, a branched-chain amino acid, inhibits food intake, whereas valine has no effect, illustrating the importance of this particular amino acid. The target of rapamycin pathway appears to be important in CNS amino acid sensing, and its activity is regulated by feeding/fasting states (for review [109]). Lastly, the activation of the target of rapamycin pathway in SCN might also potentially be involved in light entrainment process [110].

## ***Carbohydrates***

Mammalian glucose metabolism *displays* pronounced diurnal variation across the light–dark cycle, with alternating cycles of gluconeogenesis and glycogen synthesis that are coordinated with the rest–activity cycle (reviewed in [81]). In addition, glucose availability has recently been described to control circadian rhythmicity in fibroblasts [17]. Intriguingly, in addition to liver, a small amount of glycogen is also synthesized and stored in brain astrocytes. Fifteen years ago, it had been hypothesized that these stores might be used/depleted during wakefulness and restored during sleep, which has been reinforced by numerous observations (for review [111]).

## ***Cellular Energy Status***

Pacemakers in peripheral organs, such as the liver, are reset by food availability. AMPK, an enzyme that responds to nutrient availability, is involved in this



entrainment. Indeed, activation of AMPK correlated with phase advances in mice after treatment with metformin, an AMPK-targeting antihyperglycemic biguanide [112]. Recently, Lamia et al showed that AMPK directly phosphorylates the core clock protein cryptochrome 1 (CRY1), thereby marking it for degradation [17]. AMPK is activated upon its phosphorylation by protein kinases such as liver kinase B1 (LKB1) or calcium-calmodulin-dependent protein kinase  $\beta$ , which sense the AMP/ATP ratio, a direct readout of the cell's metabolic state [17]. In addition to these regulatory functions in peripheral tissues, AMPK has been hypothesized to play the role of energy sensor in the hypothalamus. Both pharmacological approaches targeting AMPK either directly or indirectly through modulation of central fatty acid metabolism and central injection of adenovirus, consistently demonstrated that AMPK activity is strongly involved in regulation of feeding behavior (for review [113]). Thus, AMPK might also serve as a molecular sensor to shift the brain from energy consuming synthetic processes that occur during sleep to catabolic energy-producing processes that occur during wakefulness (for review [111]) and might contribute to sleep homeostasis [114].

Other components of the adenosine metabolic pathway have been proposed to couple the metabolic and circadian cycles. 5'-AMP, which is elevated in the blood of DD mice, is able to induce torpor [115]. In addition, intracellular adenosine 3', 5'-monophosphate (cAMP) oscillates in the mouse SCN [116], as well as in other brain areas [117], although it is important to note that mice are nonhibernating mammals. cAMP sustains the transcriptional loop of the SCN, determining canonical pacemaker properties of amplitude, phase, and period [116]. Brain cAMP levels might also be regulated during sleep deprivation [118] and regulate sleep/wake cycles [119].

Another signal linking metabolic and circadian systems has recently been described by several groups. The ratio of oxidized nicotinamide adenine dinucleotide phosphate (NAD<sup>+</sup>) to its reduced form (NADH) is related to feeding/fasting state and may entrain peripheral clocks [15, 16]. Importantly, NAD<sup>+</sup> biosynthesis varies across the light–dark cycle, suggesting that NAD<sup>+</sup> functions as an oscillating metabolite linking circadian and metabolic cycles [16]. The major node regulating NAD<sup>+</sup> biosynthesis involves the rate-limiting enzyme nicotinamide phosphoribosyl transferase (*Nampt*), which oscillates in a circadian manner and is directly regulated at the transcriptional level by CLOCK/BMAL1. Alterations in *Nampt*/NAD<sup>+</sup> modulate the nutrient-responsive deacetylase SIRT1, which plays an important role in the regulation of glucose and lipid metabolism, insulin secretion, the inflammatory response, and the circadian clock. This pathway is particularly intriguing in light of the fact that NAMPT and SIRT1 are regulated not only by the clock, but also by the nutritional status of the organism. For example, AMPK is able to modulate NAD<sup>+</sup> metabolism and SIRT1 activity [18]. In addition, *Nampt* is upregulated in response to glucose restriction in skeletal muscle in an AMPK-dependent manner [18, 120]. Thus, regulation of the clock by NAD<sup>+</sup> and SIRT1 allows for coordination of the core clock machinery with the daily cycles of fasting/feeding and rest/activity. It has also recently been demonstrated that NAMPT is secreted and is present in the circulation, though it is not yet known whether extracellular NAMPT is regulated in a circadian manner, thereby influencing downstream processes on a



systemic level. Interestingly, NAMPT concentration in cerebrospinal fluid is decreased with increasing body fat, but further investigation will be necessary to clarify this link in humans [121].

## ***Hormonal Mediators***

*Central insulin.* Strong evidence from human studies demonstrates rhythmic variation in glucose tolerance and insulin action across the day. Both insulin secretion and sensitivity are decreased in the evening (for review [81, 122]). Insulin may also modulate circadian behavior, as insulin is able to reach the brain via a saturable transporter across the BBB. Mice with a brain-specific insulin receptor (IR) deficiency and mouse models with inducible IR inactivation demonstrated that central insulin action plays an important role in the regulation of food intake, as well as peripheral glucose, and fat metabolism. These effects are mediated through phosphatidylinositol 3 kinase (PI3 kinase) and mitogen-activated protein kinase (MAPK) cascades. Activation of the PI3 kinase results in activation of protein kinase B/Akt and phosphorylation of FOXO, which is of critical importance for maintenance of energy homeostasis by the CNS. Insulin might also be involved in sleep/wake behavior [119]. Lastly, a genome-wide small interfering RNA screen in a human cellular clock model demonstrate that among the numerous pathways interconnected with clocks, the insulin-signaling pathway was overrepresented [123]. Thus, it will be interesting to examine whether insulin acts on SCN to control circadian behavior.

*Central leptin.* The fall of leptin that occurs rapidly in response to fasting also evokes profound changes in energy balance via the hypothalamus. Like insulin, leptin is also involved in the control of feeding behavior. Leptin from the periphery is transported into the brain, binds to its receptor in the hypothalamus, and activates janus kinase 2 (JAK2)/signal transducer and activator of transcription 3 (STAT3), leading to suppression of “orexigenic peptides” (e.g., NPY and AgRP), and elevation of “anorexigenic peptides” (e.g., POMC and CART) [124], thereby curtailing food intake. In the common form of obesity, resistance to leptin has been ascribed to diminished transport of leptin across the BBB and to elevated hypothalamic levels of SOCS3 and endoplasmic reticulum (ER) stress, which inhibit leptin signaling [125–127]. Furthermore, AMPK mediates both leptin’s anorexigenic effect, as well as adiponectin’s orexigenic effects [128, 129]. In addition, the study of sleep in several mouse models of obesity and diabetes has demonstrated leptin’s involvement in sleep architecture. For example, *ob/ob* mice, a genetic model of leptin deficiency, has an elevated number of arousals from sleep [130], while *db/db* mice (which harbor a mutation in a particular isoform of the long form of leptin receptor), exhibits increased overall sleep time, a dramatic increase in sleep fragmentation, attenuated diurnal rhythmicity in REM sleep and non-REM EEG delta power, and a decrease in the compensatory response to acute sleep deprivation [131].

Taken together, these data suggest that leptin resistance might be a potential link between HF feeding, alterations of diurnal behavior, and sleep rhythms.

**Central inflammation.** Adipokines and cytokines are also able to cross the BBB and may modulate sleep. For instance, interleukin-6 and tumor necrosis factor- $\alpha$  in plasma, which are increased in obesity, may also impair circadian clock gene oscillations and promote sleep (for review [32]). Hypothalamic inflammation may also cause disrupted feeding behavior and obesity [132, 133], although a more “acute” central inflammation may preferentially lead to anorectic behavior. Thus, it is tempting to speculate that some inflammatory factors might directly target the SCN. It is known that, for instance, intracerebroventricular injection of recombinant receptor-activator of NF- $\kappa$ B ligand (RANKL) triggers c-Fos activation in the SCN [134]. Mice intraperitoneally injected with LPS exhibited abnormal diurnal activity, while deleting RANKL receptor in brain abolish this phenomenon [134].

Obesity and nutrient overflow result in conditions that increase demand on the ER in metabolic tissues including liver, adipocytes, and pancreas, resulting in a persistent inflammatory state [135], as well as hypothalamus, thereby altering feeding behavior. Interestingly, a group of transcripts strongly upregulated during wakefulness code for proteins involved in the ER stress response, chaperones, and heat-shock proteins. During waking, the expression of proteins implicated in stress responses increases, suggesting that absence of sleep could be a stress for brain cells (for review [92, 93]). For instance, the ER chaperone protein BiP, a key protein involved in the ER stress response, is expressed in a circadian manner [104, 136]. Thus, disrupted synchrony of stress response, gene expression may potentially alter circadian and sleep disturbances.

### ***Lipids, Endotoxins and Hormones from the Gut***

Several gut satiety factors produced in response to fat ingestion might also contribute to food entrainment. For instance, duodenal infusion of fat stimulates small intestinal mucosal cells to produce the lipid messenger oleoylethanolamide enabling CD36-mediated uptake of dietary oleic acid and thus promoting satiety [137]. Interestingly, this factor shows diurnal variation in cerebrospinal fluid of rats [138]. Moreover, plasma lipid N-acylphosphatidylethanolamines (NAPEs) are also secreted into circulation from the small intestine in response to ingested fat [139]. Interestingly, systemic administration of circulating NAPE decreases food intake and locomotor activity in rats [139]. Furthermore, mice fed with a HFD also display enhanced metabolic endotoxemia induced by the death of gram-negative bacteria within gut that participate in the occurrence of metabolic disorders [140]. The endotoxin LPS exhibits a diurnal variation that is disrupted by HF feeding [140]. As described above, inflammatory molecules such as LPS may induce sleep, suppress biological clock genes, and promote anorexia. Among gastrointestinal hormones, incretins also show circadian variation [141]. Furthermore, the reversibility of insulin resistance observed after biliopancreatic diversion may be related to an improvement in the circadian

control or pattern of incretin production [141]. Ghrelin, a stomach-derived hormone, which participates in meal initiation, also displays circadian rhythms, and the amplitude of its rhythm is reduced in obesity [142, 143]. Indeed in healthy subjects, high levels of ghrelin are detected in the early morning, when eating is precluded by sleep; however, this peak is not present in obese subjects [143]. Of interest, in addition to its orexigenic role, ghrelin has been shown to stimulate locomotor activity in anticipation of meals [144] and to increase slow-wave sleep [145], thereby playing an important role in the control of circadian behavior and perhaps even sleep architecture.

## Conclusions

During recent years, much progress has been made in the dissection of the neurobehavioral basis of feeding, sleep, and circadian timing. In addition, numerous epidemiological as well as experimental genetic studies have demonstrated that metabolic networks are under extensive circadian control and that alterations in the circadian clock promote the development of obesity and metabolic diseases. However, further investigation will be necessary to understand on a molecular level how perturbations of the internal clock system and sleep constitute risk factors for metabolic disorders. Finally, it will be important to determine how nutrient affects the circadian system and the molecular control of behavior. Efforts to dissect the molecular mediators that coordinate circadian, metabolic, and cardiovascular systems may ultimately lead to both improved therapeutics and preventive interventions.

**Acknowledgements** We thank members of the Bass, Takahashi, Turek, and Allada laboratories for helpful discussions, and especially M. Flourakis for his help with the figures. This work was supported by grants from Alfediam to E.M.; NIDDK (T32 DK007169) to K.M.R.; NIH (PO1 AG011412 and R01HL097817-01), ADA, Chicago Biomedical Consortium Searle Funds, and JDRF to J.B., and the University of Chicago DRTC (P60 DK020595).

## Disclosures

**J.B. is a member of the scientific advisory board of a cofounder of ReSet Therapeutics Inc. J.B. is also an advisor and receives support from Amylin Pharmaceuticals.**

## References

1. King, D. P., Zhao, Y., Sangoram, A. M., Wilsbacher, L. D., Tanka, M., Antoch, M. P., et al. (1997). Positional cloning of the mouse circadian clock gene. *Cell*, 89(4), 641–653.
2. Vitaterna, M. H., King, D. P., Chang, A. M., Kornhauser, J. M., Lowrey, P. L., McDonald, J. D., et al. (1994). Mutagenesis and mapping of a mouse gene, clock, essential for circadian behavior. *Science*, 264(5159), 719–725.
3. Green, C. B., Takahashi, J. S., & Bass, J. (2008). The meter of metabolism. *Cell*, 134(5), 728–742.
4. Takahashi, J. S., Hong, H. K., Ko, C. H., & McDearmon, E. L. (2008). The genetics of mammalian circadian order and disorder: implications for physiology and disease. *Nature Review Genetics*, 9(10), 764–775.

5. Martinek, S., Inonog, S., Manoukian, A. S., & Young, M. W. (2001). A role for the segment polarity gene *shaggy/GSK-3* in the *Drosophila* circadian clock. *Cell*, 105(6), 769–779.
6. Spengler, M. L., Kuropatwinski, K. K., Schumer, M., & Antoch, M. P. (2009). A serine cluster mediates BMAL1-dependent CLOCK phosphorylation and degradation. *Cell Cycle*, 8(24), 4138–4146.
7. Yoshitane, H., Takao, T., Satomi, Y., Du, N. H., Okano, T., & Fukada, Y. (2009). Roles of CLOCK phosphorylation in suppression of E-box-dependent transcription. *Molecular and Cellular Biology*, 29(13), 3675–3686.
8. Kornmann, B., Schaad, O., Bujard, H., Takahashi, J. S., & Schibler, U. (2007). System-driven and oscillator-dependent circadian transcription in mice with a conditionally active liver clock. *PLoS Biology*, 5(2), e34.
9. Wang, J., Yin, L., & Lazar, M. A. (2006). The orphan nuclear receptor Rev-erb alpha regulates circadian expression of plasminogen activator inhibitor type 1. *The Journal of Biological Chemistry*, 281(45), 33842–33848.
10. Raghuram, S., Stayrook, K. R., Huang, P., et al. (2007). Identification of heme as the ligand for the orphan nuclear receptors REV-ERBalpha and REV-ERBbeta. *Nature Structural & Molecular Biology*, 14(12), 1207–1213.
11. Yin, L., Wu, N., Curtin, J. C., et al. (2007). Rev-erbalpha, a heme sensor that coordinates metabolic and circadian pathways. *Science*, 318(5857), 1786–1789.
12. Wang, Y., Kumar, N., Solt, L. A., et al. (2010). Modulation of retinoic acid receptor-related orphan receptor alpha and gamma activity by 7-oxygenated sterol ligands. *The Journal of Biological Chemistry*, 285(7), 5013–5025.
13. Fontaine, C., & Staels, B. (2007). The orphan nuclear receptor Rev-erbalpha: A transcriptional link between circadian rhythmicity and cardiometabolic disease. *Current Opinion in Lipidology*, 18(2), 141–146.
14. Bislinger, S. J., & Tontonoz, P. (2008). Integration of metabolism and inflammation by lipid-activated nuclear receptors. *Nature*, 454(7203), 470–477.
15. Nakahata, Y., Sahar, S., Astarita, G., Kaluzova, M., & Sassone-Corsi, P. (2009). Circadian control of the NAD<sup>+</sup> salvage pathway by CLOCK-SIRT1. *Science*, 324(5927), 654–657.
16. Ramsey, K. M., Yoshino, J., Brace, C. S., et al. (2009). Circadian clock feedback cycle through NAMPT-mediated NAD<sup>+</sup> biosynthesis. *Science*, 324(5927), 651–654.
17. Lamia, K. A., Sachdeva, U. M., DiTacchio, L., et al. (2009). AMPK regulates the circadian clock by cryptochrome phosphorylation and degradation. *Science*, 326(5951), 437–440.
18. Canto, C., Gerhart-Hines, Z., Feige, J. N., et al. (2009). AMPK regulates energy expenditure by modulating NAD(+) metabolism and SIRT1 activity. *Nature*, 458, 1056.
19. Bunger, M. K., Wilsbacher, L. D., Moran, S. M., et al. (2000). Mop3 is an essential component of the master circadian pacemaker in mammals. *Cell*, 103(7), 1009–1017.
20. DeBruyne, J. P., Weaver, D. R., & Reppert, S. M. (2007). CLOCK and NPAS2 have overlapping roles in the suprachiasmatic circadian clock. *Nature Neuroscience*, 10(5), 543–545.
21. DeBruyne, J. P., Weaver, D. R., & Reppert, S. M. (2007). Peripheral circadian oscillators require CLOCK. *Current Biology*, 17(14), R538–R539.
22. Bae, K., Jin, X., Maywood, E. S., Hastings, M. H., Reppert, S. M., & Weaver, D. R. (2001). Differential functions of mPer1, mPer2, and mPer3 in the SCN circadian clock. *Neuron*, 30(2), 525–536.
23. Cermakian, N., Monaco, L., Pando, M. P., Dierich, A., & Sassone-Corsi, P. (2001). Altered behavioral rhythms and clock gene expression in mice with a targeted mutation in the *Period1* gene. *The Embo Journal*, 20(15), 3967–3974.
24. van der Horst, G. T., Muijtjens, M., Kobayashi, K., et al. (1999). Mammalian Cry1 and Cry2 are essential for maintenance of circadian rhythms. *Nature*, 398(6728), 627–630.
25. Vitaterna, M. H., Selby, C. P., Todo, T., et al. (1999). Differential regulation of mammalian period genes and circadian rhythmicity by cryptochromes 1 and 2. *Proceedings of the National Academy of Sciences of the United States of America*, 96(21), 12114–12119.
26. Zheng, B., Albrecht, U., Kaasik, K., et al. (2001). Nonredundant roles of the mPer1 and mPer2 genes in the mammalian circadian clock. *Cell*, 105(5), 683–694.

27. Godinho, S. I., Maywood, E. S., Shaw, L., et al. (2007). The after-hours mutant reveals a role for Fbx13 in determining mammalian circadian period. *Science*, 316(5826), 897–900.
28. Siepka, S. M., Yoo, S. H., Park, J., et al. (2007). Circadian mutant overtime reveals F-box protein FBXL3 regulation of cryptochrome and period gene expression. *Cell*, 129(5), 1011–1023.
29. Liu, C., Li, S., Liu, T., Borjigin, J., & Lin, J. D. (2007). Transcriptional coactivator PGC-1 $\alpha$  integrates the mammalian clock and energy metabolism. *Nature*, 447(7143), 477–481.
30. Yamazaki, S., Numano, R., Abe, M., et al. (2000). Resetting central and peripheral circadian oscillators in transgenic rats. *Science*, 288(5466), 682–685.
31. Yoo, S. H., Yamazaki, S., Lowrey, P. L., et al. (2004). PERIOD2:LUCIFERASE real-time reporting of circadian dynamics reveals persistent circadian oscillations in mouse peripheral tissues. *Proceedings of the National Academy of Sciences of the United States of America*, 101(15), 5339–5346.
32. Maury, E., Ramsey, K. M., & Bass, J. (2010). Circadian rhythms and metabolic syndrome: From experimental genetics to human disease. *Circulation Research*, 106(3), 447–462.
33. McIntosh, B. E., Hogenesch, J. B., & Bradfield, C. A. (2010). Mammalian Per-Arnt-Sim proteins in environmental adaptation. *Annual Review of Physiology*, 72, 625–645.
34. Xu, K., Zheng, X., & Sehgal, A. (2008). Regulation of feeding and metabolism by neuronal and peripheral clocks in *Drosophila*. *Cell Metabolism*, 8(4), 289–300.
35. Lamia, K. A., Storch, K. F., & Weitz, C. J. (2008). Physiological significance of a peripheral tissue circadian clock. *Proceedings of the National Academy of Sciences of the United States of America*, 105(39), 15172–15177.
36. Gangwisch, J. E., Malaspina, D., Boden-Albala, B., & Heymsfield, S. B. (2005). Inadequate sleep as a risk factor for obesity: analyses of the NHANES I. *Sleep*, 28(10), 1289–1296.
37. Kawakami, N., Takatsuka, N., & Shimizu, H. (2004). Sleep disturbance and onset of type 2 diabetes. *Diabetes Care*, 27(1), 282–283.
38. Knutson, K. L., Spiegel, K., Penev, P., & Van Cauter, E. (2007). The metabolic consequences of sleep deprivation. *Sleep Medicine Reviews*, 11(3), 163–178.
39. Knutson, K. L., & Van Cauter, E. (2008). Associations between sleep loss and increased risk of obesity and diabetes. *Annals of the New York Academy of Sciences*, 1129, 287–304.
40. Spiegel, K., Tasali, E., Leproult, R., & Van Cauter, E. (2009). Effects of poor and short sleep on glucose metabolism and obesity risk. *Nature Reviews. Endocrinology*, 5(5), 253–261.
41. Yaggi, H. K., Araujo, A. B., & McKinlay, J. B. (2006). Sleep duration as a risk factor for the development of type 2 diabetes. *Diabetes Care*, 29(3), 657–661.
42. Watanabe, M., Kikuchi, H., Tanaka, K., & Takahashi, M. (2010). Association of short sleep duration with weight gain and obesity at 1-year follow-up: A large-scale prospective study. *Sleep*, 33(2), 161–167.
43. Danielsen, Y. S., Pallesen, S., Stormark, K. M., Nordhus, I. H., & Bjorvatn, B. (2010). The relationship between school day sleep duration and body mass index in Norwegian children (aged 10–12). *International Journal of Pediatric Obesity*, 5(3), 214–220.
44. Lumeng, J. C., Somashekar, D., Appugliese, D., Kaciroti, N., Corwyn, R. F., & Bradley, R. H. (2007). Shorter sleep duration is associated with increased risk for being overweight at ages 9 to 12 years. *Pediatrics*, 120(5), 1020–1029.
45. de Sousa, A. G., Cercato, C., Mancini, M. C., & Halpern, A. (2008). Obesity and obstructive sleep apnea-hypopnea syndrome. *Obesity Reviews*, 9(4), 340–354.
46. Burioka, N., Koyanagi, S., Endo, M., et al. (2008). Clock gene dysfunction in patients with obstructive sleep apnoea syndrome. *The European Respiratory Journal*, 32(1), 105–112.
47. Kok, S. W., Meinders, A. E., Overeem, S., et al. (2002). Reduction of plasma leptin levels and loss of its circadian rhythmicity in hypocretin (orexin)-deficient narcoleptic humans. *The Journal of Clinical Endocrinology and Metabolism*, 87(2), 805–809.
48. Laposky, A. D., Bass, J., Kohsaka, A., & Turek, F. W. (2008). Sleep and circadian rhythms: Key components in the regulation of energy metabolism. *FEBS Letters*, 582(1), 142–151.
49. Knutsson, A. (2003). Health disorders of shift workers. *Occupational Medicine (London)*, 53(2), 103–108.

50. Ribeiro, D. C., Hampton, S. M., Morgan, L., Deacon, S., & Arendt, J. (1998). Altered post-prandial hormone and metabolic responses in a simulated shift work environment. *Journal of Endocrinology*, 158(3), 305–310.
51. Janszky, I., & Ljung, R. (2008). Shifts to and from daylight saving time and incidence of myocardial infarction. *The New England Journal of Medicine*, 359(18), 1966–1968.
52. Scheer, F. A., Hilton, M. F., Mantzoros, C. S., & Shea, S. A. (2009). Adverse metabolic and cardiovascular consequences of circadian misalignment. *Proceedings of the National Academy of Sciences of the United States of America*, 106(11), 4453–4458.
53. Ekmekcioglu, C., & Touitou, Y. (2010). Chronobiological aspects of food intake and metabolism and their relevance on energy balance and weight regulation. *Obesity Reviews* Epub ahead of print.
54. Farshchi, H. R., Taylor, M. A., & Macdonald, I. A. (2005). Deleterious effects of omitting breakfast on insulin sensitivity and fasting lipid profiles in healthy lean women. *The American Journal of Clinical Nutrition*, 81(2), 388–396.
55. Ekmekcioglu, C., Touitou, Y. Chronobiological aspects of food intake and metabolism and their relevance on energy balance and weight regulation. *Obes Rev*
56. Kohsaka, A., Laposky, A. D., & Ramsey, K. M., et al. (2007). High-fat diet disrupts behavioral and molecular circadian rhythms in mice. *Cell Metabolism*, 6(5), 414–421.
57. Arble, D. M., Bass, J., Laposky, A. D., Vitaterna, M. H., & Turek, F. W. (2009). Circadian timing of food intake contributes to weight gain. *Obesity (Silver Spring)*, 17(11), 2100–2102.
58. Uebele, V. N., Gotter, A. L., & Nuss, C. E., et al. (2009). Antagonism of T-type calcium channels inhibits high-fat diet-induced weight gain in mice. *The Journal of Clinical Investigation*, 119(6), 1659–1667.
59. Carpen, J. D., von Schantz, M., Smits, M., Skene, D. J., & Archer, S. N. (2006). A silent polymorphism in the PER1 gene associates with extreme diurnal preference in humans. *Journal of Human Genetics*, 51(12), 1122–1125.
60. Ptacek, L. J., Jones, C. R., & Fu, Y. H. (2007). Novel insights from genetic and molecular characterization of the human clock. *Cold Spring Harbor Symposia on Quantitative Biology*, 72, 273–277.
61. Takano, A., Uchiyama, M., Kajimura, N., et al. (2004). A missense variation in human casein kinase I epsilon gene that induces functional alteration and shows an inverse association with circadian rhythm sleep disorders. *Neuropsychopharmacology*, 29(10), 1901–1909.
62. Xu, Y., Padiath, Q. S., Shapiro, R. E., et al. (2005). Functional consequences of a CK1delta mutation causing familial advanced sleep phase syndrome. *Nature*, 434(7033), 640–644.
63. Toh, K. L., Jones, C. R., He, Y., et al. (2001). An hPer2 phosphorylation site mutation in familial advanced sleep phase syndrome. *Science*, 291(5506), 1040–1043.
64. Antoch, M. P., Song, E. J., Chang, A. M., et al. (1997). Functional identification of the mouse circadian Clock gene by transgenic BAC rescue. *Cell*, 89(4), 655–667.
65. Naylor, E., Bergmann, B. M., Krauski, K., et al. (2000). The circadian Clock mutation alters sleep homeostasis in the mouse. *Journal of Neuroscience*, 20(21), 8138–8143.
66. Okamura, H., Miyake, S., Sumi, Y., et al. (1999). Photic induction of mPer1 and mPer2 in cry-deficient mice lacking a biological clock. *Science*, 286(5449), 2531–2534.
67. Wisor, J. P., O'Hara, B. F., Terao, A., et al. (2002). A role for cryptochromes in sleep regulation. *BMC Neuroscience*, 3, 20.
68. Laposky, A., Easton, A., Dugovic, C., Walisser, J., Bradfield, C., & Turek, F. (2005). Deletion of the mammalian circadian clock gene BMAL1/Mop3 alters baseline sleep architecture and the response to sleep deprivation. *Sleep*, 28(4), 395–409.
69. Saper, C. B., Scammell, T. E., & Lu, J. (2005). Hypothalamic regulation of sleep and circadian rhythms. *Nature*, 437(7063), 1257–1263.
70. Wulff, K., Porcheret, K., Cussans, E., & Foster, R. G. (2009). Sleep and circadian rhythm disturbances: Multiple genes and multiple phenotypes. *Current Opinion in Genetics & Development*, 19(3), 237–246.



71. Bouatia-Naji, N., Bonnefond, A., Cavalcanti-Proenca, C., et al. (2009). A variant near MTNR1B is associated with increased fasting plasma glucose levels and type 2 diabetes risk. *Nature Genetics*, 41(1), 89–94.
72. Dupuis, J., Langenberg, C., Prokopenko, I., et al. (2010). New genetic loci implicated in fasting glucose homeostasis and their impact on type 2 diabetes risk. *Nature Genetics*, 42(2), 105–116.
73. Turek, F. W., Joshu, C., Kohsaka, A., et al. (2005). Obesity and metabolic syndrome in circadian Clock mutant mice. *Science*, 308(5724), 1043–1045.
74. Rudic, R. D., McNamara, P., Curtis, A. M., et al. (2004). BMAL1 and CLOCK, two essential components of the circadian clock, are involved in glucose homeostasis. *PLoS Biology*, 2(11), e377.
75. Shimba, S., Ishii, N., Ohta, Y., et al. (2005). Brain and muscle Arnt-like protein-1 (BMAL1), a component of the molecular clock, regulates adipogenesis. *Proceedings of the National Academy of Sciences of the United States of America*, 102(34), 12071–12076.
76. Yang, S., Liu, A., Weidenhammer, A., et al. (2009). The role of mPer2 clock gene in glucocorticoid and feeding rhythms. *Endocrinology*, 150(5), 2153–2160.
77. Green, C. B., Douris, N., Kojima, S., et al. (2007). Loss of Nocturnin, a circadian deadenylation, confers resistance to hepatic steatosis and diet-induced obesity. *Proceedings of the National Academy of Sciences of the United States of America*, 104(23), 9888–9893.
78. Ralph, M. R., Foster, R. G., Davis, F. C., & Menaker, M. (1990). Transplanted suprachiasmatic nucleus determines circadian period. *Science*, 247(4945), 975–978.
79. Andretic, R., Franken, P., & Tafti, M. (2008). Genetics of sleep. *Annual Review of Genetics*, 42, 361–388.
80. Easton, A., Meerlo, P., Bergmann, B., & Turek, F. W. (2004). The suprachiasmatic nucleus regulates sleep timing and amount in mice. *Sleep*, 27(7), 1307–1318.
81. Schmidt, C., Collette, F., Leclercq, Y., et al. (2009). Homeostatic sleep pressure and responses to sustained attention in the suprachiasmatic area. *Science*, 324(5926), 516–519.
82. Ramsey, K. M., Marcheva, B., Kohsaka, A., & Bass, J. (2007). The clockwork of metabolism. *Annual Review of Nutrition*, 27, 219–240.
83. Vanitallie, T. B. (2006). Sleep and energy balance: Interactive homeostatic systems. *Metabolism*, 55(10 Suppl. 2), S30–S35.
84. Farooqi, I. S., Bullmore, E., Keogh, J., Gillard, J., O'Rahilly, S., & Fletcher, P. C. (2007). Leptin regulates striatal regions and human eating behavior. *Science*, 317(5843), 1355.
85. Fulton, S., Pissios, P., Manchon, R. P., et al. (2006). Leptin regulation of the mesoaccumbens dopamine pathway. *Neuron*, 51(6), 811–822.
86. Fuller, P. M., Lu, J., & Saper, C. B. (2008). Differential rescue of light- and food-entrainable circadian rhythms. *Science*, 320(5879), 1074–1077.
87. Gooley, J. J., Schomer, A., & Saper, C. B. (2006). The dorsomedial hypothalamic nucleus is critical for the expression of food-entrainable circadian rhythms. *Nature Neuroscience*, 9(3), 398–407.
88. Mieda, M., Williams, S. C., Richardson, J. A., Tanaka, K., & Yanagisawa, M. (2006). The dorsomedial hypothalamic nucleus as a putative food-entrainable circadian pacemaker. *Proceedings of the National Academy of Sciences of the United States of America*, 103(32), 12150–12155.
89. Storch, K. F., & Weitz, C. J. (2009). Daily rhythms of food-anticipatory behavioral activity do not require the known circadian clock. *Proceedings of the National Academy of Sciences of the United States of America*, 106(16), 6808–6813.
90. Sutton, G. M., Perez-Tilve, D., Nogueiras, R., et al. (2008). The melanocortin-3 receptor is required for entrainment to meal intake. *Journal of Neuroscience*, 28(48), 12946–12955.
91. Challet, E. (2010). Interactions between light, mealtime and calorie restriction to control daily timing in mammals. *Journal of Comparative Physiology B, Biochemical, Systemic, and Environmental Physiology*, 180(5), 631–644.
92. Heller, H. C., & Ruby, N. F. (2004). Sleep and circadian rhythms in mammalian torpor. *Annual Review of Physiology*, 66, 275–289.



93. Cirelli, C. (2006). Cellular consequences of sleep deprivation in the brain. *Sleep Medicine Reviews*, 10(5), 307–321.
94. Cirelli, C. (2009). The genetic and molecular regulation of sleep: From fruit flies to humans. *Nature Reviews. Neuroscience*, 10(8), 549–560.
95. Lakin-Thomas, P. L., & Brody, S. (1985). Circadian rhythms in *Neurospora crassa*: interactions between clock mutations. *Genetics*, 109(1), 49–66.
96. Lam, T. K., Schwartz, G. J., & Rossetti, L. (2005). Hypothalamic sensing of fatty acids. *Nature Neuroscience*, 8(5), 579–584.
97. Sandoval, D., Cota, D., & Seeley, R. J. (2008). The integrative role of CNS fuel-sensing mechanisms in energy balance and glucose regulation. *Annual Review of Physiology*, 70, 513–535.
98. Chakravarthy, M. V., Zhu, Y., Lopez, M., et al. (2007). Brain fatty acid synthase activates PPARalpha to maintain energy homeostasis. *The Journal of Clinical Investigation*, 117(9), 2539–2552.
99. Basterfield, L., Lumley, L. K., & Mathers, J. C. (2009). Wheel running in female C57BL/6J mice: impact of oestrus and dietary fat and effects on sleep and body mass. *International Journal of Obesity (London)*, 33(2), 212–218.
100. Wang, J., & Lazar, M. A. (2008). Bifunctional role of Rev-erbalpha in adipocyte differentiation. *Molecular and Cellular Biology*, 28(7), 2213–2220.
101. Anan, F., Masaki, T., Fukunaga, N., et al. (2007). Pioglitazone shift circadian rhythm of blood pressure from non-dipper to dipper type in type 2 diabetes mellitus. *European Journal of Clinical Investigation*, 37(9), 709–714.
102. Diabetes Atherosclerosis Intervention Study Investigators. (2001). Effect of fenofibrate on progression of coronary-artery disease in type 2 diabetes: The Diabetes Atherosclerosis Intervention Study, a randomised study. *Lancet*, 357(9260), 905–910.
103. Chew, G. T., Watts, G. F., Davis, T. M., et al. (2008). Hemodynamic effects of fenofibrate and coenzyme Q10 in type 2 diabetic subjects with left ventricular diastolic dysfunction. *Diabetes Care*, 31(8), 1502–1509.
104. Keech, A., Simes, R. J., Barter, P., et al. (2005). Effects of long-term fenofibrate therapy on cardiovascular events in 9795 people with type 2 diabetes mellitus (the FIELD study): Randomised controlled trial. *Lancet*, 366(9500), 1849–1861.
105. Cretenat, G., Le Clech, M., & Gachon, F. (2010). Circadian clock-coordinated 12 Hr period rhythmic activation of the IRE1alpha pathway controls lipid metabolism in mouse liver. *Cell Metabolism*, 11(1), 47–57.
106. Bjedov, I., Toivonen, J. M., Kerr, F., et al. (2010). Mechanisms of life span extension by rapamycin in the fruit fly *Drosophila melanogaster*. *Cell Metabolism*, 11(1), 35–46.
107. Grandison, R. C., Piper, M. D., & Partridge, L. (2009). Amino-acid imbalance explains extension of lifespan by dietary restriction in *Drosophila*. *Nature*, 462(7276), 1061–1064.
108. Froy, O., Chapnik, N., & Miskin, R. (2008). Relationship between calorie restriction and the biological clock: Lessons from long-lived transgenic mice. *Rejuvenation Research*, 11(2), 467–471.
109. Minami, Y., Kasukawa, T., Kakazu, Y., et al. (2009). Measurement of internal body time by blood metabolomics. *Proceedings of the National Academy of Sciences of the United States of America*, 106(24), 9890–9895.
110. Sandoval, D. A., Obici, S., & Seeley, R. J. (2009). Targeting the CNS to treat type 2 diabetes. *Nature Reviews. Drug Discovery*, 8(5), 386–398.
111. Cao, R., Lee, B., Cho, H. Y., Saklayen, S., & Obrietan, K. (2008). Photic regulation of the mTOR signaling pathway in the suprachiasmatic circadian clock. *Molecular and Cellular Neurosciences*, 38(3), 312–324.
112. Scharf, M. T., Naidoo, N., Zimmerman, J. E., & Pack, A. I. (2008). The energy hypothesis of sleep revisited. *Progress in Neurobiology*, 86(3), 264–280.
113. Um, J. H., Yang, S., Yamazaki, S., et al. (2007). Activation of 5'-AMP-activated kinase with diabetes drug metformin induces casein kinase Iepsilon (CKIepsilon)-dependent degradation of clock protein mPer2. *The Journal of Biological Chemistry*, 282(29), 20794–20798.

114. Ronnett, G. V., & Aja, S. (2008). AMP-activated protein kinase in the brain. *International Journal of Obesity (London)*, 32(Suppl. 4), S42–S48.
115. Chikahisa, S., Fujiki, N., Kitaoka, K., Shimizu, N., & Sei, H. (2009). Central AMPK contributes to sleep homeostasis in mice. *Neuropharmacology*, 57(4), 369–374.
116. Zhang, J., Kaasik, K., Blackburn, M. R., & Lee, C. C. (2006). Constant darkness is a circadian metabolic signal in mammals. *Nature*, 439(7074), 340–343.
117. O'Neill, J. S., Maywood, E. S., Chesham, J. E., Takahashi, J. S., & Hastings, M. H. (2008). cAMP-dependent signaling as a core component of the mammalian circadian pacemaker. *Science*, 320(5878), 949–953.
118. Eckel-Mahan, K. L., Phan, T., Han, S., et al. (2008). Circadian oscillation of hippocampal MAPK activity and cAMP: implications for memory persistence. *Nature Neuroscience*, 11(9), 1074–1082.
119. Vecsey, C. G., Baillie, G. S., Jaganath, D., et al. (2009). Sleep deprivation impairs cAMP signalling in the hippocampus. *Nature*, 461(7267), 1122–1125.
120. Crocker, A., Shahidullah, M., Levitan, I. B., & Sehgal, A. (2010). Identification of a neural circuit that underlies the effects of octopamine on sleep:wake behavior. *Neuron*, 65(5), 670–681.
121. Fulco, M., Cen, Y., Zhao, P., et al. (2008). Glucose restriction inhibits skeletal myoblast differentiation by activating SIRT1 through AMPK-mediated regulation of Nampt. *Developmental Cell*, 14(5), 661–673.
122. Hallschmid, M., Randevara, H., Tan, B. K., Kern, W., & Lehnert, H. (2009). Relationship between cerebrospinal fluid visfatin (PBEF/Nampt) levels and adiposity in humans. *Diabetes*, 58(3), 637–640.
123. Konner, A. C., Klockener, T., & Bruning, J. C. (2009). Control of energy homeostasis by insulin and leptin: Targeting the arcuate nucleus and beyond. *Physiology & Behavior*, 97(5), 632–638.
124. Zhang, E. E., Liu, A. C., Hirota, T., et al. (2009). A genome-wide RNAi screen for modifiers of the circadian clock in human cells. *Cell*, 139(1), 199–210.
125. Ahima, R. S., & Lazar, M. A. (2008). Adipokines and the peripheral and neural control of energy balance. *Molecular Endocrinology*, 22(5), 1023–1031.
126. Bjorbaek, C., Elmquist, J. K., Frantz, J. D., Shoelson, S. E., & Flier, J. S. (1998). Identification of SOCS-3 as a potential mediator of central leptin resistance. *Molecular Cell*, 1(4), 619–625.
127. Mori, H., Hanada, R., Hanada, T., et al. (2004). Socs3 deficiency in the brain elevates leptin sensitivity and confers resistance to diet-induced obesity. *Nature Medicine*, 10(7), 739–743.
128. Ozcan, L., Ergin, A. S., Lu, A., et al. (2009). Endoplasmic reticulum stress plays a central role in development of leptin resistance. *Cell Metabolism*, 9(1), 35–51.
129. Kubota, N., Yano, W., Kubota, T., et al. (2007). Adiponectin stimulates AMP-activated protein kinase in the hypothalamus and increases food intake. *Cell Metabolism*, 6(1), 55–68.
130. Minokoshi, Y., Alquier, T., Furukawa, N., et al. (2004). AMP-kinase regulates food intake by responding to hormonal and nutrient signals in the hypothalamus. *Nature*, 428(6982), 569–574.
131. Laposky, A. D., Shelton, J., Bass, J., Dugovic, C., Perrino, N., & Turek, F. W. (2006). Altered sleep regulation in leptin deficient mice. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology*, 290, R894–R903.
132. Laposky, A. D., Bradley, M. A., Williams, D. L., Bass, J., & Turek, F. W. (2008). Sleep-wake regulation is altered in leptin-resistant (db/db) genetically obese and diabetic mice. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology*, 295(6), R2059–R2066.
133. Kleinridders, A., Schenten, D., Konner, A. C., et al. (2009). MyD88 signaling in the CNS is required for development of fatty acid-induced leptin resistance and diet-induced obesity. *Cell Metabolism*, 10(4), 249–259.
134. Zhang, X., Zhang, G., Zhang, H., Karin, M., Bai, H., & Cai, D. (2008). Hypothalamic IKK $\beta$ /NF- $\kappa$ B and ER stress link overnutrition to energy imbalance and obesity. *Cell*, 135(1), 61–73.

135. Hanada, R., Leibbrandt, A., Hanada, T., et al. (2009). Central control of fever and female body temperature by RANKL/RANK. *Nature*, 462(7272), 505–509.
136. Hotamisligil, G. S. (2010). Endoplasmic reticulum stress and the inflammatory basis of metabolic disease. *Cell*, 140(6), 900–917.
137. Shaw, P. J., Cirelli, C., Greenspan, R. J., & Tononi, G. (2000). Correlates of sleep and waking in *Drosophila melanogaster*. *Science*, 287(5459), 1834–1837.
138. Schwartz, G. J., Fu, J., Astarita, G., et al. (2008). The lipid messenger OEA links dietary fat intake to satiety. *Cell Metabolism*, 8(4), 281–288.
139. Murillo-Rodriguez, E., Desarnaud, F., & Prospero-Garcia, O. (2006). Diurnal variation of arachidonylethanolamine, palmitoylethanolamide and oleoylethanolamide in the brain of the rat. *Life Sciences*, 79(1), 30–37.
140. Gillum, M. P., Zhang, D., Zhang, X. M., et al. (2008). N-acylphosphatidylethanolamine, a gut-derived circulating factor induced by fat ingestion, inhibits food intake. *Cell*, 135(5), 813–824.
141. Cani, P. D., Amar, J., Iglesias, M. A., et al. (2007). Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes*, 56(7), 1761–1772.
142. Mingrone, G., Nolfi, G., Gissey, G. C., et al. (2009). Circadian rhythms of GIP and GLP1 in glucose-tolerant and in type 2 diabetic patients after biliopancreatic diversion. *Diabetologia*, 52(5), 873–881.
143. Cummings, D. E., Weigle, D. S., Frayo, R. S., et al. (2002). Plasma ghrelin levels after diet-induced weight loss or gastric bypass surgery. *New England Journal of Medicine*, 346(21), 1623–1630.
144. Yildiz, B. O., Suchard, M. A., Wong, M. L., McCann, S. M., & Licinio, J. (2004). Alterations in the dynamics of circulating ghrelin, adiponectin, and leptin in human obesity. *Proceedings of the National Academy of Sciences of the United States of America*, 101(28), 10434–10439.
145. Blum, I. D., Patterson, Z., Khazall, R., et al. (2009). Reduced anticipatory locomotor responses to scheduled meals in ghrelin receptor deficient mice. *Neuroscience*, 164(2), 351–359.
146. Weikel, J. C., Wichniak, A., Ising, M., et al. (2003). Ghrelin promotes slow-wave sleep in humans. *American Journal of Physiology. Endocrinology and Metabolism*, 284(2), E407–E415.



# Chapter 14

## Obesity and Cardiac Dysfunction

Gary Sweeney, Sheldon E. Litwin, and Evan Dale Abel

### Introduction

The increasing global incidence of obesity has been predicted to contribute to a significant increase in the prevalence of cardiovascular disease. The impact of obesity on cardiovascular disease is complex, because many factors associated with obesity are established risk factors for cardiovascular disease. Thus, obesity is associated with dyslipidemia, insulin resistance, diabetes, hypertension, and sleep-disordered breathing, all of which will increase the likelihood of cardiovascular disease. Dyslipidemia will increase the likelihood of coronary atherosclerosis and ischemic heart disease. Sleep-disordered breathing and hypertension will increase left ventricular hypertrophy (LVH), and diabetes is associated with left ventricular (LV) dysfunction (termed diabetic cardiomyopathy), which occurs in diabetes in the absence of cardiac hypertrophy or ischemia. Moreover, diabetes is independently associated with hypertension, LVH, and accelerated atherosclerosis, all of which can incite cardiac injury and dysfunction. Thus, any analysis of cardiac dysfunction in obesity will need to take into account changes that can be directly attributable to obesity, vs. changes that are secondary to these comorbidities. In practice, it is not always possible to separate obesity-induced changes from those that are due to comorbidities, and throughout this chapter we will attempt to retain this perspective. An additional layer of complexity arises from observations, after established cardiovascular disease develops; individuals with obesity appear to have a survival advantage relative to those with normal body weight. The mechanistic basis for this “obesity paradox” is incompletely understood. However, increasing understanding of the mechanisms that are responsible for this phenomenon might lead to strategies for the identification of targets that could be manipulated to reverse cardiac dysfunction from a variety of causes.

---

E.D. Abel (✉)

Division of Endocrinology, Metabolism and Diabetes, University of Utah  
School of Medicine, Salt Lake City, UT, USA  
e-mail: dale.abel@hmbg.utah.edu

This chapter will review the epidemiology of heart failure in obesity in humans and comorbidities that exacerbate cardiac dysfunction. We will discuss the obesity paradox and review evidence linking weight loss and improvement in cardiac function. Insights into potential mechanisms that have been gained from animal studies will be summarized, and the extent to which these studies have shed insight into the human condition will be discussed. Finally, we will review potential pathogenic mechanisms that warrant further investigation.

## **Epidemiology and Pathophysiology of Cardiac Dysfunction in Human Obesity**

In most studies, body mass index (BMI) has been the index of obesity that has been relied upon most heavily. A number of groups have proposed the use of indexes that more directly measure abdominal obesity in order to avoid the potential for misclassification in subjects with low muscle mass but increased abdominal fat (so called “sarcopenic obesity”), or at the opposite end of the spectrum, those with high muscle mass and low fat mass. We recently reviewed the topic and concluded that measures of abdominal obesity add modestly to the predictive value of BMI in large populations, but they are likely to be of fairly small incremental value in day-to-day clinical practice [1, 2].

### ***Evidence for a Causative Role of Obesity in Heart Failure***

There is a large body of evidence linking obesity with structural and functional changes in the heart. Many of these changes, such as LV hypertrophy, left atrial (LA) enlargement, and subclinical impairment of LV systolic and diastolic function, are believed to be precursors to more overt forms of cardiac dysfunction and heart failure. Thus, the view has been widely held that long-standing obesity will eventually lead to heart failure. There are no prospective studies in humans that have directly ascertained the progression of cardiac dysfunction in relation to obesity duration and, as such, most data from human studies have come as cross-sectional data sets with inherent limitations regarding using these data to prove a direct role of obesity in the development of heart failure [3–6]. An important concern in many of these studies is the imprecision with which heart failure is diagnosed both clinically as well as on death certificates. For example, the common association of obesity with dyspnea and edema [7] and coexistent pulmonary pathologies and right heart failure could easily be mistaken for diastolic heart failure. Moreover, obesity is frequently associated with other risk factors for developing heart failure (e.g., hypertension, diabetes, hyperlipidemia, sleep-disordered breathing, etc.) and office-based blood pressure measurements in obese patients may underestimate the burden of hypertension as would be detected using 24 h

ambulatory monitoring. The best available longitudinal, population-based data came from the Framingham Heart Study [8]. Participants were stratified by BMI at the time of enrollment and then followed for incident heart failure (diagnosed by adjudicated clinical criteria). The main findings were that increased BMI was associated with an increased risk of heart failure in both men and women and that this risk was graded across categories of increasing BMI. In the subset of patients who had echocardiography within 30 days of the heart failure diagnosis, the majority had reduced LV ejection fractions. Although the investigators adjusted for the presence of cardiovascular risk factors, the possibility exists that these statistical adjustments could still underestimate the actual prevalence of myocardial infarctions in this cohort, as many infarcts occur in the absence of conventional risk factors. Thus, the possibility that coronary artery disease contributed to the development of heart failure has not been excluded. Indeed, central obesity is both a risk marker and a risk factor for the development and progression of coronary artery disease [9].

### ***Obesity-Associated Changes in Cardiac Function***

*LV systolic function:* A variety of techniques in many studies have been used to evaluate LV systolic function in obesity. Findings have ranged from depressed to normal to supranormal (reviewed in [9]) ejection fraction (EF). The range of findings might reflect heterogeneous populations and the possibility of differences in the prevalence of comorbidities such as hypertension, diabetes, and vascular disease, which can independently contribute to LV dysfunction. Technical differences could also contribute to the heterogeneity of these findings. For example, increased endocardial shortening that is a common finding in concentric LV hypertrophy may be a consequence of the increased excursion of the endocardium in a thick walled ventricle [10, 11]. Thus, even if the EF is normal, myocardial function is often reduced when it is measured with more sensitive methods such as midwall LV fractional shortening, systolic velocity measured with tissue Doppler, or systolic strain rate. Studies using these more sensitive approaches have tended to report the existence of subclinical contractile abnormalities in obese individuals (reviewed in [9]). Invasive studies have also led to the conclusion that myocardial contractility is reduced [12].

Changes such as LV hypertrophy and mild abnormalities of myocardial systolic function have been observed in obese children and adolescents [13, 14], and the severity of the dysfunction is comparable to that seen in obese subjects in their 30s, 40s, and 50s. Thus, it is unclear whether the relatively mild alterations of myocardial systolic function progress with longer durations of obesity. Alpert and colleagues have argued that duration of obesity is the factor that determines the likelihood of developing systolic dysfunction and heart failure [3]. Although this is an attractive hypothesis, not all studies have found such a relationship [15]. Moreover, no longitudinal studies in obese subjects are available to delineate the natural history of the contractile abnormalities in obesity. Lastly, obesity in older individuals appears to pose less of a mortality risk than it does in younger subjects



[14]. Thus, it remains to be definitively established that long-term obesity leads to heart failure independent of coronary disease or other morbidities.

*LV diastolic function:* Studies that have evaluated LV diastolic filling and diastolic function in obesity have also given variable results. Mitral inflow velocities measured with pulsed wave Doppler give information about LV relaxation rate and atrial contractile strength, but the effects of altered loading conditions (i.e., LA pressure) tend to dominate over the effects of LV relaxation abnormalities. Some studies have found reduced early diastolic (E wave) velocities, while others have found them to be unchanged (reviewed in [9]). Similarly, E wave deceleration times have been reported to be increased [16] or unchanged [17] in obese subjects. Lastly, late diastolic (A wave) velocities have been reported to be increased or unchanged reviewed in Abel et al. [9]. Prolongation of the isovolumic relaxation time is probably the most consistent diastolic abnormality seen in obesity [18–20]. Because increased age predictably is associated with reduced E velocity, prolonged E deceleration time, and increased A velocity, controlling for age is mandatory in the assessment of mitral flow patterns.

Over the last decade, echocardiographic techniques have been developed that allow for the quantification of relatively load-independent indexes of myocardial diastolic function [21, 22]. These are based mainly on tissue Doppler imaging – a robust method of recording the velocity and amplitude of myocardial movements with high temporal resolution. From the parent tissue Doppler velocities, strain and strain rate at different locations in the heart can be derived. Using these approaches, at least two groups have reported evidence of reduced early diastolic tissue velocities and diastolic strain rate in obese compared to normal weight subjects [15, 17]. It is believed that decreases in these parameters predominantly reflect slowing in the rate of LV relaxation. Recently, speckle-tracking techniques have been added to the ultrasound-based methods for assessing myocardial function, but they have not yet been studied in obesity.

Limited studies are available that compare intracardiac pressures in obese and normal weight subjects. Resting pulmonary capillary wedge pressures were found to be normal in obese subjects [23]. However, compared to normal weight control subjects, the obese subjects had an exaggerated rise in wedge pressure during passive leg raising or during exercise. These data were interpreted as showing reduced distensibility of the central circulation. Noninvasive methods have also been used to estimate resting LV filling pressures in obese subjects. The ratio of the mitral E wave velocity to the early diastolic mitral annular tissue velocity ( $E'$ ) is a well-validated index of pulmonary capillary wedge pressure [24, 25].  $E/E'$  measured at rest has been reported to be normal ( $<10$ ) in obese subjects [15]. Serum levels of brain natriuretic peptide (BNP) are widely used in the clinical evaluation of patients with known or suspected heart failure. Elevated levels of this hormone are indicative of volume overload and higher LV filling pressures. Interestingly, obese subjects have lower levels of serum BNP than normal weight controls with similar pulmonary capillary wedge pressure [26]. Moreover, BNP levels are consistently reported to be in the normal range or below normal in the majority of obese subjects [27]. Thus, the bulk of evidence points to the conclusion that even though obesity

is associated with diastolic dysfunction at the myocardial level, LV filling pressures remain normal at rest.

**RV function:** One recent study found that right ventricular (RV) ejection fraction was not altered in obese subjects [28]. However, those with BMI > 35 kg/m<sup>2</sup> had reduced RV function compared with a reference population, as evidenced by reduced systolic tissue Doppler velocities and reduced measures of systolic strain and strain rate. Surprisingly, these changes occurred irrespective of the presence of sleep apnea. Similar but lesser degrees of reduced systolic function were present in overweight and mildly obese groups. Differences in RV diastolic velocities were also seen in obese vs. a reference population. BMI remained independently related to RV changes after adjusting for age, log insulin, and mean arterial pressures. In obese patients, these changes were associated with reduced exercise capacity, but not the duration of obesity or the severity of sleep apnea. Two other studies showed preserved RV systolic function in obesity [29]. However, one of these did find altered RV diastolic filling characteristics [30].

**Vascular function:** Suffice it to say that obesity, especially abdominal obesity, is a well-defined risk factor for the development of atherosclerotic coronary artery disease [31–33]. However, since abdominal obesity is part of the diagnostic criteria for the metabolic syndrome, it is nearly impossible to dissect out the independent contributions of the different components of this syndrome. In addition, endothelial dysfunction is widely present in obesity and the metabolic syndrome [34–36]. Thus, both macro- and microvascular abnormalities likely contribute to the many structural and functional problems of the heart in obesity.

## ***Obesity-Associated Changes in Cardiac Structure***

**Left ventricular hypertrophy:** There is broad consensus that obesity is independently associated with LV hypertrophy. In examining studies that report these changes, it is important to be cognizant of the methods used to normalize cardiac mass, which is usually determined noninvasively by echocardiography, radionuclide studies, or magnetic resonance imaging (MRI). Normalizing heart weight to body surface area (BSA) tends to underestimate LV hypertrophy because BSA increases more than LV weight [37–39]. Because of this, many investigators now choose to index heart size to lean body mass, height, or height raised to the power of 2.7 [38, 39].

Most recent studies support a slight predominance of concentric (increase in LV wall thickness > increase in LV cavity size) vs. eccentric hypertrophy (increase in LV cavity size > increase in LV wall thickness) in obese individuals. The pathophysiological mechanisms that account for the presence of LV hypertrophy and that determine differences in the pattern of LV geometry in humans are incompletely understood, but are likely influenced by the differences in comorbidities such as hypertension or sleep apnea. The pattern of hypertrophy that is present may be clinically meaningful since accurate phenotypic characterization might provide insight into the underlying mechanisms, which might allow more specific and targeted therapeutic approaches.

*Right ventricular cardiac hypertrophy:* A few studies have focused on the right ventricle in uncomplicated obesity. Wong et al. reported that RV cavity size and wall thickness were mildly increased in obese subjects as compared to a normal weight reference group [28]. Alpert et al. reached similar conclusions [40, 41]. Although it seems likely that RV enlargement could result from obstructive sleep apnea and chronic pulmonary hypertension (see below), the available published data surprisingly do not consistently support this hypothesis [28, 30]. In fact, in one study the majority of obese subjects did not have enough tricuspid valve regurgitation to even estimate pulmonary artery pressures, and those that did have tricuspid regurgitation generally had normal pressures [28].

*Left atrial size in obesity:* Many studies have shown increased LA dimensions in obese subjects as compared to normal weight control subjects reviewed in [9]. Unlike LV mass, LA size is usually not indexed to body size, so this finding could be misleading. However, the most commonly reported measure of LA size, the uniaxial anterior–posterior dimension, is a well-accepted, reproducible measure of LA size that has known clinical relevance with respect to long-term event rate, survival, and the risk of developing atrial fibrillation [42, 43]. The mechanisms of increased LA size appear to be similar to those causing LVH: increasing BMI, hypertension, volume overload, and possibly LV diastolic filling abnormalities. Interestingly, obese subjects in the Framingham heart study were found to have an increased risk of developing atrial fibrillation and this risk was entirely explained by the increase in LA size [44].

*Valvular heart disease in obesity:* Only limited data exist regarding the direct effects of obesity on the heart valves. Nevertheless, the topic of valvular disease in obesity has received increased attention in recent years because of the finding that anorexic drugs used to facilitate weight loss are associated with mitral and aortic valve regurgitation [45]. Somewhat counterintuitively, a widely cited paper that evaluated the frequency of valvular abnormalities in a relatively large cohort of subjects undergoing echocardiography showed a lower prevalence of valvular regurgitation in obese than in normal weight subjects [46]. The problem of difficult echocardiographic imaging windows in obese subjects complicates the quantitative assessment of valvular disease in this population.

*Cardiac tissue composition in obesity:* Relatively few studies have compared the biochemical and structural composition of the heart in obese and normal subjects. This is not too surprising given the large obstacles inherent in obtaining human cardiac tissue – particularly from control subjects without organic heart disease. Thus, we have largely been forced to rely on data from animal models that may not accurately reflect the human condition. However, autopsy series present the opportunity to study heart tissue from obese and nonobese subjects and, in general, these studies have shown cardiac hypertrophy plus a variable extent of coronary artery disease (reviewed in [9]). However, studies relying on autopsy may be biased towards the presence of coexisting conditions and/or unexpected causes of death.

*Cardiac adiposity:* Autopsy studies have long demonstrated that obesity is associated with increased accumulation of intramyocardial lipid. Recent studies

using magnetic resonance spectroscopy (MRS) have extended these observations and reveal that obesity and insulin resistance are associated with increased intramyocellular triglyceride content in the heart, the abundance of which correlates negatively with LV contractile function and positively with LV hypertrophy [47, 48]. It is not clear if triglyceride accumulation in the heart is pathogenic per se or may be a biomarker either of underlying pathology or of the presence of other lipid signaling intermediates that may lead to adverse consequences on cardiac structure and function. Molecular mechanisms contributing to cardiac lipotoxicity were recently reviewed [49]. Human epicardial fat (EF) mass is a common finding in severe obesity and is highly correlated with risk of cardiac disease [50–52]. This depot is visceral-like in nature, releasing more FFA and proinflammatory cytokines and less adiponectin than subcutaneous fat [50]. Because of the close proximity of epicardial fat to the heart and coronary vessels, there is great potential to negatively impact cardiac function and atherogenesis. Lacobellis and colleagues have argued that the amount of epicardial fat parallels the amount of visceral adipose tissue and that the amount of epicardial fat is correlated with the severity of LV hypertrophy [53, 54]. Recent studies have also quantified decreases in epicardial fat mass upon weight loss, bariatric surgery, or exercise training [55–57]. Taken together, correlations between epicardial fat and obesity or myocardial function have been established and there is growing interest in the direct contribution of epicardial fat-derived factors in the pathogenesis of heart failure.

### *The Obesity Paradox*

While it is widely accepted that obesity increases the risk of developing heart disease, a growing number of recent reports document a statistically significant survival benefit in obese patients once they have been diagnosed with cardiovascular diseases (reviewed in [9]). Evidence continues to accumulate supporting the concept of an obesity paradox. Among 22,576 treated hypertensive patients with known CAD, the hazard ratio for all-cause mortality was 25–30% lower in overweight and obese patients [58]. The better survival was all the more impressive because the obese subjects had less improvement in blood pressure than the lean subjects. A meta-analysis of more than 250,000 subjects enrolled in 40 cohort studies found that total and cardiovascular mortality were lower in overweight and obese subjects, but the same benefit was not observed in severely obese subjects with BMI >35 kg/m<sup>2</sup> [59]. Lastly, Gala and colleagues assessed mortality at a mean follow-up of 4.4 years in 2,392 patients with peripheral arterial disease. They found that compared with underweight subjects, those with normal weight, overweight, and obesity had progressive reductions in mortality [60]. A number of smaller studies showing similar survival advantages in patients with heart failure, following myocardial infarction, unstable angina, or percutaneous coronary interventions, and patients on hemodialysis were recently reviewed by us [9]. Taken together, these data show an impressive and consistent protective effect of obesity in multiple

different cardiovascular disease states. Thus, although obesity is widely accepted as a risk factor for coronary heart disease and heart failure, accumulating evidence strongly supports a protective role of obesity once patients have developed cardiovascular disease.

Although a great deal of data support the existence of a protective effect of obesity, some authors have questioned the existence of the obesity paradox [61, 62]. These authors have suggested that there is a “U-shaped” outcome curve according to BMI for patients with heart failure, in which mortality is greatest in underweight patients; lower in normal, overweight, and mildly obese patients; but higher again in more severely obese patients [62, 63]. It has also been posited that more intense treatment regimens are applied in obese patients and that this might explain their increased survival post-MI [62]. However, this seems doubtful given the widely accepted and well-publicized guidelines for the treatment of cardiovascular disease. One recent study has suggested that overweight and obese individuals were in fact protected from short-term death, yet have a long-term mortality risk that is similar to normal weight individuals [64]. The conclusion that obesity may both elicit cardiac disease and protect from cardiovascular death clearly now requires further mechanistic analyses at cellular, molecular, and systematic levels. If we can identify the beneficial component, it might be possible to harness the effect for therapeutic purposes.

## **Comorbidities that Contribute to Cardiac Dysfunction in Obesity**

### ***Diabetes (Hyperglycemia)***

The development of insulin resistance and type 2 diabetes in obese individuals [65, 66], the significant contribution of diabetes to cardiovascular disease [67–69], and potential mechanisms by which diabetes may lead to cardiac dysfunction [70] have been extensively reviewed. Nevertheless, the precise role of diabetes in producing structural changes in the adult heart is quite controversial. As with many aspects of cardiovascular disease in obesity, it is a challenge to separate the direct effects of obesity from its frequent traveling companions. The presence of diabetes or measures of insulin resistance were not predictive of LV mass in the mainly Caucasian, middle-aged women studied by Avelar et al. [71]. In other populations, such as those in the Strong Heart Study (native Americans of the western U.S.) and the Framingham heart study, diabetes or the metabolic syndrome appears to be a significant risk factor for LV hypertrophy [34, 72, 73]. In the population involved in the Strong Heart Study, diabetes is almost universal and may have a genetic underpinning. Thus, it is likely that multiple predisposing factors for LV hypertrophy and genetic modifiers may summate in a complex and nonlinear fashion that produces different end effects, depending upon the population under study. Advanced glycation end products (AGE) mediate the detrimental influence of

hyperglycemia on many diabetic complications and Laakso's group conducted a comprehensive 18-year follow-up study in Finnish subjects which was the first to demonstrate that AGE can predict total, cardiovascular disease and coronary heart disease mortality in nondiabetic women [74]. Furthermore, it has been suggested that in cardiomyocytes, AGE regulate the response to ischemia-reperfusion injury via modulation of cardiac energy metabolism [75]. Ren's group recently demonstrated that a 12-week high-fat diet in rats induced obesity and elevated serum AGE levels which correlated with increased *O*-Glc-NAc modifications and apoptosis in cardiomyocytes [76], in a similar manner to the apoptosis induced by hyperglycemia [77]. Many of the changes in cardiac metabolism, discussed in the section on animal models that have recently been described in obesity, were long recognized to occur in animals and humans with type 1 and type 2 diabetes. Thus, the development of diabetes in individuals with obesity is likely to have synergistic and deleterious effects on cardiac metabolism and function.

### ***Pressure/Volume Overload***

Hypertension is highly prevalent in obese subjects (>60%) and it is probably the most common cause of LV hypertrophy in the general population. It is generally accepted that pressure overload leads to a concentric pattern of hypertrophy [78]. Given the relatively high frequency of concentric LV geometry in obese patients, it is very likely that hypertension plays a major role. Even if daytime or office blood pressures are normal, 24 h blood pressure recordings show that obese patients often have a "nondipping" pattern in which they lose the normal nocturnal decline in blood pressure [79]. Sleep disordered breathing may explain this phenomenon (see below). Thus, the true prevalence of hypertension in obesity may be even higher than what has been estimated in the literature. Higher systolic blood pressures, even if they are not in the hypertensive range, are associated with a greater extent of LV hypertrophy in obesity [71]. Several studies have shown synergistic effects between increasing BMI and increasing systolic blood pressure [38, 71].

Obese individuals have expanded central blood volume [23]. In addition, stroke volume and cardiac output are both increased (reviewed in [9]). These changes in blood volume and cardiac output are most likely due to the increased metabolic demand that results from increases in both lean and fat mass. The large fat depots in obesity produce a low-resistance vascular circuit that may further increase cardiac output. There may also be changes in renal absorption of salt and water. The combination of these factors is proposed to produce a form of volume overload similar to that which occurs with regurgitant valvular heart disease, beriberi, or arterial-venous fistulas. Chronic volume overload or high output failure is classically felt to produce an eccentric form of cardiac hypertrophy with enlarged cardiac chambers, but normal wall thickness [78]. However, dilated cardiomyopathy of any etiology will produce a similar cardiac geometry. Even in high output failure, the cardiac output may decline in the end stage of the disease. Several authors have

argued that volume overload is a primary mechanism contributing to the hypertrophy in obesity [80–82]. Unfortunately, this relatively straightforward and logical hypothesis is complicated by the fact that multiple recent studies have shown a predominance of concentric geometry in obese patients (reviewed in [9]).

## ***Sleep Apnea***

Sleep disordered breathing is increasingly recognized as an important cause of cardiovascular abnormalities in obesity [83]. Obstructive sleep apnea is very common in obesity and is nearly universal in severe obesity. There are multiple routes by which sleep apnea could lead to LV hypertrophy, including: exacerbation of nighttime and daytime hypertension, increased sympathetic tone, chronic hypoxemia, and exaggerated swings in intrathoracic pressure during obstructive episodes [83]. Although some studies have failed to show a relationship between sleep apnea and LV hypertrophy, the majority do support such an association (reviewed in [9]). One of the confounding factors in these analyses results from difficulties in the quantification of the severity of sleep apnea. Most studies have relied upon the apnea–hypopnea index (the number of apneic and hypopneic episodes per hour of sleep). However, the *number* of such episodes may not be the actual cause of hypertrophy. Rather, growing data suggest that *nocturnal oxygen desaturation* may be the true culprit [71, 84]. Cloward and colleagues found that the use of continuous positive airway pressure at night for 6 months was associated with a reduction in LV wall thickness [85]. Avelar et al. reported that in a predominantly Caucasian female population with severe obesity, the degree of LV hypertrophy was related to BMI, systolic blood pressure, and the severity of nocturnal hypoxemia [71]. The majority of these patients had concentric LV hypertrophy. In this study, a diagnosis of hypertension or an average nocturnal O<sub>2</sub> saturation <85% each had synergistic interactions with increasing BMI.

## **Mechanistic Insights Gained from Studies in Animal Models of Obesity**

Many groups have used animal models (mainly rodents) to elucidate mechanism for changes in cardiac structure and function in obesity and diabetes. Because obesity leads to impaired glucose tolerance and alterations in circulating lipids (triglycerides and FFA), it is challenging to separate changes that are attributable to metabolic disturbances associated with obesity vs. obesity-associated hemodynamic changes. In one sense this is not necessarily a problem given that in humans the consequences of obesity on cardiac structure and function are also multifactorial. A number of approaches and models have been developed to address mechanisms. These include diet-induced obesity (DIO) models, genetic mutants that develop obesity, the majority of which have alterations in leptin signaling (e.g., *db/db* mice and the *fa/fa* rat) or



leptin generation (e.g., *ob/ob* mice), and transgenic/knockout models that target specific pathways that are deemed to play an important role in cardiac dysfunction in obesity. Animal models of obesity and diabetes have been the subject of many recent reviews [86, 87]. In these models the impact of obesity and their associated changes in metabolism on cardiac function in nonstressed hearts and following ischemic insults *ex vivo* and *in vivo* have been evaluated. In most instances, obesity has been shown to be associated with subtle changes in LV function in nonstressed hearts and with decreased recovery from ischemia and reperfusion injury, particularly when studied *in vivo* [9]. Pathogenic mechanisms that have been investigated in these models include the role of changes in myocardial substrate utilization, changes in myocardial insulin sensitivity, lipotoxicity, and mitochondrial dysfunction.

*Altered cardiac substrate metabolism:* In most genetic models of obesity, as well as in DIO models, myocardial substrate utilization shifts towards increased utilization of fatty acids (FA) and decreased utilization of glucose [70]. These changes are present within 2 weeks of high-fat feeding and in genetic mutants as early as weaning [88, 89]. In both of these instances, they occur prior to the development of significant changes in systemic glucose homeostasis, in the absence of major changes in circulating concentrations of fatty acids or triglycerides, and prior to the onset of obesity in DIO models. The earliest changes that have been described are reduced glucose transporter translocation, which accounts initially for reduced glucose uptake. This in turn leads to reciprocal increase in FA uptake and oxidation. At this early stage, there are no discernible defects in the ability of insulin to activate PI3K and Akt signaling. Similar findings were recently reported in cardiac muscle biopsies obtained from humans with obesity and insulin resistance [90]. Recent studies have also suggested that DIO leads to activation of inflammatory pathways within cardiomyocytes that in turn can alter metabolic signaling pathways such as activation of AMPK [91].

As high-fat feeding persists, there is increased expression of PPAR- $\alpha$  targets, presumably on the basis of increased availability of fatty acid ligands [88]. PPAR- $\alpha$  is a ligand-regulated transcription factor that increases the expression of most genes involved in fatty acid uptake and mitochondrial fatty acid oxidation. As high-fat feeding persists, an imbalance between FA uptake and oxidation occurs leading to accumulation of triglycerides [92]. Similar changes have been described in humans with obesity (i.e., increased FA oxidation, decreased glucose oxidation, and increased cardiac accumulation of triglycerides) [47, 93]. Moreover, activation of PPAR- $\alpha$  targets and increased intramuscular triglycerides were also observed in cardiac muscle samples obtained from subjects with heart failure and diabetes [94]. An increase in myocardial FA utilization is also associated with increased myocardial oxygen consumption and reduced cardiac efficiency [88, 95]. One basis for this is the increased oxygen cost of oxidizing fatty acids relative to glucose. Decreased cardiac efficiency has been described in humans with obesity and insulin resistance [93]. Reduced cardiac efficiency has been postulated to contribute to reduced ability of these hearts to adapt to hemodynamic (e.g., pressure overload) or ischemic stress.

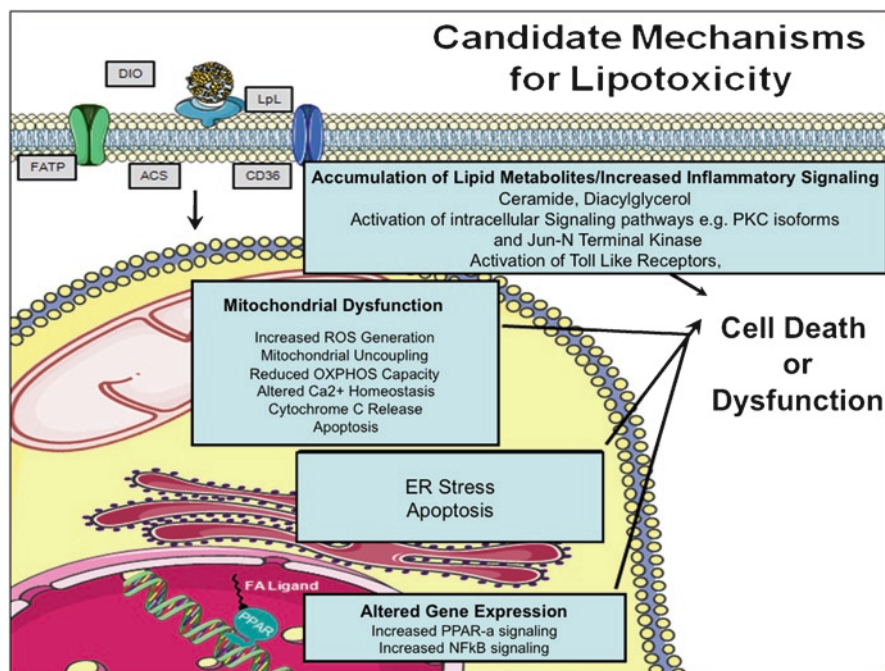
*Mitochondrial uncoupling in obesity:* A recently described mechanism for increased myocardial oxygen consumption is mitochondrial uncoupling, which has been described in *ob/ob* and *db/db* mice [96, 97] and in UCP-DTA mice [98], which

are transgenic mice that were genetically engineered to lose brown adipose tissue, and which develops mild obesity and impaired glucose tolerance. However, mitochondrial uncoupling was not observed after short-term high-fat feeding [88]. Thus, mitochondrial uncoupling might represent a fairly late adaptation in the continuum from overfeeding to obesity to diabetes. When mitochondrial uncoupling occurs in murine models, the mechanism appears to be induced by fatty acid-induced ROS generation, which in turn activates uncoupling proteins [96, 97]. This ROS-induced mitochondrial uncoupling is observed in murine models after the onset of obesity, glucose intolerance, and generalized as well as myocardial insulin resistance. It is not known if mitochondrial uncoupling exists in humans with obesity, and if these changes contribute to impaired myocardial energetics. However, studies using MRS, in relatively inadequately controlled individuals with type 2 diabetes, revealed reduced ATP/PCr ratios that were consistent with diminished high-energy cardiac reserves [99]. Moreover, there was an inverse correlation between circulating concentrations of FFA and the ATP/PCr ratios. Given the existence of evidence linking fatty acids to mitochondrial uncoupling, these observations indirectly suggest that FA-mediated mitochondrial uncoupling might contribute to diminished myocardial energetics, at least in individuals with type 2 diabetes. It is important to note that in overweight and better-controlled diabetic subjects, no changes in PCr/ATP ratios were observed using MRS [100]. However, a recent study performed in atrial appendages obtained from humans with type 2 diabetes revealed mitochondrial dysfunction and evidence of mitochondrial ROS overproduction that correlated with HbA1C concentrations as low as 6.5 [101]. Taken together, the available evidence in humans supports the existence of mitochondrial dysfunction in type 2 diabetes. Future studies in obese individuals with normal glucose tolerance or impaired glucose tolerance without diabetes will therefore be needed to determine if obesity per se will lead to similar changes in mitochondrial energetics as have been described in human studies.

*Mitochondrial dysfunction:* In addition to mitochondrial uncoupling, mitochondrial dysfunction associated with altered mitochondrial morphology, loss of subunits of the electron transport chain, and decreased mitochondrial respiratory capacity has been described in cardiac mitochondria isolated from *db/db* and *ob/ob* mouse hearts [96, 97]. Many of these changes were also observed in the hearts of mice with severe myocardial insulin resistance on the basis of genetic disruption of insulin signaling in cardiomyocytes implicating important links between insulin signaling and mitochondrial oxidative capacity [102]. Eight weeks of high-fat feeding in Wistar rats also led to cardiac dysfunction that was associated with the development of dysmorphic mitochondria and intramyocellular lipid accumulation [103]. However, similar changes were not observed following high-fat feeding in mice implying species-specific differences in the susceptibility of mitochondria to damage – induced by caloric excess [88]. In DIO or genetic models of obesity, cardiac dysfunction develops in parallel with these changes in mitochondrial function and substrate utilization. However, the degree of cardiac dysfunction described is dependent upon the technique utilized to determine cardiac function. Most dramatic changes are observed in isolated perfused heart preparations or when load-independent parameters are

measured following LV catheterization. Load-dependent analyses such as echocardiography or MRI will often reveal preserved or even increased systolic function in the early stages of diet-induced or genetic obesity [70]. These findings might reflect neurohumoral adaptations that sustain cardiac function early in the evolution of obesity. However, as obesity persists or diabetes ensues, these techniques will ultimately reveal evidence of systolic and diastolic LV dysfunction.

**Lipotoxicity:** Lipotoxicity has been proposed as a mechanism that impairs myocardial contractile function in obesity. These observations have been based upon the correlation of increased myocardial triglyceride content with decreased cardiac function or decreased recovery from myocardial ischemia. However, the mechanisms by which lipotoxicity impairs cardiac function are incompletely understood, but have been recently reviewed [49]. As summarized in Fig. 1, mechanisms that have been postulated to lead to lipotoxic cardiac injury include mitochondrial dysfunction as discussed above, endoplasmic reticulum stress, increased transcriptional activation of inflammatory or profibrotic mediators such as NF $\kappa$ B, and accumulation of lipid-derived intermediates such as ceramide and diacyl glycerol that activate signaling pathways that may lead to cellular dysfunction or cell death.



**Fig. 1** Mechanisms by which lipotoxicity may lead to cardiac dysfunction. Increased uptake of fatty acids into cardiomyocytes following diet-induced obesity (DIO), which is associated with increased plasma membrane localization of CD36, or in transgenic mouse models with over-expression of a membrane anchored lipoprotein lipase (LPL), fatty acid transporter isoform 1 (fatp1) or acyl CoA synthase (Acs). Lipotoxic heart injury develops as a result of the impact of lipid excess in multiple subcellular compartments as depicted in the figure

Studies in Zucker Fatty (*fa/fa*) revealed a correlation between intramyocellular ceramide content and cardiomyocyte apoptosis [104]. Treatment of these rats with rosiglitazone, which normalized systemic metabolic homeostasis, was associated with improved LV function and reduced apoptosis that correlated with a lowering of ceramide content. Studies in cultured cells have provided evidence that although ceramide exposure may induce apoptosis, inhibition of de novo ceramide synthesis in cells exposed to increased fatty acids does not completely inhibit apoptosis, therefore suggesting that ceramide-independent pathways exist that contribute to FA-induced myocardial apoptosis [49].

It is important to note that most of the adverse cardiac consequences of high-fat feeding occur when diets are provided that lead to obesity and insulin resistance. In contrast, isocaloric high-fat diets, even those that are rich in saturated fats, have been shown to reduce adverse LV remodeling that occurs in response to pressure overload hypertrophy or that develops after coronary artery ligation [49]. The mechanisms for the cardioprotective effects of lipids in these studies are incompletely understood. However, these studies serve to underscore the complexity of the cardiac adaptations to lipid excess and the complex interactions between obesity, insulin resistance, and glucose intolerance in leading to cardiac dysfunction.

*Transgenic models of lipotoxicity:* A number of transgenic models have been generated that seek to mimic aspects of obesity-associated cardiac dysfunction. These models, which have been recently reviewed [49], include models of lipotoxicity that were generated by overexpression of acyl CoA synthase – *acs11*, Fatty acid transporter 1 (*fatp1*), membrane anchored lipoprotein lipase, or PPAR- $\alpha$ . All of these models develop increased myocardial lipid content and cardiac dysfunction. The nature of the accumulated lipid varies between models. For example, LPL overexpression in addition to increased concentration of derivatives of long-chain FA such as ceramides also exhibits cholesterol accumulation [105]. Differences also exist in the nature of the contractile dysfunction that develops. Thus, high-level overexpression of *acs11* leads to systolic dysfunction and premature mortality that is associated with ceramide accumulation [106]. In contrast, overexpression of PPAR- $\alpha$  in cardiomyocytes leads to LV contractile dysfunction that is associated with low rates of glucose oxidation and high rates of FA oxidation [107, 108]. Placing these animals on a diet that is enriched in medium and short-chained fatty acids ameliorated these effects, leading to the conclusion that toxic effects of PPAR- $\alpha$  overexpression might be mediated by long-chain fatty acids or their metabolites. Moreover, crossing these mice with CD36 null animals normalized contractile dysfunction indicating that CD36 mediates uptake of lipids that precipitate lipotoxic cardiac dysfunction [109]. Indeed, studies in *fa/fa* rats or in rats fed a high-fat diet revealed increased plasma membrane translocation of CD36, supporting a critical role for increased uptake of lipid via CD36 as a mediator of lipotoxicity [110]. CD36 null mice were shown to have delayed cardiac aging, which was attributable to reduction in life-long lipid uptake by the heart [111]. Treatment of mice that harbor a membrane-targeted lipoprotein lipase with the drug myriocin (an inhibitor of de novo ceramide biosynthesis) partially but not completely reversed contractile dysfunction [105], which indicates that, in addition to ceramide,

additional mediators of lipotoxicity-associated myocardial dysfunction must exist. In contrast to most transgenic models of lipotoxicity that develop systolic dysfunction, mice that overexpress *fatp1* develop diastolic dysfunction [112]. In summary, many transgenic models of lipotoxicity have provided direct evidence linking excess myocardial lipid uptake or utilization with cardiac dysfunction. The molecular mechanisms for these changes are incompletely understood, and the contribution of each of these pathways to obesity-related cardiac dysfunction remains to be fully elucidated.

## Postulated Mechanisms for Cardiac Dysfunction in Obesity

In this section, we will review a number of well-studied pathways or mechanisms that in theory could contribute to cardiac dysfunction in obesity. The discussion will serve to underscore the potential roles of these pathways in obesity-related cardiac dysfunction. Although many of the observations raise important hypotheses regarding the pathophysiology of obesity-related cardiac dysfunction, they remain to be definitively shown to contribute to obesity-associated cardiac dysfunction in humans or appropriate animal models.

### *Neurohumoral Activation*

Numerous studies show evidence that obese subjects have activation of the sympathetic nervous system. This appears to result at least in part from the effects of sleep-disordered breathing (reviewed in [9]). Increased sympathetic tone may contribute to the high incidence of concentric LV geometry because of hemodynamic factors such as elevated blood pressure and increased cardiac contractility. In addition, catecholamines may have direct hypertrophic effects that are independent of hemodynamic factors. There also appears to be activation of the renin-angiotensin system in obesity [113]. These two pathways are interrelated and are both prohypertrophic via direct signaling effects and hemodynamic effects (i.e., vasoconstriction and elevation of blood pressure). As mentioned above, sympathetic activation likely results from sleep apnea and other indirect factors. In contrast, activation of the renin-angiotensin system may occur directly via signals from adipose tissue. Engeli et al. have suggested that adipose tissue contains the major components of a local renin-angiotensin system [114]. Furthermore, increased activity of this system has been implicated in human obesity hypertension [114, 115]. One proposed mechanism for such an effect is increased secretion of angiotensinogen from adipocytes, especially those in visceral fat [116]. Angiotensin is thought to cause sympathoexcitation, so there may be additive effects of renin-angiotensin and sympathetic activation with respect to blood pressure elevation and cardiac remodeling in obesity [115].

## ***Effects of Adiponectin on the Heart***

Adiponectin levels are decreased in obesity. There is currently great research and clinical interest in the endocrine effects of adipokines on cardiac tissue and their role in heart failure (reviewed in [9] and [117, 118] and there is now considerable interest in the suggestion that adiponectin may represent a fruitful therapeutic target in heart failure [119, 120]. Although the bulk of circulating adiponectin is secreted from adipocytes, it can also be synthesized and secreted by cardiomyocytes [121], and subsequent autocrine or paracrine effects may play an important role in adiponectin's cardioprotective effects. Indeed, altered adiponectin content was observed postmortem in patients who suffered myocardial infarction or dilated cardiomyopathy [122].

Germline knockouts of the adiponectin gene have been particularly informative in terms of elucidating the interaction between adiponectin and cardiovascular injury [123] and reviewed in [9]. The relevance of this model stems from the fact that levels of adiponectin are invariably reduced in animals and humans with obesity [124]. Several *in vivo* studies using adiponectin KO mice have shown that pressure overload-induced concentric hypertrophy was enhanced in adiponectin-deficient mice and led to increased mortality that was corrected by adiponectin replacement [125, 126]. It has been suggested that the modulating effects of adiponectin on LV remodeling might manifest only when additional hypertrophic signaling pathways are activated. Administration of adiponectin 30 min before, during, or 15 min after surgery-induced ischemia reperfusion diminished the infarct size, apoptosis, and TNF- $\alpha$  production in both wild type and adiponectin KO mice [127] and adiponectin protected against coronary artery ligation-induced apoptosis, myocyte hypertrophy, and interstitial fibrosis [128], at least in part via AMPK-dependent signaling, suppressing iNOS and superoxide production or altered glucose metabolism [129]. One study has demonstrated that 30  $\mu$ g/ml full-length adiponectin (fAd) reduced hypoxia-reoxygenation-induced apoptosis (measured only by TUNEL assay) in primary neonatal cardiomyocytes [127]. We have characterized the expression profile and role of AdipoR1 and R2 in mediating signaling and metabolic effects of globular (g) Ad and fAd in both neonatal and adult rat cardiomyocytes [130]. Furthermore, myocardial necrosis in obese mice with viral myocarditis was attenuated upon induction of endogenous cardiac adiponectin expression, suggesting this may represent a compensatory protective response [131–133].

A number of clinical studies have established correlations between plasma adiponectin levels and various aspects and severity of heart failure [134–137] and reviewed in [9], with the majority, but not all, suggesting a cardioprotective effect. Genetic studies have also suggested an important role of adiponectin in heart failure, with increased LV mass observed in uncomplicated obese subjects carrying the G/G genotype at position 276 of the human adiponectin gene, which tracks with lower concentrations of adiponectin [138]. Indeed, adiponectin has been proposed as a biomarker, which might serve as a suitable screening test facilitating early intervention and prevention of heart failure [139] and reviewed in [9]. In particular, given our appreciation of the significant roles played by the various multimeric forms of adiponectin [140], it will be most interesting to observe if any aspects of CVD correlate most strongly with oligomeric, hexameric, or trimeric forms.



Various potential mechanistic explanations for cardioprotective effects of adiponectin are now beginning to emerge. Adiponectin is known to mediate potent metabolic effects in skeletal muscle and liver [124, 140] and an understanding of direct metabolic effects on cardiomyocytes is now emerging. Adiponectin caused a small but significant increase in glucose and fatty acid uptake and induced AMPK phosphorylation in cultured neonatal rat cardiomyocytes and mouse HL-1 cells [121]. Another study demonstrated a role for the C-terminal globular fragment of adiponectin in regulating cardiac fatty acid oxidation in rabbit hearts in the immediate newborn period [141]. We have recently examined the effect of specific forms of adiponectin on metabolism in neonatal rat cardiomyocytes and found that both full-length and globular adiponectin elicited an acute increase in glucose uptake and oxidation [130]. After prolonged treatment, increased fatty acid uptake and oxidation predominated and correlated with decreased PDH activity and glucose oxidation. Based on these studies to date, it has been speculated that acute administration of adiponectin post-MI may confer beneficial metabolic effects. Collectively, the effects described above were mediated via both AdipoR1 and AdipoR2 and occurred via signaling mechanisms involving AMPK, ACC, and Akt [142]. AdipoR1 is the major form in cardiomyocytes and skeletal muscle [143], whereas AdipoR2 is predominantly expressed in liver. With respect to the heart, two recent studies demonstrated expression of both AdipoR isoforms that were upregulated by rosiglitazone or STZ-induced diabetes [144, 145]. A decrease in cardiac AdipoR1 expression has been detected after CAL-induced MI in mice [146]. A potential pathophysiological role for alterations in AdipoR is supported by a positive correlation between receptor expression and insulin resistance [147] or plasma insulin levels [148, 149], lower AdipoR1 and AdipoR2 expression in patients with a family history of diabetes [147], and altered skeletal muscle AdipoR expression in diabetic humans or mice (reviewed in [9]). There is now great interest in the role of the newly discovered adaptor protein containing pleckstrin homology domain, phosphotyrosine binding (PTB) domain, and leucine zipper motif (APPL1) in adiponectin action. Adiponectin stimulates APPL1 interaction with its receptors and this mediates downstream signaling and metabolic events [150]. We also now appreciate that APPL1 acts as a novel and critical regulator of the crosstalk between adiponectin and insulin signaling pathways [150–152]. AMPK is well established as the key regulator of many adiponectin effects [150]. Finally, given our growing appreciation of the distinct signaling and physiological roles played by the various multimeric forms of adiponectin [140, 153], it will likely prove interesting to observe, with or without pre-existing hyperglycemia, if distinct aspects of cardiac remodeling are regulated by globular or high molecular weight hexameric or trimeric forms.

### ***Effects of Leptin on the Heart***

Obese individuals typically have elevated circulating levels of leptin which may impact upon myocardial function via direct peripheral effects or via secondary central nervous system-mediated responses as recently reviewed [118, 154, 155].



Leptin-deficient *ob/ob* mice exhibit a higher degree of cardiomyocyte apoptosis, which can be restored to normal levels by leptin administration [156]. Leptin can also induce generation of reactive oxygen species in the heart [157]. Another characteristic marker of heart failure, depressed cardiac response to  $\beta$ -adrenergic agonists, is also observed in *ob/ob* mice and can be corrected by leptin replacement therapy [158]. Several reports have suggested that leptin directly induced hypertrophy in both human and rodent cardiomyocytes (reviewed in [9]), although one report concluded that leptin mediates antihypertrophic effects [159]. In general, the *ob/ob* mouse model is rather unique (plasma leptin levels are increased in almost all other obese rodent models and in humans [160] and may not precisely reflect the alterations observed in human obesity. Another caveat of this model for the study of cardiac hypertrophy is the presence of low arterial pressure, while the opposite is found in most forms of obesity [161]. In summary, it would appear that leptin mediates a myriad of effects, which can impinge upon development or progression of heart failure and it is likely that too much or too little is detrimental.

The prevailing hypothesis is that leptin resistance occurs in obese humans, and while this may certainly be true of the hypothalamus, there is current interest in the theory that selective leptin resistance occurs in obesity [162, 163] such that whether the effects of leptin on the heart are enhanced or suppressed is uncertain. This concept arose from observations that while the effects of leptin on satiety and energy metabolism were resistant, the sympathoexcitatory effects were maintained (reviewed in [9]). It was recently suggested that leptin resistance in obesity is restricted to the metabolic actions of leptin [162] and impaired leptin signaling was observed in one study using ventricular cardiomyocytes isolated from rats subjected to 10-week dietary sucrose feeding to generate hyperleptinemia and insulin resistance [164]. Notably, leptin administration in mice has been shown to reduce infarct size and enhance functional recovery following coronary artery ligation [165, 166].

The mechanisms via which leptin directly influences cardiac function are important to delineate, with a view to therapeutic targeting. Leptin acts via a family of receptor (*ob.R*) isoforms [167] and mediates a wide range of physiological effects [168, 169]. Recently, we have focused on leptin-induced cytoskeletal rearrangement in mediating direct effects of leptin since cytoskeletal alterations have previously been implicated in the pathophysiology of heart failure [170, 171] and have also been implicated in regulation of neonatal cardiomyocyte hypertrophy by leptin [172]. We have now shown that leptin, induced actin remodeling in neonatal cardiac fibroblasts in parallel with temporal activation of the Rho/ROCK signaling pathway [173]. Pharmacological inhibition of Rho (using C3 transferase) or ROCK (Y-27632) prevented leptin-stimulated polymerization of actin and cell surface MT1-MMP (matrix metalloproteinase) content. Leptin treatment also enhanced extracellular activation of a known MT1-MMP substrate, MMP2, which was again attenuated in the presence of C3 transferase and Y-27632. Hence, our results suggest that leptin directly regulates myocardial matrix remodeling in cardiac fibroblasts by regulating the cell surface localization of MT1-MMP, via Rho/ROCK-dependent actin polymerization, and MMP-2 activation.

Leptin-deficient *ob/ob* mice are well characterized as an obese animal model that exhibits cardiac remodeling and depressed function [174–176]. Using this approach,

several studies have now indicated a permissive role of leptin in regulating cardiac function [158, 174–178]. However, a precisely defined or consistent effect of leptin or leptin deficiency on cardiac structure and function has not been fully elucidated. In *ob/ob* mice, obesity ensues first (~5 weeks) which is then followed by diabetes several weeks later. Buchanan and colleagues [89] reported that LV function varied with age; for example, LV contraction and relaxation ( $dP/dt$ ) were high in young animals (4–5 weeks of age), despite the presence of significant obesity. In 10–11-week-old female *ob/ob* mice, Christoffersen and colleagues reported evidence of diastolic dysfunction manifested by reduced E/A ratios [179]. Similar findings have been reported in *db/db* (leptin receptor-deficient) mice with evidence of systolic and diastolic function by echocardiography that was absent in 6-week-old (around onset of hyperglycemia), but present in 12-week-old mice [180].

### *Changes in Extracellular Matrix and Fibrosis*

The ECM provides the support essential for maintaining alignment of myofibrils within the myocardium. A healthy ECM facilitates storage of energy produced during systole and contributes to relengthening of myocytes during diastole [181, 182]. Alterations in the composition and structure of the ECM can play an important role in heart failure [181, 182]. There is a constant turnover of ECM in the myocardium, approximately 0.6% of total per day, and proper maintenance of ECM composition is vital in the healthy heart [181]. Thus, it is important to delineate the potential mechanisms contributing to these changes. With respect to obesity, the concept that adipokines regulate myocardial ECM is of great potential importance [183, 184]. The main components of the cardiac ECM are often described as structural (collagen/elastic fibers) and adhesive (fibronectin, laminin). Turnover of these proteins is also regulated by a family of degradative enzymes termed matrix metalloproteinases (MMPs) and their endogenous inhibitors, TIMPs [185–187].

The changes occurring in myocardial ECM during remodeling are summarized below. Cardiac fibroblasts, which account for ~70% of cells in the heart, are thought to be the principal site for synthesis of ECM components and regulation of their turnover by secretion of MMPs and TIMPs. However, cardiomyocytes also make an important and clearly quite distinct contribution. Fibrillar collagens type I and III comprise ~85 and ~11% of total myocardial collagen in healthy hearts, respectively [185, 186]. Collagen content does not always accurately reflect changes in function and it has been suggested that the distribution and orientation of collagen fibrils is a major determinant of ventricular stiffness [186–188]. Indeed, it is now accepted that during progressive remodeling in heart failure, the highly organized architecture of the ECM is replaced with a thickened and poorly organized collagen weave [189]. Early compensatory remodeling is not usually associated with significant alterations in collagen content; however, alterations in collagen (replacement of type III with type I collagen) have been clearly documented in later stages of various models of HF [187]. In particular, increased fibrosis has been

described in the hearts of *ob/ob* mice [190, 191] and in Zucker (*fa/fa*) rats [104, 192]. Changes in MMP expression and activity have a distinct pattern during progression of HF: early compensatory remodeling is not typically associated with any alteration in MMPs [193, 194]. However, with deterioration in function in both human and animal models, an increase in MMP2 and MMP9 activity is observed [193–198]. Thus, elevated MMP activity in the left ventricle of the heart is viewed as an early trigger of pathologic remodeling leading to end-stage HF [199]. As decompensation progresses, increases in MMP1 and MMP3 are also seen [200]. Chemical inhibition of MMPs has also been shown to attenuate LV dilation and preserve function after coronary ligation [201, 202]. Four distinct TIMP isoforms have been identified and, although, for example, all TIMPs can inhibit MMP2 and MMP9, TIMP4 is cardiac specific, is the most abundant form in the myocardium, and has a much greater substrate-binding affinity than other TIMPs [203, 204].

### ***Cardiomyocyte Apoptosis***

Myocyte apoptosis has been suggested to be an important etiological component of heart failure, particularly in the transition from compensatory remodeling to heart failure [205]. Endomyocardial biopsies from patients with dilated or ischemic cardiomyopathy and end-stage heart failure demonstrated apoptotic cardiomyocyte death [206, 207] and there was increased susceptibility to hypoxia-induced apoptosis in cardiomyocytes isolated from failing human hearts [208]. It has been postulated that there is great therapeutic potential to preserve cardiac function by reducing apoptosis. Therefore, furthering our current understanding of mechanisms regulating myocyte apoptosis in obesity and diabetes is essential [209]. Accordingly, inhibiting caspases can both reduce the occurrence of heart failure or slow its progression after MI (reviewed in [9]) as well as improve contractile function [210]. Note that besides the obvious role of apoptotic cell death in myocyte loss, activation of several proteases in the apoptotic cascade can cleave various contractile proteins ( $\alpha$ -actin,  $\alpha$ -actinin,  $\alpha/\beta$ -myosin heavy chain, tropomyosin, and troponins [211, 212] leading to deterioration in contractile function of the existing myocytes.

The potential mechanisms by which obesity [9] and diabetes [70] may lead to cardiomyocyte apoptosis have been well studied, yet the precise mechanisms remain controversial [34, 71–73]. Studies in Zucker (*fa/fa*) rats have shown increased apoptosis that was reduced by treatment with a thiazolidinedione, which acts at least in part via increasing adiponectin [104]. Studies using TUNEL and caspase-3 activity assays demonstrated increased levels of apoptosis in young obese *ob/ob* and *db/db* mice compared with controls. In older obese and diabetic mice, more DNA damage and less DNA repair were noted and the degree of apoptosis became more enhanced and correlated with decreased survival. This suggests a potential role for apoptosis in myocardial dysfunction and early mortality in these models and that coincident diabetes may play an important aggravating role [156]. The significance of the

combined effects of obesity and diabetes, rather than either alone, is further highlighted by studies in isolated working hearts from *db/db* mice. These hearts exhibit reduced recovery following ischemia and reperfusion after diabetes develops, but recovery is normal in the prediabetic stage [213]. Furthermore, studies in older Zucker rats demonstrated reduced recovery from ischemia, which could be prevented by treating these animals with a TZD [214–216]. Additional studies performed in vivo using coronary artery ligation with Zucker rats, *db/db* mice, and mice with DIO show that combined obesity and insulin resistance is associated with increased infarct size impaired recovery of cardiac function [217–220].

## ***Inflammation***

The link between obesity and inflammation is of relevance to heart failure since both acute inflammatory events and chronic low-grade inflammation can impact structure and function of the myocardium [221–223]. Several studies have established clear correlations between markers of inflammation, such as C-reactive protein, and mortality in patients with heart failure [224, 225]. Whereas proinflammatory cytokines such as IL-6, IL-1 $\beta$ , ANP, and TNF- $\alpha$  increase, there is not a corresponding increase in anti-inflammatory cytokines such as IL-10 and TGF- $\beta$  [226–228]. Not only the myocardium but also infiltrating cells such as macrophages, leukocytes, and platelets can contribute to the inflamed environment. There are now many reports indicating that these various proinflammatory cytokines can play a role in the myocardial remodeling process by directly influencing aspects such as hypertrophy, apoptosis, fibrosis, and ultimately contractility [229, 230]. Many of the adverse effects of proinflammatory cytokines involve activation of signaling pathways such as NF $\kappa$ B, a major mediator of inflammation in the failing myocardium. There is also currently great interest in the connection between innate immunity and inflammation in heart failure with toll-like receptors playing a central role [231, 232]. Although the potential for targeting inflammatory components in the treatment of heart failure has been mooted previously, studies to date employing anti-TNF- $\alpha$  therapeutic approaches have thus far proven ineffective in treating heart failure. This has somewhat tempered enthusiasm regarding the possibility of targeting the imbalance in inflammatory cytokines, although other aspects of the cytokine imbalance besides TNF- $\alpha$  may still prove to be effective targets for therapeutic interventions [227, 233]. However, targeting inflammation is likely to be further improved as we understand more about the pathophysiological role of novel hormones and cytokines together with specific myocardial consequences of their effects [234]. One example is lipocalin-2, a novel hormone recently shown to be released by infiltrating polymorphonuclear cells and to regulate the inflammatory response induced by ischemia reperfusion [235], while a second example is the recent interest in preventing unwanted T-cell activation which contributes to both local and systemic inflammation [236].

## Clinical Considerations

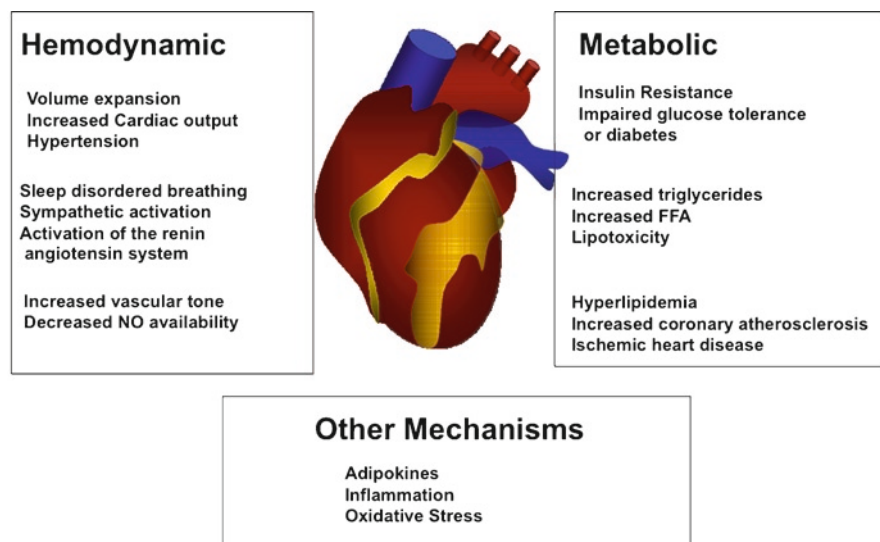
Obesity is clearly a risk factor for developing coronary atherosclerosis. In a large registry of > 100,000 patients presenting with first non-ST segment elevation myocardial infarctions, there was a linear association between BMI and earlier age of presentation. The most obese patients presented almost 15 years earlier than non-obese patients [237]. In a study of 1,314 patients undergoing coronary artery bypass surgery, subjects with higher BMI had more angiographic evidence of progression of atherosclerosis [238]. This relationship was seen in subjects who were randomized to low doses of lovastatin, but not in those assigned to more aggressive lipid-lowering therapy. The authors concluded that obesity is associated with more rapid progression of atherosclerosis, but that this can be attenuated with pharmacological lipid-lowering therapy. Nissen and colleagues studied progression of atherosclerosis with coronary intravascular ultrasound in 839 patients participating in a weight loss trial using the endocannabinoid receptor antagonist, rimonabant [239]. Patients treated with rimonabant lost more weight and had greater reductions in waist circumference and hs-CRP than the placebo group. However, the primary end point of change in percent atheroma volume was not different at the 18-month time point between the two groups. Thus, over a relatively short time frame, modest weight loss was apparently unable to slow the progression of atherosclerosis.

Many studies have shown that weight loss improves blood pressure, diabetes, sleep apnea, hyperlipidemia, and sympathetic/parasympathetic tone (reviewed in [9]). If sustained, these improvements would be expected to translate into benefits for cardiac structure and function [240, 241]. In support of this hypothesis, several studies have reported beneficial effects of weight loss on cardiac structure and LV function. Weight loss achieved via lifestyle modifications or through bariatric surgery have both been consistently associated with reductions in LV dimensions, LV wall thickness, LV mass, and LA dimension (reviewed in [9]). Perhaps more importantly, patients can exercise longer and they report less dyspnea and chest pain after significant weight loss [242]. It is widely believed that cardiovascular and other health benefits accrue when weight loss is achieved by reduced caloric intake and increased exercise. In fact, even modest amounts of weight loss or increased exercise may produce salutary effects. The removal of subcutaneous fat by liposuction does not appear to produce beneficial metabolic changes [243]. Pharmacotherapy with the two currently approved drugs for obesity treatment, orlistat (a gastrointestinal lipase inhibitor) and sibutramine (a monoamine reuptake inhibitor), has both been associated with weight loss, improvements in serum lipids, and insulin sensitivity [244–246]. Orlistat treatment usually reduces blood pressure, while sibutramine is commonly associated with increased heart rate and blood pressure [245, 247]. Neither drug has significant effects on cardiac dimensions, valvular function, or pulmonary artery pressures when given for up to 6 months [247, 248]. The full extent and duration of benefits that can be achieved when weight loss is induced by pharmacotherapy or bariatric surgery is not clear at this point in time. Nonetheless, bariatric surgery in general produces much greater amounts of weight loss than lifestyle modification or pharmacotherapy, and

these losses are usually sustained for much longer [249]. Given the rapid increase in the prevalence of severe obesity worldwide [250, 251] and the growing popularity of surgical treatments, further studies of the cardiovascular outcomes after bariatric surgery are clearly warranted [252].

## Conclusions

The dramatic and rapid increase in the worldwide prevalence of obesity has profound public health implications. The full scope of the problem is hard to realize because many obesity-related complications require decades to become manifest. Therefore, it is critical to increase our understanding of the pathophysiological bases for the numerous and multisystem disorders that are associated with or caused by obesity. We have focused in this chapter on the clinical phenotypes of cardiac dysfunction in obesity, discussed insights gained from animal models, and presented additional mechanisms that could contribute to heart failure in obesity. Figure 2 summarizes many of the processes that might link obesity to various aspects of cardiac remodeling and the eventual development of ventricular dysfunction. Current evidence is consistent with the conclusion that obesity influences cardiac remodeling, directly and indirectly, via its closely associated comorbidities



## Multiple Mechanisms Contribute to Cardiac Dysfunction in Obesity

**Fig. 2** Summary of multiple mechanisms that lead to or may contribute to cardiac dysfunction in obesity

such as hypertension, diabetes, sleep disordered breathing, renal dysfunction, atherosclerosis, and endothelial dysfunction. This complex web of interrelated processes begins with the onset of obesity, which increasingly occurs in childhood or adolescence. However, the cumulative effects on the heart may not become clinically apparent for as much as 50 years or more. Thus, it is likely that the present day impact of obesity on the cardiovascular system represents the tip of an iceberg. Our ability to unravel the impact of obesity on human health is complicated by the growing body of data showing that obesity may have protective as well as deleterious effects on the cardiovascular system. Multiple studies showing improved survival in obese patients with established cardiovascular disease have led to the concept of “the obesity paradox.” Understanding the nature of the beneficial and the detrimental components of obesity is arguably one of the most pressing agendas for our current biomedical and scientific communities.

## References

1. Gelber, R. P., Gaziano, J. M., Orav, E. J., Manson, J. E., Buring, J. E., & Kurth, T. (2008). Measures of obesity and cardiovascular risk among men and women. *Journal of the American College of Cardiology*, 52(8), 605–615.
2. Litwin, S. E. (2008). Which measures of obesity best predict cardiovascular risk? *Journal of the American College of Cardiology*, 52(8), 616–619.
3. Alpert, M. A., Lambert, C. R., Panayiotou, H., et al. (1995). Relation of duration of morbid obesity to left ventricular mass, systolic function, and diastolic filling, and effect of weight loss. *The American Journal of Cardiology*, 76(16), 1194–1197.
4. Wilhelmsen, L., Rosengren, A., Eriksson, H., & Lappas, G. (2001). Heart failure in the general population of men—morbidity, risk factors and prognosis. *Journal of Internal Medicine*, 249(3), 253–261.
5. He, J., Ogden, L. G., Bazzano, L. A., Vupputuri, S., Loria, C., & Whelton, P. K. (2001). Risk factors for congestive heart failure in US men and women: NHANES I epidemiologic follow-up study. *Archives of Internal Medicine*, 161(7), 996–1002.
6. Chen, Y. T., Vaccarino, V., Williams, C. S., Butler, J., Berkman, L. F., & Krumholz, H. M. (1999). Risk factors for heart failure in the elderly: A prospective community-based study. *The American Journal of Medicine*, 106(6), 605–612.
7. Caruana, L., Petrie, M. C., Davie, A. P., & McMurray, J. J. (2000). Do patients with suspected heart failure and preserved left ventricular systolic function suffer from “diastolic heart failure” or from misdiagnosis? A prospective descriptive study. *BMJ (Clinical Research Ed.)*, 321(7255), 215–218.
8. Kenchaiah, S., Evans, J. C., Levy, D., et al. (2002). Obesity and the risk of heart failure. *The New England Journal of Medicine*, 347(5), 305–313.
9. Abel, E. D., Litwin, S. E., & Sweeney, G. (2008). Cardiac remodeling in obesity. *Physiological Reviews*, 88(2), 389–419.
10. Aurigemma, G. P., Silver, K. H., Priest, M. A., & Gaasch, W. H. (1995). Geometric changes allow normal ejection fraction despite depressed myocardial shortening in hypertensive left ventricular hypertrophy. *Journal of the American College of Cardiology*, 26(1), 195–202.
11. de Simone, G., Devereux, R. B., Koren, M. J., Mensah, G. A., Casale, P. N., & Laragh, J. H. (1996). Midwall left ventricular mechanics. An independent predictor of cardiovascular risk in arterial hypertension. *Circulation*, 93(2), 259–265.



12. Garavaglia, G. E., Messerli, F. H., Nunez, B. D., Schmieder, R. E., & Grossman, E. (1988). Myocardial contractility and left ventricular function in obese patients with essential hypertension. *The American Journal of Cardiology*, 62(9), 594–597.
13. Chinali, M., de Simone, G., Roman, M. J., et al. (2006). Impact of obesity on cardiac geometry and function in a population of adolescents: The Strong Heart Study. *Journal of the American College of Cardiology*, 47(11), 2267–2273.
14. von Haehling, S., Doehner, W., & Anker, S. D. (2006). Obesity and the heart a weighty issue. *Journal of the American College of Cardiology*, 47(11), 2274–2276.
15. Wong, C. Y., O'Moore-Sullivan, T., Leano, R., Byrne, N., Beller, E., & Marwick, T. H. (2004). Alterations of left ventricular myocardial characteristics associated with obesity. *Circulation*, 110(19), 3081–3087.
16. Pascual, M., Pascual, D. A., Soria, F., et al. (2003). Effects of isolated obesity on systolic and diastolic left ventricular function. *Heart (British Cardiac Society)*, 89(10), 1152–1156.
17. Peterson, L. R., Waggoner, A. D., Schechtman, K. B., et al. (2004). Alterations in left ventricular structure and function in young healthy obese women: Assessment by echocardiography and tissue Doppler imaging. *Journal of the American College of Cardiology*, 43(8), 1399–1404.
18. Morricone, L., Malavazos, A. E., Coman, C., Donati, C., Hassan, T., & Caviezel, F. (2002). Echocardiographic abnormalities in normotensive obese patients: Relationship with visceral fat. *Obesity Research*, 10(6), 489–498.
19. Berkalp, B., Cesur, V., Corapcioglu, D., Erol, C., & Baskal, N. (1995). Obesity and left ventricular diastolic dysfunction. *International Journal of Cardiology*, 52(1), 23–26.
20. Wikstrand, J., Pettersson, P., & Bjorntorp, P. (1993). Body fat distribution and left ventricular morphology and function in obese females. *Journal of Hypertension*, 11(11), 1259–1266.
21. Mottram, P. M., & Marwick, T. H. (2005). Assessment of diastolic function: What the general cardiologist needs to know. *Heart (British Cardiac Society)*, 91(5), 681–695.
22. Ho, C. Y., & Solomon, S. D. (2006). A clinician's guide to tissue Doppler imaging. *Circulation*, 113(10), e396–e398.
23. Kaltman, A. J., & Goldring, R. M. (1976). Role of circulatory congestion in the cardiorespiratory failure of obesity. *The American Journal of Medicine*, 60(5), 645–653.
24. Dokainish, H., Zoghbi, W. A., Lakkis, N. M., et al. (2004). Optimal noninvasive assessment of left ventricular filling pressures: A comparison of tissue Doppler echocardiography and B-type natriuretic peptide in patients with pulmonary artery catheters. *Circulation*, 109(20), 2432–2439.
25. Ommen, S. R., Nishimura, R. A., Appleton, C. P., et al. (2000). Clinical utility of Doppler echocardiography and tissue Doppler imaging in the estimation of left ventricular filling pressures: A comparative simultaneous Doppler-catheterization study. *Circulation*, 102(15), 1788–1794.
26. Mehra, M. R., Uber, P. A., Park, M. H., et al. (2004). Obesity and suppressed B-type natriuretic peptide levels in heart failure. *Journal of the American College of Cardiology*, 43(9), 1590–1595.
27. Das, S. R., Drazner, M. H., Dries, D. L., et al. (2005). Impact of body mass and body composition on circulating levels of natriuretic peptides: Results from the Dallas Heart Study. *Circulation*, 112(14), 2163–2168.
28. Wong, C. Y., O'Moore-Sullivan, T., Leano, R., Hukins, C., Jenkins, C., & Marwick, T. H. (2006). Association of subclinical right ventricular dysfunction with obesity. *Journal of the American College of Cardiology*, 47(3), 611–616.
29. Her, C., Cerabona, T., Bairamian, M., & McGoldrick, K. E. (2006). Right ventricular systolic function is not depressed in morbid obesity. *Obesity Surgery*, 16(10), 1287–1293.
30. Otto, M. E., Belohlavek, M., Khandheria, B., Gilman, G., Svatikova, A., & Somers, V. (2004). Comparison of right and left ventricular function in obese and nonobese men. *The American Journal of Cardiology*, 93(12), 1569–1572.
31. Kortelainen, M. L., & Sarkioja, T. (2001). Visceral fat and coronary pathology in male adolescents. *International Journal of Obesity and Related Metabolic Disorders*, 25(2), 228–232.
32. Morricone, L., Donati, C., Hassan, T., Cioffi, P., & Caviezel, F. (2002). Relationship of visceral fat distribution to angiographically assessed coronary artery disease: Results in subjects with or

- without diabetes or impaired glucose tolerance. *Nutrition, Metabolism, and Cardiovascular Diseases*, 12(5), 275–283.
33. Allison, M. A., & Michael Wright, C. (2004). Body morphology differentially predicts coronary calcium. *International Journal of Obesity and Related Metabolic Disorders*, 28(3), 396–401.
  34. Van Gaal, L. F., Mertens, I. L., & De Block, C. E. (2006). Mechanisms linking obesity with cardiovascular disease. *Nature*, 444(7121), 875–880.
  35. Diamant, M., & Tushuizen, M. E. (2006). The metabolic syndrome and endothelial dysfunction: Common highway to type 2 diabetes and CVD. *Current Diabetes Reports*, 6(4), 279–286.
  36. Skilton, M. R., & Celermajer, D. S. (2006). Endothelial dysfunction and arterial abnormalities in childhood obesity. *International Journal of Obesity*, 30(7), 1041–1049.
  37. Lauer, M. S., Anderson, K. M., Larson, M. G., & Levy, D. (1994). A new method for indexing left ventricular mass for differences in body size. *The American Journal of Cardiology*, 74(5), 487–491.
  38. de Simone, G., Devereux, R. B., Roman, M. J., Alderman, M. H., & Laragh, J. H. (1994). Relation of obesity and gender to left ventricular hypertrophy in normotensive and hypertensive adults. *Hypertension*, 23(5), 600–606.
  39. de Simone, G., Kizer, J. R., Chinali, M., et al. (2005). Normalization for body size and population-attributable risk of left ventricular hypertrophy: The Strong Heart Study. *American Journal of Hypertension*, 18(2 pt 1), 191–196.
  40. Alpert, M. A., Terry, B. E., & Kelly, D. L. (1985). Effect of weight loss on cardiac chamber size, wall thickness and left ventricular function in morbid obesity. *The American Journal of Cardiology*, 55(6), 783–786.
  41. Alpert, M. A., Terry, B. E., Mulekar, M., et al. (1997). Cardiac morphology and left ventricular function in normotensive morbidly obese patients with and without congestive heart failure, and effect of weight loss. *The American Journal of Cardiology*, 80(6), 736–740.
  42. Gerdts, E., Wachtell, K., Omvik, P., et al. (2007). Left atrial size and risk of major cardiovascular events during antihypertensive treatment: Losartan intervention for endpoint reduction in hypertension trial. *Hypertension*, 49(2), 311–316.
  43. Kizer, J. R., Bella, J. N., Palmieri, V., et al. (2006). Left atrial diameter as an independent predictor of first clinical cardiovascular events in middle-aged and elderly adults: The Strong Heart Study (SHS). *American Heart Journal*, 151(2), 412–418.
  44. Wang, T. J., Parise, H., Levy, D., et al. (2004). Obesity and the risk of new-onset atrial fibrillation. *JAMA*, 292(20), 2471–2477.
  45. Connolly, H. M., Crary, J. L., McGoon, M. D., et al. (1997). Valvular heart disease associated with fenfluramine-phentermine. *The New England Journal of Medicine*, 337(9), 581–588.
  46. Singh, J. P., Evans, J. C., Levy, D., et al. (1999). Prevalence and clinical determinants of mitral, tricuspid, and aortic regurgitation (the Framingham Heart Study). *The American Journal of Cardiology*, 83(6), 897–902.
  47. Szczepaniak, L. S., Dobbins, R. L., Metzger, G. J., et al. (2003). Myocardial triglycerides and systolic function in humans: In vivo evaluation by localized proton spectroscopy and cardiac imaging. *Magnetic Resonance in Medicine*, 49(3), 417–423.
  48. McGavock, J. M., Lingvay, I., Zib, I., et al. (2007). Cardiac steatosis in diabetes mellitus: A <sup>1</sup>H-magnetic resonance spectroscopy study. *Circulation*, 116(10), 1170–1175.
  49. Wende, A. R., & Abel, E. D. (2010). Lipotoxicity in the heart. *Biochimica et Biophysica Acta*, 1801(3), 311–319.
  50. Rabkin, S. W. (2007). Epicardial fat: Properties, function and relationship to obesity. *Obesity Reviews*, 8(3), 253–261.
  51. Sarin, S., Wenger, C., Marwaha, A., et al. (2008). Clinical significance of epicardial fat measured using cardiac multislice computed tomography. *The American Journal of Cardiology*, 102(6), 767–771.
  52. Silaghi, A., Piercecchi-Marti, M. D., Grino, M., et al. (2008). Epicardial adipose tissue extent: Relationship with age, body fat distribution, and coronaropathy. *Obesity (Silver Spring)*, 16(11), 2424–2430.
  53. Iacobellis, G., Ribaudo, M. C., Leto, G., et al. (2002). Influence of excess fat on cardiac morphology and function: Study in uncomplicated obesity. *Obesity Research*, 10(8), 767–773.

54. Iacobellis, G., & Leonetti, F. (2005). Epicardial adipose tissue and insulin resistance in obese subjects. *The Journal of Clinical Endocrinology and Metabolism*, 90(11), 6300–6302.
55. Iacobellis, G., Singh, N., Wharton, S., & Sharma, A. M. (2008). Substantial changes in epicardial fat thickness after weight loss in severely obese subjects. *Obesity (Silver Spring)*, 16(7), 1693–1697.
56. Kim, M. K., Tomita, T., Kim, M. J., Sasai, H., Maeda, S., & Tanaka, K. (2009). Aerobic exercise training reduces epicardial fat in obese men. *Journal of Applied Physiology*, 106(1), 5–11.
57. Willens, H. J., Byers, P., Chirinos, J. A., Labrador, E., Hare, J. M., & de Marchena, E. (2007). Effects of weight loss after bariatric surgery on epicardial fat measured using echocardiography. *The American Journal of Cardiology*, 99(9), 1242–1245.
58. Uretsky, S., Messerli, F. H., Bangalore, S., et al. (2007). Obesity paradox in patients with hypertension and coronary artery disease. *The American Journal of Medicine*, 120(10), 863–870.
59. Romero-Corral, A., Montori, V. M., Somers, V. K., et al. (2006). Association of bodyweight with total mortality and with cardiovascular events in coronary artery disease: A systematic review of cohort studies. *Lancet*, 368(9536), 666–678.
60. Galal, W., van Gestel, Y. R., Hoeks, S. E., et al. (2008). The obesity paradox in patients with peripheral arterial disease. *Chest*, 134(5), 925–930.
61. Mehta, R. H., Califf, R. M., Garg, J., et al. (2007). The impact of anthropomorphic indices on clinical outcomes in patients with acute ST-elevation myocardial infarction. *European Heart Journal*, 28(4), 415–424.
62. Diercks, D. B., Roe, M. T., Mulgund, J., et al. (2006). The obesity paradox in non-ST-segment elevation acute coronary syndromes: Results from the can rapid risk stratification of unstable angina patients suppress adverse outcomes with early implementation of the American College of Cardiology/American Heart Association Guidelines Quality Improvement Initiative. *American Heart Journal*, 152(1), 140–148.
63. Habbu, A., Lakkis, N. M., & Dokainish, H. (2006). The obesity paradox: Fact or fiction? *The American Journal of Cardiology*, 98(7), 944–948.
64. Nigam, A., Wright, R. S., Allison, T. G., et al. (2006). Excess weight at time of presentation of myocardial infarction is associated with lower initial mortality risks but higher long-term risks including recurrent re-infarction and cardiac death. *International Journal of Cardiology*, 110(2), 153–159.
65. Maggio, C. A., & Pi-Sunyer, F. X. (2003). Obesity and type 2 diabetes. *Endocrinology and Metabolism Clinics of North America*, 32(4), 805–822, viii.
66. Kahn, B. B., & Flier, J. S. (2000). Obesity and insulin resistance. *The Journal of Clinical Investigation*, 106(4), 473–481.
67. Mensah, G. A., Mokdad, A. H., Ford, E., et al. (2004). Obesity, metabolic syndrome, and type 2 diabetes: Emerging epidemics and their cardiovascular implications. *Cardiology Clinics*, 22(4), 485–504.
68. Nilsson, P. M. (2005). Diabetes and obesity: New data on mechanisms and intervention trials. *Expert Review of Cardiovascular Therapy*, 3(2), 243–247.
69. Tsujino, T., Kawasaki, D., & Masuyama, T. (2006). Left ventricular diastolic dysfunction in diabetic patients: Pathophysiology and therapeutic implications. *American Journal of Cardiovascular Drugs*, 6(4), 219–230.
70. Boudina, S., & Abel, E. D. (2007). Diabetic cardiomyopathy revisited. *Circulation*, 115(25), 3213–3223.
71. Avelar, E., Cloward, T. V., Walker, J. M., et al. (2007). Left ventricular hypertrophy in severe obesity: Interactions among blood pressure, nocturnal hypoxemia, and body mass. *Hypertension*, 49(1), 34–39.
72. Rutter, M. K., Parise, H., Benjamin, E. J., et al. (2003). Impact of glucose intolerance and insulin resistance on cardiac structure and function: Sex-related differences in the Framingham Heart Study. *Circulation*, 107(3), 448–454.
73. Palmieri, V., Bella, J. N., Arnett, D. K., et al. (2001). Effect of type 2 diabetes mellitus on left ventricular geometry and systolic function in hypertensive subjects: Hypertension Genetic Epidemiology Network (HyperGEN) study. *Circulation*, 103(1), 102–107.

74. Kilhovd, B. K., Juutilainen, A., Lehto, S., et al. (2005). High serum levels of advanced glycation end products predict increased coronary heart disease mortality in nondiabetic women but not in nondiabetic men: A population-based 18-year follow-up study. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 25(4), 815–820.
75. Bucciarelli, L. G., Kaneko, M., Ananthakrishnan, R., et al. (2006). Receptor for advanced-glycation end products: Key modulator of myocardial ischemic injury. *Circulation*, 113(9), 1226–1234.
76. Li, S. Y., Liu, Y., Sigmon, V. K., McCort, A., & Ren, J. (2005). High-fat diet enhances visceral advanced glycation end products, nuclear O-Glc-Nac modification, p38 mitogen-activated protein kinase activation and apoptosis. *Diabetes, Obesity & Metabolism*, 7(4), 448–454.
77. Li, S. Y., Sigmon, V. K., Babcock, S. A., & Ren, J. (2007). Advanced glycation endproduct induces ROS accumulation, apoptosis, MAP kinase activation and nuclear O-GlcNAcylation in human cardiac myocytes. *Life Sciences*, 80(11), 1051–1056.
78. Grossman, W., Jones, D., & McLaurin, L. P. (1975). Wall stress and patterns of hypertrophy in the human left ventricle. *The Journal of Clinical Investigation*, 56(1), 56–64.
79. Kotsis, V., Stabouli, S., Bouldin, M., Low, A., Toumanidis, S., & Zakopoulos, N. (2005). Impact of obesity on 24-hour ambulatory blood pressure and hypertension. *Hypertension*, 45(4), 602–607.
80. Alpert, M. A., & Hashimi, M. W. (1993). Obesity and the heart. *The American Journal of the Medical Sciences*, 306(2), 117–123.
81. Lavie, C. J., & Messerli, F. H. (1986). Cardiovascular adaptation to obesity and hypertension. *Chest*, 90(2), 275–279.
82. Contaldo, F., Pasanisi, F., Finelli, C., & de Simone, G. (2002). Obesity, heart failure and sudden death. *Nutrition, Metabolism, and Cardiovascular Diseases*, 12(4), 190–197.
83. Quan, S. F., & Gersh, B. J. (2004). Cardiovascular consequences of sleep-disordered breathing: Past, present and future: Report of a workshop from the National Center on Sleep Disorders Research and the National Heart, Lung, and Blood Institute. *Circulation*, 109(8), 951–957.
84. de Simone, G. (2007). Morbid obesity and left ventricular geometry. *Hypertension*, 49(1), 7–9.
85. Cloward, T. V., Walker, J. M., Farney, R. J., & Anderson, J. L. (2003). Left ventricular hypertrophy is a common echocardiographic abnormality in severe obstructive sleep apnea and reverses with nasal continuous positive airway pressure. *Chest*, 124(2), 594–601.
86. Bugger, H., & Abel, E. D. (2009). Rodent models of diabetic cardiomyopathy. *Disease Model & Mechanisms*, 2(9–10), 454–466.
87. Hsueh, W., Abel, E. D., Breslow, J. L., et al. (2007). Recipes for creating animal models of diabetic cardiovascular disease. *Circulation Research*, 100(10), 1415–1427.
88. Wright, J. J., Kim, J., Buchanan, J., et al. (2009). Mechanisms for increased myocardial fatty acid utilization following short-term high-fat feeding. *Cardiovascular Research*, 82(2), 351–360.
89. Buchanan, J., Mazumder, P. K., Hu, P., et al. (2005). Reduced cardiac efficiency and altered substrate metabolism precedes the onset of hyperglycemia and contractile dysfunction in two mouse models of insulin resistance and obesity. *Endocrinology*, 146(12), 5341–5349.
90. Cook, S. A., Varela-Carver, A., Mongillo, M., et al. (2010). Abnormal myocardial insulin signalling in type 2 diabetes and left-ventricular dysfunction. *European Heart Journal*, 31(1), 100–111.
91. Ko, H. J., Zhang, Z., Jung, D. Y., et al. (2009). Nutrient stress activates inflammation and reduces glucose metabolism by suppressing AMP-activated protein kinase in the heart. *Diabetes*, 58(11), 2536–2546.
92. Coort, S. L., Hasselbaink, D. M., Koonen, D. P., et al. (2004). Enhanced sarcolemmal FAT/CD36 content and triacylglycerol storage in cardiac myocytes from obese Zucker rats. *Diabetes*, 53(7), 1655–1663.
93. Peterson, L. R., Herrero, P., Schechtman, K. B., et al. (2004). Effect of obesity and insulin resistance on myocardial substrate metabolism and efficiency in young women. *Circulation*, 109(18), 2191–2196.
94. Sharma, S., Adroge, J. V., Golfman, L., et al. (2004). Intramyocardial lipid accumulation in the failing human heart resembles the lipotoxic rat heart. *The FASEB Journal*, 18(14), 1692–1700.

95. Boudina, S., Abel, E. D. (2006). Mitochondrial uncoupling: A key contributor to reduced cardiac efficiency in diabetes. *Physiology (Bethesda)*, 21, 250–258.
96. Boudina, S., Sena, S., Theobald, H., et al. (2007). Mitochondrial energetics in the heart in obesity-related diabetes: Direct evidence for increased uncoupled respiration and activation of uncoupling proteins. *Diabetes*, 56(10), 2457–2466.
97. Boudina, S., Sena, S., O'Neill, B. T., Tathireddy, P., Young, M. E., & Abel, E. D. (2005). Reduced mitochondrial oxidative capacity and increased mitochondrial uncoupling impair myocardial energetics in obesity. *Circulation*, 112(17), 2686–2695.
98. Duncan, J. G., Fong, J. L., Medeiros, D. M., Finck, B. N., & Kelly, D. P. (2007). Insulin-resistant heart exhibits a mitochondrial biogenic response driven by the peroxisome proliferator-activated receptor- $\alpha$ /PGC-1 $\alpha$  gene regulatory pathway. *Circulation*, 115(7), 909–917.
99. Scheuermann-Freestone, M., Madsen, P. L., Manners, D., et al. (2003). Abnormal cardiac and skeletal muscle energy metabolism in patients with type 2 diabetes. *Circulation*, 107(24), 3040–3046.
100. Rijzewijk, L. J., van der Meer, R. W., Lamb, H. J., et al. (2009). Altered myocardial substrate metabolism and decreased diastolic function in nonischemic human diabetic cardiomyopathy: Studies with cardiac positron emission tomography and magnetic resonance imaging. *Journal of the American College of Cardiology*, 54(16), 1524–1532.
101. Anderson, E. J., Kypson, A. P., Rodriguez, E., Anderson, C. A., Lehr, E. J., & Neuffer, P. D. (2009). Substrate-specific derangements in mitochondrial metabolism and redox balance in the atrium of the type 2 diabetic human heart. *Journal of the American College of Cardiology*, 54(20), 1891–1898.
102. Boudina, S., Bugger, H., Sena, S., et al. (2009). Contribution of impaired myocardial insulin signaling to mitochondrial dysfunction and oxidative stress in the heart. *Circulation*, 119(9), 1272–1283.
103. Ouwens, D. M., Boer, C., Fodor, M., et al. (2005). Cardiac dysfunction induced by high-fat diet is associated with altered myocardial insulin signalling in rats. *Diabetologia*, 48(6), 1229–1237.
104. Zhou, Y. T., Grayburn, P., Karim, A., et al. (2000). Lipotoxic heart disease in obese rats: Implications for human obesity. *Proceedings of the National Academy of Sciences of the United States of America*, 97(4), 1784–1789.
105. Park, T. S., Hu, Y., Noh, H. L., et al. (2008). Ceramide is a cardiotoxin in lipotoxic cardiomyopathy. *Journal of Lipid Research*, 49(10), 2101–2112.
106. Chiu, H. C., Kovacs, A., Ford, D. A., et al. (2001). A novel mouse model of lipotoxic cardiomyopathy. *The Journal of Clinical Investigation*, 107(7), 813–822.
107. Finck, B. N., Lehman, J. J., Leone, T. C., et al. (2002). The cardiac phenotype induced by PPAR $\alpha$  overexpression mimics that caused by diabetes mellitus. *The Journal of Clinical Investigation*, 109(1), 121–130.
108. Finck, B. N., Han, X., Courtois, M., et al. (2003). A critical role for PPAR $\alpha$ -mediated lipotoxicity in the pathogenesis of diabetic cardiomyopathy: Modulation by dietary fat content. *Proceedings of the National Academy of Sciences of the United States of America*, 100(3), 1226–1231.
109. Yang, J., Sambandam, N., Han, X., et al. (2007). CD36 deficiency rescues lipotoxic cardiomyopathy. *Circulation Research*, 100(8), 1208–1217.
110. Schwenk, R. W., Luiken, J. J., Bonen, A., Glatz, J. F. (2008). Regulation of sarcolemmal glucose and fatty acid transporters in cardiac disease. *Cardiovascular Research*, 79(2), 249–258.
111. Koonen, D. P., Febbraio, M., Bonnet, S., et al. (2007). CD36 expression contributes to age-induced cardiomyopathy in mice. *Circulation*, 116(19), 2139–2147.
112. Chiu, H. C., Kovacs, A., Blanton, R. M., et al. (2005). Transgenic expression of fatty acid transport protein 1 in the heart causes lipotoxic cardiomyopathy. *Circulation Research*, 96(2), 225–233.
113. Ruano, M., Silvestre, V., Castro, R., et al. (2005). Morbid obesity, hypertensive disease and the renin-angiotensin-aldosterone axis. *Obesity Surgery*, 15(5), 670–676.
114. Engeli, S., Negrel, R., & Sharma, A. M. (2000). Physiology and pathophysiology of the adipose tissue renin-angiotensin system. *Hypertension*, 35(6), 1270–1277.



115. Davy, K. P., & Hall, J. E. (2004). Obesity and hypertension: Two epidemics or one? *American Journal of Physiology*, 286(5), R803–R813.
116. van Harmelen, V., Elizalde, M., Ariapart, P., et al. (2000). The association of human adipose angiotensinogen gene expression with abdominal fat distribution in obesity. *International Journal of Obesity and Related Metabolic Disorders*, 24(6), 673–678.
117. Goldstein, B. J., Scalia, R. G., & Ma, X. L. (2009). Protective vascular and myocardial effects of adiponectin. *Nature Clinical Practice. Cardiovascular medicine*, 6(1), 27–35.
118. Sweeney, G. (2010). Cardiovascular effects of leptin. *Nature Reviews. Cardiology*, 7(1), 22–29.
119. Ouchi, N., Shibata, R., & Walsh, K. (2006). Targeting adiponectin for cardioprotection. *Expert Opinion on Therapeutic Targets*, 10(4), 573–581.
120. Ouchi, N., Shibata, R., & Walsh, K. (2006). Cardioprotection by adiponectin. *Trends in Cardiovascular Medicine*, 16(5), 141–146.
121. Pineiro, R., Iglesias, M. J., Gallego, R., et al. (2005). Adiponectin is synthesized and secreted by human and murine cardiomyocytes. *FEBS Letters*, 579(23), 5163–5169.
122. Takahashi, T., Saegusa, S., Sumino, H., et al. (2005). Adiponectin, T-cadherin and tumour necrosis factor- $\alpha$  in damaged cardiomyocytes from autopsy specimens. *The Journal of International Medical Research*, 33(2), 236–244.
123. Fujita, K., Maeda, N., Sonoda, M., et al. (2008). Adiponectin protects against angiotensin II-induced cardiac fibrosis through activation of PPAR- $\alpha$ . *Arteriosclerosis, Thrombosis, and Vascular Biology*, 28(5), 863–870.
124. Kadowaki, T., & Yamauchi, T. (2005). Adiponectin and adiponectin receptors. *Endocrine Reviews*, 26(3), 439–451.
125. Liao, Y., Takashima, S., Maeda, N., et al. (2005). Exacerbation of heart failure in adiponectin-deficient mice due to impaired regulation of AMPK and glucose metabolism. *Cardiovascular Research*, 67(4), 705–713.
126. Shibata, R., Ouchi, N., Ito, M., et al. (2004). Adiponectin-mediated modulation of hypertrophic signals in the heart. *Nature Medicine*, 10(12), 1384–1389.
127. Shibata, R., Sato, K., Pimentel, D. R., et al. (2005). Adiponectin protects against myocardial ischemia-reperfusion injury through AMPK- and COX-2-dependent mechanisms. *Nature Medicine*, 11(10), 1096–1103.
128. Shibata, R., Izumiya, Y., Sato, K., et al. (2007). Adiponectin protects against the development of systolic dysfunction following myocardial infarction. *Journal of Molecular and Cellular Cardiology*, 42(6), 1065–1074.
129. Tao, L., Gao, E., Jiao, X., et al. (2007). Adiponectin cardioprotection after myocardial ischemia/reperfusion involves the reduction of oxidative/nitrative stress. *Circulation*, 115(11), 1408–1416.
130. Palanivel, R., Fang, X., Park, M., et al. (2007). Globular and full-length adiponectin mediate specific changes in glucose and fatty acid uptake and metabolism in cardiomyocytes. *Cardiovascular Research*, 75(1), 148–157.
131. Kanda, T., Saegusa, S., Takahashi, T., et al. (2007). Reduced-energy diet improves survival of obese KKAY mice with viral myocarditis: Induction of cardiac adiponectin expression. *International Journal of Cardiology*, 119, 310–318.
132. Takahashi, T., Yu, F., Saegusa, S., et al. (2006). Impaired expression of cardiac adiponectin in leptin-deficient mice with viral myocarditis. *International Heart Journal*, 47(1), 107–123.
133. Takahashi, T., Saegusa, S., Sumino, H., et al. (2005). Adiponectin replacement therapy attenuates myocardial damage in leptin-deficient mice with viral myocarditis. *The Journal of International Medical Research*, 33(2), 207–214.
134. Celik, T., & Yaman, H. (2009). Elevated adiponectin levels in patients with chronic heart failure: An independent predictor of mortality or a marker of cardiac cachexia? *International Journal of Cardiology in press*.
135. Dieplinger, B., Gegenhuber, A., Poelz, W., Haltmayer, M., & Mueller, T. (2009). Prognostic value of increased adiponectin plasma concentrations in patients with acute destabilized heart failure. *Clinical Biochemistry*, 42(10–11), 1190–1193.

136. Kimura, K., Miura, S., Iwata, A., et al. (2009). Association between cardiac function and metabolic factors including adiponectin in patients with acute myocardial infarction. *Journal of Cardiology*, 53(1), 65–71.
137. Laoutaris, I. D., Vasiliadis, I. K., Dritsas, A., et al. (2009). High plasma adiponectin is related to low functional capacity in patients with chronic heart failure. *International Journal of Cardiology in press*.
138. Iacobellis, G., Petrone, A., Leonetti, F., & Buzzetti, R. (2006). Left ventricular mass and +276 G/G single nucleotide polymorphism of the adiponectin gene in uncomplicated obesity. *Obesity (Silver Spring)*, 14(3), 368–372.
139. Okamoto, H. (2009). Can adiponectin be a novel metabolic biomarker for heart failure? *Circulation Journal*, 73(6), 1012–1013.
140. Fang, X., & Sweeney, G. (2006). Mechanisms regulating energy metabolism by adiponectin in obesity and diabetes. *Biochemical Society Transactions*, 34(pt 5), 798–801.
141. Onay-Besikci, A., Altarejos, J. Y., & Lopaschuk, G. D. (2004). gAd-globular head domain of adiponectin increases fatty acid oxidation in newborn rabbit hearts. *The Journal of Biological Chemistry*, 279(43), 44320–44326.
142. Yamauchi, T., Kamon, J., Ito, Y., et al. (2003). Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. *Nature*, 423(6941), 762–769.
143. Fang, X., Palanivel, R., Zhou, X., et al. (2005). Hyperglycemia- and hyperinsulinemia-induced alteration of adiponectin receptor expression and adiponectin effects in L6 myoblasts. *Journal of Molecular Endocrinology*, 35(3), 465–476.
144. Ding, G., Qin, Q., He, N., et al. (2007). Adiponectin and its receptors are expressed in adult ventricular cardiomyocytes and upregulated by activation of peroxisome proliferator-activated receptor gamma. *Journal of Molecular and Cellular Cardiology*, 43(1), 73–84.
145. Guo, Z., Xia, Z., Yuen, V. G., & McNeill, J. H. (2007). Cardiac expression of adiponectin and its receptors in streptozotocin-induced diabetic rats. *Metabolism*, 56(10), 1363–1371.
146. Saito, Y., Fujioka, D., Kawabata, K. I., et al. (2007). Statin reverses reduction of adiponectin receptor expression in infarcted heart and in TNF{alpha}-treated cardiomyocytes in association with improved glucose uptake. *American Journal of Physiology. Heart and Circulatory Physiology*, 293, H390–H397.
147. Civitarese, A. E., Jenkinson, C. P., Richardson, D., et al. (2004). Adiponectin receptors gene expression and insulin sensitivity in non-diabetic Mexican Americans with or without a family history of Type 2 diabetes. *Diabetologia*, 47(5), 816–820.
148. Tsuchida, A., Yamauchi, T., Ito, Y., et al. (2004). Insulin/Foxo1 pathway regulates expression levels of adiponectin receptors and adiponectin sensitivity. *The Journal of Biological Chemistry*, 279(29), 30817–30822.
149. Inukai, K., Nakashima, Y., Watanabe, M., et al. (2004). Regulation of adiponectin receptor gene expression in diabetic mice. *American Journal of Physiology. Endocrinology and Metabolism*, 288, E876–E882.
150. Mao, X., Kikani, C. K., Riojas, R. A., et al. (2006). APPL1 binds to adiponectin receptors and mediates adiponectin signalling and function. *Nature Cell Biology*, 8(5), 516–523.
151. Cheng, K. K., Iglesias, M. A., Lam, K. S., et al. (2009). APPL1 potentiates insulin-mediated inhibition of hepatic glucose production and alleviates diabetes via Akt activation in mice. *Cell Metabolism*, 9(5), 417–427.
152. Wang, C., Mao, X., Wang, L., et al. (2007). Adiponectin sensitizes insulin signaling by reducing p70 S6 kinase-mediated serine phosphorylation of IRS-1. *The Journal of Biological Chemistry*, 282(11), 7991–7996.
153. Pajvani, U. B., Hawkins, M., Combs, T. P., et al. (2004). Complex distribution, not absolute amount of adiponectin, correlates with thiazolidinedione-mediated improvement in insulin sensitivity. *The Journal of Biological Chemistry*, 279(13), 12152–12162.
154. Luo, J. D., Zhang, G. S., & Chen, M. S. (2005). Leptin and cardiovascular diseases. *Timely Topics in Medicine. Cardiovascular Diseases*, 9, E34.
155. Rahmouni, K., & Haynes, W. G. (2004). Leptin and the cardiovascular system. *Recent Progress in Hormone Research*, 59, 225–244.



156. Barouch, L. A., Gao, D., Chen, L., et al. (2006). Cardiac myocyte apoptosis is associated with increased DNA damage and decreased survival in murine models of obesity. *Circulation Research*, 98(1), 119–124.
157. Xu, F. P., Chen, M. S., Wang, Y. Z., et al. (2004). Leptin induces hypertrophy via endothelin-1-reactive oxygen species pathway in cultured neonatal rat cardiomyocytes. *Circulation*, 110(10), 1269–1275.
158. Minhas, K. M., Khan, S. A., Raju, S. V., et al. (2005). Leptin repletion restores depressed {beta}-adrenergic contractility in ob/ob mice independently of cardiac hypertrophy. *The Journal of Physiology*, 565(pt 2), 463–474.
159. Barouch, L. A., Berkowitz, D. E., Harrison, R. W., O'Donnell, C. P., & Hare, J. M. (2003). Disruption of leptin signaling contributes to cardiac hypertrophy independently of body weight in mice. *Circulation*, 108(6), 754–759.
160. Ren, J. (2004). Leptin and hyperleptinemia – from friend to foe for cardiovascular function. *The Journal of Endocrinology*, 181(1), 1–10.
161. Mark, A. L., Shaffer, R. A., Correia, M. L., Morgan, D. A., Sigmund, C. D., & Haynes, W. G. (1999). Contrasting blood pressure effects of obesity in leptin-deficient ob/ob mice and agouti yellow obese mice. *Journal of Hypertension*, 17(12 pt 2), 1949–1953.
162. Correia, M. L., & Rahmouni, K. (2006). Role of leptin in the cardiovascular and endocrine complications of metabolic syndrome. *Diabetes, Obesity & Metabolism*, 8(6), 603–610.
163. Martin, S. S., Qasim, A., & Reilly, M. P. (2008). Leptin resistance: A possible interface of inflammation and metabolism in obesity-related cardiovascular disease. *Journal of the American College of Cardiology*, 52(15), 1201–1210.
164. Hintz, K. K., Aberle, N. S., & Ren, J. (2003). Insulin resistance induces hyperleptinemia, cardiac contractile dysfunction but not cardiac leptin resistance in ventricular myocytes. *International Journal of Obesity and Related Metabolic Disorders*, 27(10), 1196–1203.
165. Smith, C. C., Mocanu, M. M., Davidson, S. M., Wynne, A. M., Simpkin, J. C., & Yellon, D. M. (2006). Leptin, the obesity-associated hormone, exhibits direct cardioprotective effects. *British Journal of Pharmacology*, 149(1), 5–13.
166. Abe, Y., Ono, K., Kawamura, T., et al. (2007). Leptin induces elongation of cardiac myocyte and causes eccentric left ventricular dilatation with compensation. *American Journal of Physiology. Heart and Circulatory Physiology*, 292, H2387–H2396.
167. Sweeney, G. (2002). Leptin signaling. *Cellular Signalling*, 14(8), 655–663.
168. Ahima, R. S., & Flier, J. S. (2000). Leptin. *Annual Review of Physiology*, 62, 413–437.
169. Schulze, P. C., & Kratzsch, J. (2005). Leptin as a new diagnostic tool in chronic heart failure. *Clinica Chimica Acta*, 362(1–2), 1–11.
170. Giganti, A., & Friederich, E. (2003). The actin cytoskeleton as a therapeutic target: State of the art and future directions. *Progress in Cell Cycle Research*, 5, 511–525.
171. Lin, G., Craig, G. P., Zhang, L., et al. (2007). Acute inhibition of Rho-kinase improves cardiac contractile function in streptozotocin-diabetic rats. *Cardiovascular Research*, 75(1), 51–58.
172. Zeidan, A., Javadov, S., & Karmazyn, M. (2006). Essential role of Rho/ROCK-dependent processes and actin dynamics in mediating leptin-induced hypertrophy in rat neonatal ventricular myocytes. *Cardiovascular Research*, 72(1), 101–111.
173. Schram, K., Wong, M. M., Palanivel, R., No, E. K., Dixon, I. M., & Sweeney, G. (2008). Increased expression and cell surface localization of MT1-MMP plays a role in stimulation of MMP-2 activity by leptin in neonatal rat cardiac myofibroblasts. *Journal of Molecular and Cellular Cardiology*, 44(5), 874–881.
174. Ren, J., & Ma, H. (2008). Impaired cardiac function in leptin-deficient mice. *Current Hypertension Reports*, 10(6), 448–453.
175. Trivedi, P. S., & Barouch, L. A. (2008). Cardiomyocyte apoptosis in animal models of obesity. *Current Hypertension Reports*, 10(6), 454–460.
176. Yang, R., & Barouch, L. A. (2007). Leptin signaling and obesity: Cardiovascular consequences. *Circulation Research*, 101(6), 545–559.
177. Dong, F., Zhang, X., Yang, X., et al. (2006). Impaired cardiac contractile function in ventricular myocytes from leptin-deficient ob/ob obese mice. *The Journal of Endocrinology*, 188(1), 25–36.

178. Barouch, L., Gao, D., Chen, L., Miller, K. L., Xy, W., Phan, A. C., et al. (2006). Cardiac myocytes apoptosis is associated with increased DNA damage and decreased survival in murine models of obesity. *Circulation Research*, 98(1), 119–124.
179. Christoffersen, C., Bollano, E., Lindegaard, M. L., et al. (2003). Cardiac lipid accumulation associated with diastolic dysfunction in obese mice. *Endocrinology*, 144(8), 3483–3490.
180. Semeniuk, L. M., Kryski, A. J., & Severson, D. L. (2002). Echocardiographic assessment of cardiac function in diabetic db/db and transgenic db/db-hGLUT4 mice. *American Journal of Physiology. Heart and Circulatory Physiology*, 283(3), H976–H982.
181. Fedak, P. W., Verma, S., Weisel, R. D., & Li, R. K. (2005). Cardiac remodeling and failure from molecules to man (Part II). *Cardiovascular Pathology*, 14(2), 49–60.
182. Miner, E. C., & Miller, W. L. (2006). A look between the cardiomyocytes: The extracellular matrix in heart failure. *Mayo Clinic Proceedings*, 81(1), 71–76.
183. Felkin, L. E., Birks, E. J., George, R., et al. (2006). A quantitative gene expression profile of matrix metalloproteinases (MMPs) and their inhibitors (TIMPs) in the myocardium of patients with deteriorating heart failure requiring left ventricular assist device support. *The Journal of Heart and Lung Transplantation*, 25(12), 1413–1419.
184. Deschamps, A. M., & Spinale, F. G. (2006). Pathways of matrix metalloproteinase induction in heart failure: Bioactive molecules and transcriptional regulation. *Cardiovascular Research*, 69(3), 666–676.
185. Fedak, P., Verma, S., Weisel, R. D., & Li, R. K. (2005). Cardiac remodeling and failure: From molecules to man (Part II). *Cardiovascular Pathology*, 14(2), 49–60.
186. Fedak, P., Verma, S., Weisel, R. D., & Li, R. K. (2005). Cardiac remodeling and failure: From molecules to man (Part I). *Cardiovascular Pathology*, 14(1), 1–11.
187. Graham, H. K., Horn, M., & Trafford, A. W. (2008). Extracellular matrix profiles in the progression to heart failure. European Young Physiologists Symposium Keynote Lecture- Bratislava 2007. *Acta Physiologica*, 194(1), 3–21.
188. Fazel, S., Chen, L., Weisel, R. D., et al. (2005). Cell transplantation preserves cardiac function after infarction by infarct stabilization: Augmentation by stem cell factor. *The Journal of Thoracic and Cardiovascular Surgery*, 130(5), 1310.
189. Spinale, F. G. (2007). Myocardial matrix remodeling and the matrix metalloproteinases: Influence on cardiac form and function. *Physiological Reviews*, 87(4), 1285–1342.
190. Zaman, A. K., Fujii, S., Sawa, H., et al. (2001). Angiotensin-converting enzyme inhibition attenuates hypofibrinolysis and reduces cardiac perivascular fibrosis in genetically obese diabetic mice. *Circulation*, 103(25), 3123–3128.
191. Zaman, A. K., Fujii, S., Goto, D., et al. (2004). Salutary effects of attenuation of angiotensin II on coronary perivascular fibrosis associated with insulin resistance and obesity. *Journal of Molecular and Cellular Cardiology*, 37(2), 525–535.
192. Toblli, J. E., Cao, G., DeRosa, G., & Forcada, P. (2005). Reduced cardiac expression of plasminogen activator inhibitor 1 and transforming growth factor beta1 in obese Zucker rats by perindopril. *Heart (British Cardiac Society)*, 91(1), 80–86.
193. Graham, H. K., & Trafford, A. W. (2007). Spatial disruption and enhanced degradation of collagen with the transition from compensated ventricular hypertrophy to symptomatic congestive heart failure. *American Journal of Physiology. Heart and Circulatory Physiology*, 292(3), H1364–H1372.
194. Mujumdar, V. S., & Tyagi, S. C. (1999). Temporal regulation of extracellular matrix components in transition from compensatory hypertrophy to decompensatory heart failure. *Journal of Hypertension*, 17(2), 261–270.
195. Iwanaga, Y., Aoyama, T., Kihara, Y., Onozawa, Y., Yoneda, T., & Sasayama, S. (2002). Excessive activation of matrix metalloproteinases coincides with left ventricular remodeling during transition from hypertrophy to heart failure in hypertensive rats. *Journal of the American College of Cardiology*, 39(8), 1384–1391.
196. Spinale, F. G., Coker, M. L., Thomas, C. V., Walker, J. D., Mukherjee, R., & Hebban, L. (1998). Time-dependent changes in matrix metalloproteinase activity and expression during the progression of congestive heart failure: Relation to ventricular and myocyte function. *Circulation Research*, 82(4), 482–495.

197. Spinale, F. G., Zellner, J. L., Johnson, W. S., Eble, D. M., & Munyer, P. D. (1996). Cellular and extracellular remodeling with the development and recovery from tachycardia-induced cardiomyopathy: Changes in fibrillar collagen, myocyte adhesion capacity and proteoglycans. *Journal of Molecular and Cellular Cardiology*, 28(8), 1591–1608.
198. Gilbert, S. J., Wotton, P. R., Tarlton, J. F., Duance, V. C., & Bailey, A. J. (1997). Increased expression of promatrix metalloproteinase-9 and neutrophil elastase in canine dilated cardiomyopathy. *Cardiovascular Research*, 34(2), 377–383.
199. Moshal, K. S., Tyagi, N., Moss, V., et al. (2005). Early induction of matrix metalloproteinase-9 transduces signaling in human heart end stage failure. *Journal of Cellular and Molecular Medicine*, 9(3), 704–713.
200. Polyakova, V., Hein, S., Kostin, S., Ziegelhoeffer, T., & Schaper, J. (2004). Matrix metalloproteinases and their tissue inhibitors in pressure-overloaded human myocardium during heart failure progression. *Journal of the American College of Cardiology*, 44(8), 1609–1618.
201. Matsumura, S., Iwanaga, S., Mochizuki, S., Okamoto, H., Ogawa, S., & Okada, Y. (2005). Targeted deletion or pharmacological inhibition of MMP-2 prevents cardiac rupture after myocardial infarction in mice. *The Journal of Clinical Investigation*, 115(3), 599–609.
202. Rohde, L. E., Ducharme, A., Arroyo, L. H., et al. (1999). Matrix metalloproteinase inhibition attenuates early left ventricular enlargement after experimental myocardial infarction in mice. *Circulation*, 99(23), 3063–3070.
203. Troeberg, L., Tanaka, M., Wait, R., Shi, Y. E., Brew, K., & Nagase, H. (2002). *E. coli* expression of TIMP-4 and comparative kinetic studies with TIMP-1 and TIMP-2: Insights into the interactions of TIMPs and matrix metalloproteinase 2 (gelatinase A). *Biochemistry*, 41(50), 15025–15035.
204. Schulz, R. (2007). Intracellular targets of matrix metalloproteinase-2 in cardiac disease: Rationale and therapeutic approaches. *Annual Review of Pharmacology and Toxicology*, 47, 211–242.
205. Garg, S., Narula, J., & Chandrashekar, Y. (2005). Apoptosis and heart failure: Clinical relevance and therapeutic target. *Journal of Molecular and Cellular Cardiology*, 38(1), 73–79.
206. Narula, J., Haider, N., Virmani, R., et al. (1996). Apoptosis in myocytes in end-stage heart failure. *The New England Journal of Medicine*, 335(16), 1182–1189.
207. Olivetti, G., Abbi, R., Quaini, F., et al. (1997). Apoptosis in the failing human heart. *The New England Journal of Medicine*, 336(16), 1131–1141.
208. Todor, A., Sharov, V. G., Tanhehco, E. J., Silverman, N., Bernabei, A., & Sabbah, H. N. (2002). Hypoxia-induced cleavage of caspase-3 and DFF45/ICAD in human failed cardiomyocytes. *American Journal of Physiology. Heart and Circulatory Physiology*, 283(3), H990–H995.
209. Morissette, M. R., & Rosenzweig, A. (2005). Targeting survival signaling in heart failure. *Current Opinion in Pharmacology*, 5(2), 165–170.
210. Chandrashekar, Y., Sen, S., Anway, R., Shuros, A., & Anand, I. (2004). Long-term caspase inhibition ameliorates apoptosis, reduces myocardial troponin-I cleavage, protects left ventricular function, and attenuates remodeling in rats with myocardial infarction. *Journal of the American College of Cardiology*, 43(2), 295–301.
211. Sung, M. M., Schulz, C. G., Wang, W., Sawicki, G., Bautista-Lopez, N. L., & Schulz, R. (2007). Matrix metalloproteinase-2 degrades the cytoskeletal protein alpha-actinin in peroxynitrite mediated myocardial injury. *Journal of Molecular and Cellular Cardiology*, 43(4), 429–436.
212. Communal, C., Sumanda, M., de Tombe, P., Narula, J., Solaro, R. J., & Hajjar, R. J. (2002). Functional consequences of caspase activation in cardiac myocytes. *Proceedings of the National Academy of Sciences of the United States of America*, 99(9), 6252–6256.
213. Aasum, E., Hafstad, A. D., Severson, D. L., & Larsen, T. S. (2003). Age-dependent changes in metabolism, contractile function, and ischemic sensitivity in hearts from db/db mice. *Diabetes*, 52(2), 434–441.
214. Sidell, R. J., Cole, M. A., Draper, N. J., Desrois, M., Buckingham, R. E., & Clarke, K. (2002). Thiazolidinedione treatment normalizes insulin resistance and ischemic injury in the Zucker Fatty rat heart. *Diabetes*, 51(4), 1110–1117.
215. Yue, T. L., Bao, W., Gu, J. L., et al. (2005). Rosiglitazone treatment in Zucker diabetic Fatty rats is associated with ameliorated cardiac insulin resistance and protection from ischemia/reperfusion-induced myocardial injury. *Diabetes*, 54(2), 554–562.

216. Johns, D. G., Ao, Z., Eybye, M., et al. (2005). Rosiglitazone protects against ischemia/reperfusion-induced leukocyte adhesion in the Zucker diabetic fatty rat. *The Journal of Pharmacology and Experimental Therapeutics*, 315(3), 1020–1027.
217. Hoshida, S., Yamashita, N., Otsu, K., Kuzuya, T., & Hori, M. (2000). Cholesterol feeding exacerbates myocardial injury in Zucker diabetic fatty rats. *American Journal of Physiology. Heart and Circulatory Physiology*, 278(1), H256–H262.
218. Greer, J. J., Ware, D. P., & Lefer, D. J. (2006). Myocardial infarction and heart failure in the db/db diabetic mouse. *American Journal of Physiology. Heart and Circulatory Physiology*, 290(1), H146–H153.
219. Thakker, G. D., Frangogiannis, N. G., Bujak, M., et al. (2006). Effects of diet-induced obesity on inflammation and remodeling after myocardial infarction. *American Journal of Physiology. Heart and Circulatory Physiology*, 291(5), H2504–H2514.
220. Jones, S. P., Girod, W. G., Granger, D. N., Palazzo, A. J., & Lefer, D. J. (1999). Reperfusion injury is not affected by blockade of P-selectin in the diabetic mouse heart. *The American Journal of Physiology*, 277(2 pt 2), H763–H769.
221. Parish, R. C., & Evans, J. D. (2008). Inflammation in chronic heart failure. *The Annals of Pharmacotherapy*, 42(7), 1002–1016.
222. Yndestad, A., Damas, J. K., Oie, E., Ueland, T., Gullestad, L., & Aukrust, P. (2006). Systemic inflammation in heart failure—the whys and wherefores. *Heart Failure Reviews*, 11(1), 83–92.
223. Yndestad, A., Damas, J. K., Oie, E., Ueland, T., Gullestad, L., & Aukrust, P. (2007). Role of inflammation in the progression of heart failure. *Current Cardiology Reports*, 9(3), 236–241.
224. Mueller, C., Laule-Kilian, K., Christ, A., Brunner-La Rocca, H. P., & Perruchoud, A. P. (2006). Inflammation and long-term mortality in acute congestive heart failure. *American Heart Journal*, 151(4), 845–850.
225. Ingelsson, E., Arnlov, J., Sundstrom, J., & Lind, L. (2005). Inflammation, as measured by the erythrocyte sedimentation rate, is an independent predictor for the development of heart failure. *Journal of the American College of Cardiology*, 45(11), 1802–1806.
226. Bozkurt, B., Mann, D. L., & Deswal, A. (2010). Biomarkers of inflammation in heart failure. *Heart Fail Rev*, 15(4):331–41.
227. Gullestad, L., Kjekshus, J., Damas, J. K., Ueland, T., Yndestad, A., & Aukrust, P. (2005). Agents targeting inflammation in heart failure. *Expert Opinion on Investigational Drugs*, 14(5), 557–566.
228. von Eynatten, M., Hamann, A., Twardella, D., Nawroth, P. P., Brenner, H., & Rothenbacher, D. (2006). Relationship of adiponectin with markers of systemic inflammation, atherogenic dyslipidemia, and heart failure in patients with coronary heart disease. *Clinical Chemistry*, 52(5), 853–859.
229. Hilfiger-Kleiner, D., Landmesser, U., & Drexler, H. (2006). Molecular mechanisms in heart failure focus on cardiac hypertrophy, inflammation, angiogenesis, and apoptosis. *Journal of the American College of Cardiology*, 48(9 suppl), A56–A66.
230. Aukrust, P., Yndestad, A., Damas, J. K., & Gullestad, L. (2004). Inflammation and chronic heart failure-potential therapeutic role of intravenous immunoglobulin. *Autoimmunity Reviews*, 3(3), 221–227.
231. Chao, W. (2009). Toll-like receptor signaling: A critical modulator of cell survival and ischemic injury in the heart. *American Journal of Physiology. Heart and Circulatory Physiology*, 296(1), H1–H12.
232. Satoh, M., Minami, Y., Takahashi, Y., & Nakamura, M. (2008). Immune modulation: Role of the inflammatory cytokine cascade in the failing human heart. *Current Heart Failure Reports*, 5(2), 69–74.
233. Aukrust, P., Gullestad, L., Ueland, T., Damas, J. K., & Yndestad, A. (2005). Inflammatory and anti-inflammatory cytokines in chronic heart failure: Potential therapeutic implications. *Annals of Medicine*, 37(2), 74–85.
234. Heymans, S., Hirsch, E., Anker, S. D., et al. (2009). Inflammation as a therapeutic target in heart failure? A scientific statement from the Translational Research Committee of the Heart Failure Association of the European Society of Cardiology. *European Journal of Heart Failure*, 11(2), 119–129.

235. Aigner, F., Maier, H. T., Schwelberger, H. G., et al. (2007). Lipocalin-2 regulates the inflammatory response during ischemia and reperfusion of the transplanted heart. *American Journal of Transplantation*, 7(4), 779–788.
236. Yndestad, A., Holm, A. M., Muller, F., et al. (2003). Enhanced expression of inflammatory cytokines and activation markers in T-cells from patients with chronic heart failure. *Cardiovascular Research*, 60(1), 141–146.
237. Madala, M. C., Franklin, B. A., Chen, A. Y., et al. (2008). Obesity and age of first non-ST-segment elevation myocardial infarction. *Journal of the American College of Cardiology*, 52(12), 979–985.
238. Wee, C. C., Girotra, S., Weinstein, A. R., Mittleman, M. A., & Mukamal, K. J. (2008). The relationship between obesity and atherosclerotic progression and prognosis among patients with coronary artery bypass grafts the effect of aggressive statin therapy. *Journal of the American College of Cardiology*, 52(8), 620–625.
239. Nissen, S. E., Nicholls, S. J., Wolski, K., et al. (2008). Effect of rimonabant on progression of atherosclerosis in patients with abdominal obesity and coronary artery disease: The STRADIVARIUS randomized controlled trial. *JAMA*, 299(13), 1547–1560.
240. Poirier, P., Giles, T. D., Bray, G. A., et al. (2006). Obesity and cardiovascular disease: Pathophysiology, evaluation, and effect of weight loss: An update of the 1997 American Heart Association Scientific Statement on Obesity and Heart Disease from the Obesity Committee of the Council on Nutrition, Physical Activity, and Metabolism. *Circulation*, 113(6), 898–918.
241. Klein, S., Burke, L. E., Bray, G. A., et al. (2004). Clinical implications of obesity with specific focus on cardiovascular disease: A statement for professionals from the American Heart Association Council on Nutrition, Physical Activity, and Metabolism: Endorsed by the American College of Cardiology Foundation. *Circulation*, 110(18), 2952–2967.
242. Karason, K., Lindroos, A. K., Stenlof, K., & Sjostrom, L. (2000). Relief of cardiorespiratory symptoms and increased physical activity after surgically induced weight loss: Results from the Swedish Obese Subjects study. *Archives of Internal Medicine*, 160(12), 1797–1802.
243. Klein, S., Fontana, L., Young, V. L., et al. (2004). Absence of an effect of liposuction on insulin action and risk factors for coronary heart disease. *The New England Journal of Medicine*, 350(25), 2549–2557.
244. Padwal, R. S., & Majumdar, S. R. (2007). Drug treatments for obesity: Orlistat, sibutramine, and rimonabant. *Lancet*, 369(9555), 71–77.
245. Fujioka, K., Seaton, T. B., Rowe, E., et al. (2000). Weight loss with sibutramine improves glycaemic control and other metabolic parameters in obese patients with type 2 diabetes mellitus. *Diabetes, Obesity & Metabolism*, 2(3), 175–187.
246. Derosa, G., Cicero, A. F., Murdolo, G., et al. (2005). Efficacy and safety comparative evaluation of orlistat and sibutramine treatment in hypertensive obese patients. *Diabetes, Obesity & Metabolism*, 7(1), 47–55.
247. Zannad, F., Gille, B., Grentzinger, A., et al. (2002). Effects of sibutramine on ventricular dimensions and heart valves in obese patients during weight reduction. *American Heart Journal*, 144(3), 508–515.
248. Guven, A., Koksak, N., Cetinkaya, A., Sokmen, G., & Ozdemir, R. (2004). Effects of the sibutramine therapy on pulmonary artery pressure in obese patients. *Diabetes, Obesity & Metabolism*, 6(1), 50–55.
249. Sjostrom, L., Lindroos, A. K., Peltonen, M., et al. (2004). Lifestyle, diabetes, and cardiovascular risk factors 10 years after bariatric surgery. *The New England Journal of Medicine*, 351(26), 2683–2693.
250. Katzmarzyk, P. T., & Mason, C. (2006). Prevalence of class I, II and III obesity in Canada. *CMAJ*, 174(2), 156–157.
251. Hensrud, D. D., & Klein, S. (2006). Extreme obesity: A new medical crisis in the United States. *Mayo Clinic Proceedings*, 81(10 suppl), S5–S10.
252. Adams, T. D., Avelar, E., Cloward, T., et al. (2005). Design and rationale of the Utah obesity study. A study to assess morbidity following gastric bypass surgery. *Contemporary Clinical Trials*, 26(5), 534–551.

# Chapter 15

## Atherogenic Lipid Metabolism in Obesity

Sue-Anne Toh, Michael Levin, and Daniel J. Rader

### Introduction

The dyslipidemia classically associated with obesity is characterized by a metabolic atherogenic triad of (a) increased levels of triglyceride-rich, apolipoprotein B (apoB) containing lipoproteins (very low density lipoprotein [VLDL], intermediate-density lipoproteins [IDL], and remnant particles), (b) a shift in the low density lipoprotein (LDL) pool towards small, dense LDL particles, and (c) low levels of high density lipoprotein (HDL) cholesterol. All of these lipid anomalies contribute to increased cardiometabolic risk [1], and are thought to be engendered by an excess of visceral adipose tissue. Visceral adipose tissue accumulation is strongly associated with impaired adipose tissue function, such that pathogenic metabolic and immune responses that promote insulin resistance, dyslipidemia, and atherosclerosis are incited [2].

Another metabolic abnormality that is linked to atherogenic dyslipidemia in the setting of obesity is an accumulation of excess lipids in the liver [3–5]. In many patients, lipid overload in the liver is manifest clinically by transaminase elevation and evidence of fatty liver on imaging [6]. The hepatic accumulation of fat (primarily triglyceride in cytoplasmic lipid droplets) is associated with changes in hepatic metabolism that promote the development of atherogenic dyslipidemia, though the mechanisms are not yet fully understood, and it is not clear that cytoplasmic triglyceride storage itself is causally related to dyslipidemia.

There has been substantial research effort focused on elucidating the mechanisms for the link between obesity and atherogenic dyslipidemia, which appears to be intimately related to the presence of insulin resistance. This chapter details the known molecular mechanisms of adipocyte and hepatic function, as it pertains to apoB-containing lipoprotein assembly and metabolism, both in the healthy as well

---

S.-A. Toh (✉)  
Department of Medicine, National University of Singapore,  
5, Lower Kent Ridge Road, Singapore 119074  
e-mail: sue\_anne\_toh@nuhs.edu.sg







due to resistance of adipose tissue to the antilipolytic action of insulin [9] and (2) reduced hepatic sensitivity to insulin leading to resistance to the acute suppressive effects of the hormone on VLDL production [10]. Thus, defective regulation of hepatic VLDL synthesis and secretion likely reflects insulin resistance both in adipose tissue and the liver. We elaborate on each of these in turn.

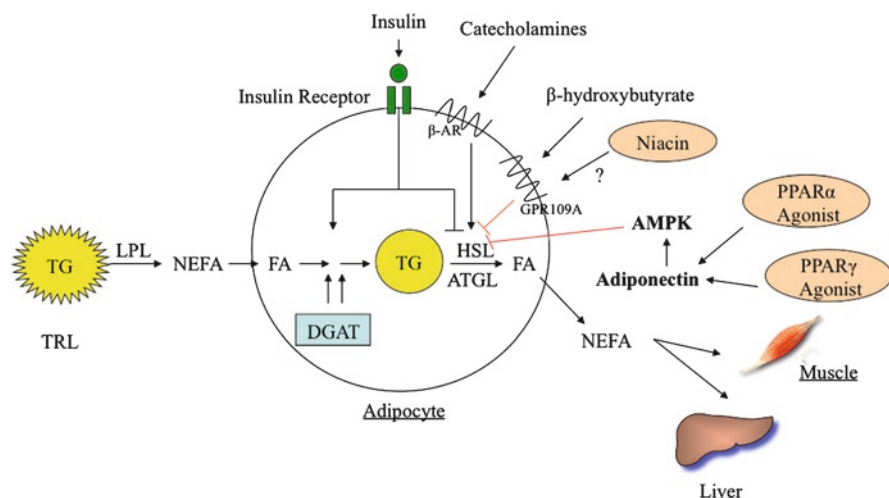
### ***Dysregulation of Fatty Acid Storage and Enhanced Lipolysis Leading to Increased Free Fatty Acid Flux from Adipose Tissue to Liver***

Adipose tissue plays a direct role in mediating plasma lipid concentrations through the uptake of plasma TG and the release of adipose-derived fatty acids into plasma. Adipose tissue insulin resistance is associated with impaired adipocyte trapping of fatty acids and excessive adipocyte lipolysis, both of which lead to increased circulating FFAs relative to tissue requirements. As described earlier, increased flux of FFAs from adipose tissue to liver leads to increased hepatic triglyceride synthesis and VLDL production, resulting in atherogenic dyslipidemia (Fig. 1).

### ***Dysregulation of Lipoprotein Lipase Activity in Obesity and Insulin Resistance***

Adipose acquires dietary fatty acids via the metabolism of triglyceride-rich lipoproteins (TRLs), especially chylomicrons, during the fed state to serve as energy storage. Endothelial-bound lipoprotein lipase (LPL) catalyzes the essential first step in the delivery of FFA to adipose for storage [11], triggering hydrolysis of TG and the release of FFA which are then largely taken up by adjacent adipocytes [12] (Fig. 2). Insulin stimulates adipose LPL expression and activity, in the fed state, directing dietary TRL-derived fatty acids preferentially to storage in adipose, whereas during fasting, it is downregulated in adipose and upregulated in muscle, directing TRL-derived fatty acid to muscle for energy utilization [13, 14].

Obesity and insulin resistance are associated with dysregulation of LPL, such that there is an overall reduction of LPL activity in obese subjects [15]. In a study of post prandial lipid metabolism, adipose tissue LPL mRNA levels, LPL activity correlated with insulin resistance, and inversely correlated with area under TG curve. In states of normal physiology, there is a large gradient between fasting and fed insulin levels. However, in states of hyperinsulinemia, such as in obesity and insulin resistance, this gradient is reduced, such that there could be attenuation of insulin's regulatory effects. Both fasting and postprandial levels of FFA are often elevated in obese and insulin resistant individuals, suggesting that the ability of insulin to upregulate LPL and effectively trap fatty acids is impaired [16].



**Fig. 2** TG-rich lipoprotein (TRL) metabolism and interactions with adipose tissue: TRL TGs are hydrolyzed by lipoprotein lipase (LPL), and nonesterified fatty acids (NEFAs) are taken up by the adipocyte and stored as TG until there is a demand for FA release by peripheral tissues. Adipocyte TGs are hydrolyzed by HSL. Stimulation of lipolysis occurs primarily via catecholamines and activation of beta-adrenergic receptors ( $\beta$ -AR) that leads to HSL activation. Insulin mediates anti-lipolytic action through the insulin receptor to promote the storage of TG within the adipocyte.  $\beta$ -hydroxybutyrate (via GPR109A) is also an inhibitor of lipolysis. Insulin resistance is associated with impaired adipocyte fatty acid trapping and excessive lipolysis. This can lead to increased circulating NEFAs relative to tissue requirements. Pharmacological agents that target adipose tissue may have potential beneficial effects on lipoprotein metabolism by promoting more efficient fatty acid trapping via upregulation of diacylglycerol acyl transferase (DGAT) activity or preventing excessive lipolysis (e.g., via inhibition of HSL by GPR109A or AMP Kinase [AMPK] activation)

### ***Dysregulation of Adipose Tissue Fatty Acid Storage in Obesity and Insulin Resistance***

Efficient TRL-derived FFA trapping is also accomplished through efficient facilitated transport of NEFAs across the endothelial barrier and the adipocyte plasma membrane [17]. Once FAs are taken up into the adipocyte, efficient FA acylation is necessary to trap them within the adipocyte and prevent back diffusion out of the cell. Ultimately, the final step in TG synthesis is catalyzed by the action of the DGAT (diacylglycerol acyl transferase) enzymes that catalyzes acylation of diacylglycerol (DAG) using a fatty acyl CoA substrate (Fig. 2). In this capacity, DGAT activity promotes TG storage while decreasing FA substrates [18], resulting in a global beneficial effect on hypertriglyceridemia, obesity, and insulin resistance [19–21].

Both the DGAT1 and DGAT 2 genes have been cloned, and are expressed in many tissues, including adipose tissue, liver, skeletal muscle, and intestines, although differences in relative abundance are evident [22, 23]. Although both

enzymes catalyze esterification and TG synthesis, they are functionally distinguished by their differences in regulation [24], phenotypic consequences when rendered deficient [19, 21] and additional functions (DGAT 1 also esterifies alcohol, waxes, and retinyl esters) [25, 26], *Dgat1*-deficient mice are resistant to diet-induced obesity [19], whereas adipose-specific *Dgat1* overexpressing mice are more obese but are not insulin resistant. The generation of *Dgat1* deficient mice with the phenotype of obesity resistance, increase energy expenditure and apparently improved glucose metabolism led to enthusiasm that DGAT inhibition may be a worthwhile therapeutic strategy [27]. However, the degree to which DGAT would have to be inhibited and whether this inhibition would cause side effects are unclear. Subsequently, *Dgat2*-deficient mice were found to have reduced fat stores and die soon after birth as a result of profound reductions in substrates for energy metabolism and impaired skin permeability [21]. Taken together, DGAT1 and DGAT2 clearly play an important role in adipocyte TG synthesis and efficient FA trapping.

### ***Enhanced Lipolysis in Obesity and Insulin Resistance States***

During the fasting state, energy is mobilized from adipose for use by other tissues, requiring lipolysis of stored TG (Fig. 2). Adipocyte hydrolysis of TG is regulated by the enzymes hormone sensitive lipase (HSL) and adipose triglyceride lipase (ATGL), intracellular, neutral lipases that catalyze hydrolysis of triacylglycerol (TAG) into DAG and monoacylglycerol plus FFAs [28]. Positive regulation of adipocyte lipolysis occurs via beta adrenergic receptor stimulation by catecholamines (Fig. 2). Insulin is a key negative regulator of HSL and adipocyte lipolysis [29]. During the fed state, insulin levels are high and adipocyte lipolysis is inhibited, thus promoting TG storage. Conversely, during fasting, insulin levels are low and adipocyte lipolysis is activated, promoting TG hydrolysis. Thus, insulin regulation imposes strict control on adipose lipolysis. In states of normal physiology, there is a large gradient between fasting and fed insulin levels, allowing for greater lipolysis during the fasting state to supply FFAs to tissues. However, in the hyperinsulinemic, obese insulin resistant state, this gradient is reduced, and lipolysis in these subjects has been shown to be less responsive to suppression by insulin [9, 30].

HSL knockout mice are not obese [28] and their adipocytes have residual TG lipase due to ATGL [31]. ATGL, expressed predominantly in white adipose tissue but also present in brown adipose tissue, is localized to the lipid droplet and initiates TG hydrolysis, generating DAG and NEFAs [41]. In vitro, overexpression of ATGL-produced enhanced lipolysis, whereas the inhibition of ATGL using antisense methods reduced lipolysis [32]. In vivo, mouse studies have demonstrated that the inhibition of ATGL led to decreased TG hydrolase activity [32]. Endogenous activators and inhibitors of ATGL have yet to be completely characterized. However, insulin has been shown to downregulate ATGL expression in 3T3-L1

adipocytes, whereas PPAR $\gamma$  activation positively regulates ATGL mRNA and protein expression in mature adipocytes in vitro and in adipose tissue in vivo, suggesting a possible role for ATGL in mediating PPAR $\gamma$ 's effects on lipid metabolism [33].

In adipocytes, the AMP-activated protein kinase (AMPK) system acts to suppress the activation of lipolysis through increasing cAMP [34]. The AMPK system acts as a sensor of cellular energy status, and becomes activated in situations of energy consumption. It is activated by increases in cellular AMP:ATP ratio caused by metabolic stresses that either interfere with ATP production (e.g., deprivation of glucose or oxygen) or that accelerate ATP consumption (e.g., muscle contraction) [35]. If fatty acids released by lipolysis are not removed from the cell rapidly enough, they recycle to TG, thus consuming ATP [36]. Inhibition of lipolysis by AMPK has been proposed as a mechanism to limit this recycling [37], ensuring that the rate of lipolysis does not exceed the rate at which fatty acids can be removed or metabolized by other routes, such as fatty acid oxidation.

GPR109A is a G-protein coupled receptor on adipocytes, the activation of which inhibits lipolytic activity [38, 39]. GPR109A was initially identified as the receptor for pharmacologic doses of nicotinic acid (niacin) and was shown to mediate the acute antilipolytic effect of niacin on adipocytes (Fig. 2). The mechanism of niacin's antilipolytic effect involves reduced production of adipocyte cAMP via adenylyl cyclase and so reduced HSL activity due to reduced protein kinase A (PKA) activation [40, 41].  $\beta$ -hydroxybutyrate was subsequently identified as an endogenous ligand for GPR109A, suggesting a physiologic role in which during ketosis, liver derived  $\beta$ -hydroxybutyrate feeds back on the adipocyte, activates GPR109A and suppresses lipolysis and the release of FFAs as a safeguard against ketosis [38, 42, 43]. Adiponectin is a peptide which is secreted by adipose tissue and has been shown to have the dual effect of increasing fatty acid oxidation and improving insulin sensitivity in skeletal muscle, liver, and fat via activation of AMPK [44] (Fig. 2). The metabolic effects of adiponectin are reversed by blocking AMPK with a dominant negative mutant [44], or a chemical inhibitor [45], strongly suggesting that AMPK is an important cellular mediator of the metabolic effects of adiponectin. Low serum adiponectin is commonly associated with all the components of atherogenic dyslipidemia (increase in plasma small dense LDL (sdLDL), decrease in HDL-C, increase in triglyceride concentration). PPAR $\alpha$  agonists (fibrates), increase plasma adiponectin levels in patients with CVD, dyslipidemia, and metabolic syndrome, in proportion to the extent of change in HDL and TG levels [46]. While PPAR $\alpha$  is expressed predominantly in the liver [47], it is also expressed in adipose [48]. Thiazolidinediones (TZDs) are activators of PPAR $\gamma$  and increase plasma adiponectin levels along with favorable changes in atherogenic dyslipidemia and insulin resistance [49–52]. In addition, TZDs directly stimulate the AMPK pathway in liver and adipose tissue [53]. Thus, adiponectin and AMPK may provide a unifying link in mediating the effects of PPAR $\gamma$  agonists on the inhibition of lipolysis in the adipocyte (Fig. 2).

## Reduced Hepatic Sensitivity to Insulin Leading to Increased Hepatic Lipogenesis, VLDL-Apolipoprotein-B Assembly and Secretion

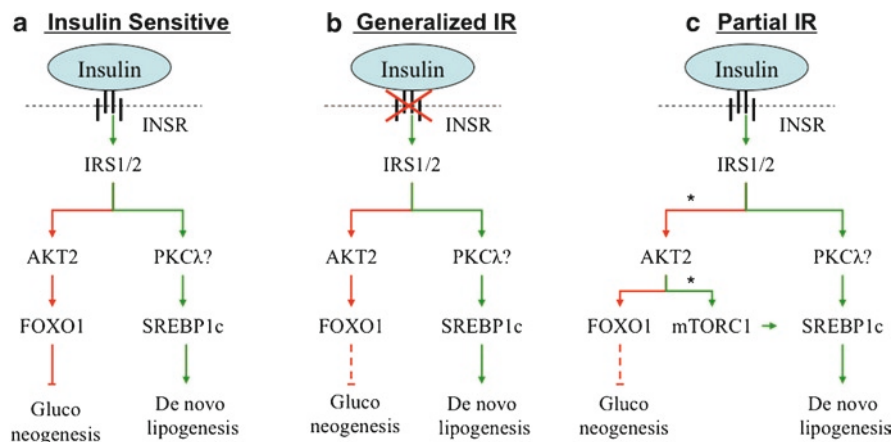
### *Dysregulation of Hepatic Lipogenesis and the Paradox of “Partial” Insulin Resistance*

In the liver, insulin is responsible for both reducing hepatic glucose production (gluconeogenesis) and increasing the production of fatty acids and triglycerides (lipogenesis). The detailed mechanisms by which insulin affects the lipogenic pathway are still being fully elucidated, although insulin has been shown to increase the transcription of sterol regulatory element binding proteins (SREBPs) [54]. SREBPs are membrane-bound transcription factors, which are proteolytically cleaved in response to low cellular cholesterol content and regulate many of the genes involved in hepatic lipid metabolism. SREBP2 is critical for cholesterol synthesis and LDL receptor regulation. SREBP-1c activation by insulin promotes fatty acid and TG synthesis.

Hepatic insulin resistance has been modeled in liver-specific insulin receptor knockout (LIRKO) mice [55]. Like humans with defects in the insulin receptor [56], these mice exhibit hyperglycemia and hyperinsulinemia, while remaining protected from hypertriglyceridemia and hepatic steatosis. This situation is consistent with both the gluconeogenic and lipogenic pathways being regulated by the insulin receptor (Fig. 3b). Signaling intermediates along the gluconeogenesis limb include serine/threonine kinase AKT2 and forkhead box O transcription factor 1 (FOXO1), while intermediates along the de novo lipogenesis arm involve SREBP-1c, and potentially PKC $\lambda$  [57].

However, in *ob/ob* mice, a leptin-deficient mouse model that develops obesity and insulin resistance, insulin efficiently activates hepatic SREBP1c, and enhances fatty acid synthesis despite the resistance of hepatic glucose production to insulin, consistent with “partial insulin resistance” of the liver [58]. Recently, more evidence has been presented that lends support to the hypothesis that a more specific intracellular defect may lead to hepatic “partial insulin resistance.” In these studies, both rodent and human models with postinsulin receptor defects exhibit hyperinsulinemia, hyperglycemia, and hypertriglyceridemia. This indicates an uncoupling (Fig. 3c) between the postinsulin receptor pathways, with the loss of insulin-mediated suppression of gluconeogenesis driven by phosphoenolpyruvate decarboxylase and glucose-6-phosphatase, but the retention of insulin’s stimulatory effect on hepatic lipogenesis catalyzed by fatty acid synthases [54, 56]. This partial insulin resistance results in combined hyperglycemia and hypertriglyceridemia, as seen in many patients with obesity and insulin resistance associated dyslipidemia.

The exact steps, branch points, and redundancies in pathways of glucose and lipid metabolism within the insulin signaling pathway that lead to “partial insulin resistance” have yet to be conclusively determined; at least two models



**Fig. 3** Proposed model for hepatic insulin resistance (IR) phenotypes. (a) Under normal circumstances, insulin stimulation of the insulin receptor (INSR) activates apparently independent arms of the signaling pathway: (1) AKT2 activation, phosphorylation of FOXO1, and inhibition of gluconeogenic gene transcription, leading to decreased glucose output and (2) activation of SREBP1c target lipogenic genes, leading to increased lipogenesis. (b) Mutations in INSR prevent activation of both ends of the pathway resulting in increased gluconeogenesis without increased lipogenesis. (c) Mutation in the AKT2 gene or inhibition of mTORC1 impairs inhibition of gluconeogenesis without abolishing lipogenic effect of insulin resulting in increased glucose and triglyceride levels. The asterisk denotes a branch point in the signaling pathway described in references [54, 56, 59]

currently exist. Brown and Goldstein, through a series of studies in mice, have shown that AKT is required for mediating both gluconeogenesis and lipogenesis [54]. They further demonstrated that mTORC1, a complex downstream of AKT, is required for stimulating the lipogenic pathway but not the gluconeogenic pathway. These results suggest that the branch point between the two pathways occurs somewhere downstream of AKT but upstream of mTORC1. These findings are consistent with that of Leavens et al. [59], who showed that genetic ablation of Akt2 (the major hepatic Akt isoform), reduces hepatic SREBP-1c mRNA levels and prevents steatosis in insulin-resistant *ob/ob* mice, thus demonstrating that Akt2 is required for hepatic lipid accumulation. However, Semple et al. showed that the loss of function mutations in human AKT2 lead only to the partially insulin resistant state of hyperglycemia and hypertriglyceridemia [56], indicating that the branch point occurs at or upstream of AKT. Part of these discrepancies may relate to the fact that the liver must integrate other signals in addition to insulin. Of particular importance is glucagon, whose stimulation of adenyl cyclase produces actions that oppose the actions of insulin, including the insulin-mediated increase in SREBP-1c mRNA [58]. The identification of the branch point and the nature of those pathways responsible for the divergent signaling to glucose output and lipogenesis remains an unresolved question. One of the implications of hepatic “partial insulin resistance” in humans is that aggressive treatment of Type 2 diabetes patients with large doses of insulin may help to control hyperglycemia, but possibly enhance hepatic TG synthesis, dyslipidemia, and hepatic steatosis.

### ***Dysregulation of Apolipoprotein-B Synthesis, VLDL Assembly and Secretion***

The availability of lipids within the lumen of the endoplasmic reticulum (ER) is an important factor in determining the secretion of VLDL and apoB via co- and post-translational mechanisms [60–62]. At least two intracellular pathways of apoB degradation have been identified, the proteasomal ER-associated degradation (ERAD) pathway and the post-ER pre-secretory proteolysis (PERPP) pathway [62]. Recently, ER stress has been tied to the obese state and implicated in the pathogenesis of atherogenic dyslipidemia. In hepatocyte cell culture and in mouse liver *in vivo*, increased fatty acid delivery and/or accumulation of TGs (or intermediary metabolites) increased ER stress [63]. Mild increases in ER stress increased apoB100 secretion, whereas prolonged ER stress reduced apoB100 secretion and resulted in greater hepatic steatosis. This implies that there are complex relationships involving the role of ER stress in the regulation of secretion of atherogenic apo B containing lipoproteins.

Another important factor involved in the assembly of apoB-containing lipoproteins is the microsomal triglyceride transfer protein (MTP), which catalyses the transfer of neutral lipids to the newly synthesized apoB protein in the ER, forming the nascent VLDL particle. The activity of MTP within the ER is a critical determinant of VLDL secretion [64]. Lack of adequate MTP results in impaired apoB lipidation and targeting of apoB to the ERAD pathway for degradation. Conversely, overexpression of MTP in the liver results in increased VLDL apoB production [65]. Hepatic expression of MTP is influenced by insulin, and there is an insulin-response element that is negatively regulated by insulin [66]. In insulin-resistant states, the hyperinsulinism may upregulate MTP expression, predisposing to hepatic VLDL oversecretion.

Thus, chronic modulation of apoB and VLDL secretion can be achieved via changes in MTP expression and activity [67]. Insulin may control the rate of hepatic VLDL production directly by influencing the rate of apoB synthesis and degradation [68], or modulation of MTP gene expression [69]. Insulin regulation of apoB secretion appears to involve the activation of the PI-3 kinase pathway, which may mediate the inhibitory effect of insulin on the VLDL assembly process [69] but the precise molecular mechanism remains unclear. There is thought to be the loss of this inhibition in insulin resistance that frequently accompanies obesity, leading to increased VLDL secretion, but as discussed, this paradigm is likely oversimplified.

### **Impaired Catabolism of Atherogenic Lipoprotein Remnants**

Metabolic studies in humans have also shown that obesity, insulin resistance and type 2 diabetes are associated with reduced catabolism of apoB-containing lipoprotein remnants. Apolipoprotein C-III (apoC-III) is an apolipoprotein constituent in VLDL and chylomicrons and regulates their catabolism. ApoC-III blocks catabolism of triglyceride-rich and remnant lipoprotein particles both by inhibiting LPL as



well as by inhibiting the LDL receptor-mediated uptake of remnant lipoproteins (Fig. 1). ApoC-III is synthesized in the liver and small intestine [70]. Insulin inhibits the transcription of the apoC-III gene [71]. Conversely, there is increased production and decreased catabolism of apoC-III in insulin resistance, resulting in higher plasma levels of apoC-III [72, 73]. In addition, as noted above, in obese insulin resistant states, the activity of LPL is also suppressed. Thus, the combination of reduced LPL and increased apoC-III in insulin resistant states leads to decreased hydrolysis and catabolism of TG-rich particles [73].

Hepatic lipase (HL) is an enzyme which hydrolyzes triglycerides in plasma lipoproteins and plays an important role in the metabolism of TRL remnants and TG-rich LDL. HL remodels VLDL remnants, converts IDL to LDL particles, and mediates the hydrolysis of TG-enriched LDL, promoting its conversion to sdLDL. HL is known to be substantially upregulated in insulin resistance in humans [74, 75], resulting in the accelerated formation of sdLDL particles [76]. The basis for increased HL in insulin resistance remains uncertain. Unlike LPL, HL is not regulated by insulin per se, although fructose feeding of hamsters, which induces insulin resistance, upregulates HL expression and plasma activity [77]. The sdLDL particles are more intrinsically atherogenic because of their enhanced susceptibility to oxidative modification and have reduced affinity for the LDL receptor which results them being less efficiently cleared from the circulation [78].

## Hypercatabolism of HDL

Low levels of HDL-C are very common in obesity [74]. However, despite this common clinical observation, the mechanisms of reduced HDL-C in obese and insulin resistant states are poorly understood. One causal factor is certainly the elevated levels of TRLs as previously discussed. There is substantial interaction between TRL and HDL in their metabolism. With the hydrolysis by LPL of chylomicron, VLDL triglycerides, and shrinkage of the particles, excess surface phospholipids and apolipoproteins (including apoA-I, apoCs, and apoE) are transferred to HDL, providing greater HDL mass [79]. Accordingly, impaired LPL activity, as often seen in obesity and insulin resistance, is one factor that results in reduced HDL-C levels.

In addition, cholesteryl esters are transferred from HDL to TRL by the cholesteryl ester transfer protein (CETP) in exchange for TG (Fig. 1). Increased levels of acceptor TRL particles, such as in obesity, drive an increased rate of CETP-mediated exchange, thus resulting in a greater siphoning of cholesterol out of HDL and reduction in HDL-C levels [80]. Interestingly, adipose tissue secretes CETP, and adipose tissue may be an important source of plasma CETP in humans [81]. Plasma CETP activity and mass are increased in obese humans [82]. In hamsters, fructose feeding significantly raised adipose CETP expression and plasma CETP levels [83]. Thus, increased production of CETP by adipose in obese states could directly result in reduced HDL-C levels, even without elevations in TRL.

Furthermore, the CETP-mediated transfer process enriches HDL with triglycerides, resulting in a TG-rich HDL that is a better substrate for HL [84].

As previously noted, HL is increased in obese and insulin-resistant states [73, 85]. Hydrolysis of HDL TG by HL leads to the formation of smaller HDL particles that are more rapidly catabolized as a result of HL-mediated lipolysis [86, 87]. The basis for elevated HL activity in obesity and insulin resistance remains uncertain. Fructose feeding of hamsters, which induces insulin resistance, upregulates HL expression and plasma activity levels [88]. Further investigation is needed to understand the relationship between obesity, insulin resistance, HL expression, and HDL metabolism. In summary, in the obese insulin resistant state increases in the plasma concentrations of TRL, CETP, and HL result in greater CETP-mediated exchange of cholesterol out of HDL and of TG into HDL and greater hydrolysis of HDL TG by HL, resulting in accelerated catabolism and lower levels of HDL cholesterol.

A relative of HL, endothelial lipase (EL), may also play an important role in contributing to reduced HDL-C in insulin resistance [89]. EL has major effects on HDL metabolism in mice: overexpression reduces HDL-C levels [90, 91] by increasing the catabolism of HDL [92], whereas inhibition [93] or genetic deletion [90, 94] of EL increases HDL-C levels by reducing the catabolic rate. In humans, rare genetic variants of the EL gene are associated with the phenotype of high HDL-C [95]. Plasma levels of EL are significantly inversely correlated with HDL-C levels even after correcting for confounding factors [89]. Importantly, EL levels were found to be significantly elevated in patients with the metabolic syndrome and highly correlated with BMI and inversely with HDL-C [89], suggesting that EL is upregulated in insulin resistance and may contribute to the low HDL-C levels seen in obesity.

Finally, adipose tissue may be an important source of cholesterol efflux to HDL. Adipose tissue contains approximately 25% of total body cholesterol in lean subjects and much more in obese subjects, and the vast majority of adipose cholesterol is unesterified [95]. Several laboratories have demonstrated efflux of cholesterol from adipocytes to HDL in vitro, and adipocytes have been demonstrated to express all of the major cholesterol efflux transporters, such as ATP-binding cassette subfamily A member 1 (ABCA1), ATP-binding cassette subfamily G member 1 (ABCG1), and scavenger receptor class B type I (SR-BI). Their functional significance has, however, not yet been demonstrated in vivo until somewhat recently. Reilly et al. demonstrated that adipocytes support transfer of cholesterol to HDL in vivo as well as in vitro and implicate ABCA1 and SR-BI, but not ABCG1, cholesterol transporters in this process [96]. It is possible, although unproven, that in obesity or insulin resistance, these transporters in adipocytes are downregulated or dysfunctional, thus resulting in reduced cholesterol efflux to HDL and contributing to reduced HDL-C levels. More work is needed in this area. Sorting out the relative importance of these mechanisms is of critical importance in developing novel strategies for raising HDL-C levels or improving its function.

## Conclusion

Atherogenic dyslipidemia (increased TRLs, increased sdLDLs, and low levels of HDL) is extremely common in obesity, and is often accompanied by insulin resistance. This constellation is associated with substantially increased risk for cardiovascular

disease in these individuals. The mechanisms connecting obesity, insulin resistance, and dyslipidemia are incompletely understood, but are thought to be driven by increased flux of FFAs from adipose tissue to liver driving hepatic overproduction of VLDL, reduced LPL, and increased apoC-III expression resulting in reduced clearance of triglyceride-rich remnants, and increased CETP, HL, and EL activity driving accelerated HDL catabolism. The present paradigm is likely oversimplified, and a more thorough understanding of the physiological and molecular mechanisms is critical for improving our approach to managing the influence of obesity on lipoprotein metabolism, and to the development of appropriate therapeutic approaches. In the next few years, information from human genetics and laboratory science may provide additional insights into candidate genes and pathways influencing dysregulation of lipid metabolism in obesity, insulin resistance, and cardiovascular disease. In this regard, further research focused on the interactions between hepatic and adipose tissue function with lipoprotein metabolism will likely yield promising therapeutic targets for the reduction of cardiometabolic risk in this patient group.

## References

1. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection. (2001). Evaluation, and treatment of high blood cholesterol in adults (adult treatment panel III). *JAMA: The Journal of the American Medical Association*, 285(19), 2486–2497.
2. Bays, H. E., González-Campoy, J. M., Bray, G. A., Kitabchi, A. E., Bergman, D. A., Schorr, A. B., et al. (2008). Pathogenic potential of adipose tissue and metabolic consequences of adipocyte hypertrophy and increased visceral adiposity. *Expert Review of Cardiovascular Therapy*, 6(3), 343–368.
3. Sparks, J. D., Collins, H. L., Sabio, I., et al. (1997). Effects of fatty acids on apolipoprotein B secretion by McArdle RH-7777 rat hepatoma cells. *Biochimica et Biophysica Acta*, 1347(1), 51–56.
4. White, A. L., Graham, D. L., LeGros, J., et al. (1992). Oleate-mediated stimulation of apolipoprotein B secretion from rat hepatoma cells: A function of the ability of apolipoprotein B to direct lipoprotein assembly and escape presecretory degradation. *The Journal of Biological Chemistry*, 267, 15657–15664.
5. Wu, X., Sakata, N., Dixon, J., & Ginsberg, H. N. (1994). Exogenous VLDL stimulates apolipoprotein B secretion from HepG2 cells by both pre- and post-translational mechanisms. *Journal of Lipid Research*, 35, 1200–1211.
6. Targher, G., Marra, F., & Marchesini, G. (2008). Increased risk of cardiovascular disease in non-alcoholic fatty liver disease: Causal effect or epiphenomenon? *Diabetologia*, 51(11), 1947–1953. Epub 2008 Sep 2.
7. Lewis, G. F., Uffelman, K. D., Szeto, L. W., Weller, B., & Steiner, G. (1995). Interaction between free fatty acids and insulin in the acute control of very low density lipoprotein production in humans. *The Journal of Clinical Investigation*, 95(1), 158–166.
8. Kissebah, A. H., Alfarsi, S., Evans, D. J. & Adams, P. W. (1982). Integrated regulation of very low density lipoprotein triglyceride and apolipoprotein-B kinetics in non-insulin dependent diabetes mellitus. *Diabetes*, 31(3), 217–225.
9. Campbell, P. J., Carlson, M. G., & Nurjhan, N. (1994). Fat metabolism in human obesity. *The American Journal of Physiology*, 266(4 Pt 1), E600–605.
10. Malmstrom, R., Packard, C. J., Caslake, M., et al. (1997). Defective regulation of triglyceride metabolism by insulin in the liver in NIDDM. *Diabetologia*, 40(4), 454–462.

11. Goldberg, I. J., & Merkel, M. (2001). Lipoprotein lipase: Physiology, biochemistry, and molecular biology. *Frontiers in Bioscience: A Journal and Virtual Library*, 6, D388–405.
12. Bamba, V., & Rader, D. J. (2007). Obesity and atherogenic dyslipidemia. *Gastroenterology*, 132(6), 2181–2190.
13. Merkel, M., Eckel, R. H., & Goldberg, I. J. (2002). Lipoprotein lipase: Genetics, lipid uptake, and regulation. *Journal of Lipid Research*, 43(12), 1997–2006.
14. Ruge, T., Svensson, M., Eriksson, J. W., & Olivecrona, G. (2005). Tissue-specific regulation of lipoprotein lipase in humans: Effects of fasting. *European Journal of Clinical Investigation*, 35(3), 194–200.
15. Yu, Y. H., & Ginsberg, H. N. (2005). Adipocyte signaling and lipid homeostasis: Sequelae of insulin-resistant adipose tissue. *Circulation Research*, 96(10), 1042–1052.
16. Panarotto, D., Remillard, P., Bouffard, L., & Maheux, P. (2002). Insulin resistance affects the regulation of lipoprotein lipase in the postprandial period and in an adipose tissue-specific manner. *European Journal of Clinical Investigation*, 32(2), 84–92.
17. Large, V., Peroni, O., Letexier, D., Ray, H., & Beylot, M. (2004). Metabolism of lipids in human white adipocyte. *Diabetes & Metabolism*, 30(4), 294–309.
18. Bagnato, C., & Igal, R. A. (2003). Overexpression of diacylglycerol acyltransferase-1 reduces phospholipid synthesis, proliferation, and invasiveness in simian virus 40-transformed human lung fibroblasts. *The Journal of Biological Chemistry*, 278(52), 52203–52211.
19. Smith, S. J., Cases, S., Jensen, D. R., et al. (2000). Obesity resistance and multiple mechanisms of triglyceride synthesis in mice lacking Dgat. *Nature Genetics*, 25(1), 87–90.
20. Chen, H. C., Smith, S. J., Ladha, Z., et al. (2002). Increased insulin and leptin sensitivity in mice lacking acyl CoA: Diacylglycerol acyltransferase 1. *The Journal of Clinical Investigation*, 109(8), 1049–1055.
21. Stone, S. J., Myers, H. M., Watkins, S. M., et al. (2004). Lipopenia and skin barrier abnormalities in DGAT2-deficient mice. *The Journal of Biological Chemistry*, 279(12), 11767–11776.
22. Oelkers, P., Behari, A., Cromley, D., Billheimer, J. T., & Sturley, S. L. (1998). Characterization of two human genes encoding acyl coenzyme A: Cholesterol acyltransferase-related enzymes. *The Journal of Biological Chemistry*, Oct 9 273(41), 26765–26771.
23. Cases, S., Stone, S. J., Zhou, P., et al. (2001). Cloning of DGAT2, a second mammalian diacylglycerol acyltransferase, and related family members. *The Journal of Biological Chemistry*, 276(42), 38870–38876.
24. Meegalla, R. L., Billheimer, J. T., & Cheng, D. (2002). Concerted elevation of acyl-coenzyme A: Diacylglycerol acyltransferase (DGAT) activity through independent stimulation of mRNA expression of DGAT1 and DGAT2 by carbohydrate and insulin. *Biochemical and Biophysical Research Communications*, 298(3), 317–323.
25. Yen, C. L., Monetti, M., Burri, B. J., & Farese, R. V., Jr. (2005). The triacylglycerol synthesis enzyme DGAT1 also catalyzes the synthesis of diacylglycerols, waxes, and retinyl esters. *Journal of Lipid Research*, 46(7), 1502–1511.
26. Orland, M. D., Anwar, K., Cromley, D., et al. (2005). Acyl coenzyme A dependent retinol esterification by acyl coenzyme A: Diacylglycerol acyltransferase 1. *Biochimica et Biophysica Acta*, 1737(1), 76–82.
27. Chen, H. C., & Farese, R. V., Jr. (2000). DGAT and triglyceride synthesis: A new target for obesity treatment? *Trends in Cardiovascular Medicine*, 10(5), 188–192.
28. Haemmerle, G., Zimmermann, R., & Zechner, R. (2003). Letting lipids go: Hormone-sensitive lipase. *Current Opinion in Lipidology*, 14(3), 289–297.
29. Langin, D. (2006). Adipose tissue lipolysis as a metabolic pathway to define pharmacological strategies against obesity and the metabolic syndrome. *Pharmacological Research*, 53(6), 482–491.
30. Skowronski, R., Hollenbeck, C. B., Varasteh, B. B., Chen, Y. D., & Reaven, G. M. (1991). Regulation of non-esterified fatty acid and glycerol concentration by insulin in normal individuals and patients with type 2 diabetes. *Diabetic Medicine: A Journal of the British Diabetic Association*, 8(4), 330–333.

31. Zimmermann, R., Strauss, J. G., Haemmerle, G., et al. (2004). Fat mobilization in adipose tissue is promoted by adipose triglyceride lipase. *Science*, 306(5700), 1383–1386.
32. Kershaw, E. E., Hamm, J. K., Verhagen, L. A., Peroni, O., Katic, M., & Flier, J. S. (2006). Adipose triglyceride lipase: Function, regulation by insulin, and comparison with adiponutrin. *Diabetes*, 55(1), 148–157.
33. Kershaw, E. E., Schupp, M., Guan, H. P., Gardner, N. P., Lazar, M. A., & Flier, J. S. (2007). PPARgamma regulates adipose triglyceride lipase in adipocytes in vitro and in vivo. *American Journal of Physiology. Endocrinology and Metabolism*, 293(6), E1736–1745.
34. Towler, M. C., & Hardie, D. G. (2007). AMP-activated protein kinase in metabolic control and insulin signaling. *Circulation Research*, 100(3), 328–341.
35. Brooks, B. J., Arch, J. R., & Newsholme, E. A. (1983). Effect of some hormones on the rate of the triacylglycerol/fatty-acid substrate cycle in adipose tissue of the mouse in vivo. *Bioscience Reports*, 3(3), 263–267.
36. Hardie, D. G., & Carling, D. (1997). The AMP-activated protein kinase—fuel gauge of the mammalian cell? *European Journal of Biochemistry/FEBS*, 246(2), 259–273.
37. Yamauchi, T., Kamon, J., Minokoshi, Y., et al. (2002). Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. *Nature Medicine*, 8(11), 1288–1295.
38. Tunaru, S., Kero, J., Schaub, A., et al. (2003). PUMA-G and HM74 are receptors for nicotinic acid and mediate its anti-lipolytic effect. *Nature Medicine*, 9(3), 352–355.
39. Offermanns, S. (2006). The nicotinic acid receptor GPR109A (HM74A or PUMA-G) as a new therapeutic target. *Trends in Pharmacological Sciences*, 27(7), 384–390.
40. Karpe, F., & Frayn, K. N. (2004). The nicotinic acid receptor – a new mechanism for an old drug. *Lancet*, 363(9424), 1892–1894.
41. Pike, N. B., & Wise, A. (2004). Identification of a nicotinic acid receptor: Is this the molecular target for the oldest lipid-lowering drug? *Current Opinion in Investigational Drugs (London, England: 2000)*, 5(3), 271–275.
42. Taggart, A. K., Kero, J., Gan, X., et al. (2005). (D)-beta-Hydroxybutyrate inhibits adipocyte lipolysis via the nicotinic acid receptor PUMA-G. *The Journal of Biological Chemistry*, 280(29), 26649–26652.
43. Wise, A., Foord, S. M., Fraser, N. J., et al. (2003). Molecular identification of high and low affinity receptors for nicotinic acid. *The Journal of Biological Chemistry*, 278(11), 9869–9874.
44. Wu, X., Motoshima, H., Mahadev, K., Stalker, T. J., Scalia, R., & Goldstein, B. J. (2003). Involvement of AMP-activated protein kinase in glucose uptake stimulated by the globular domain of adiponectin in primary rat adipocytes. *Diabetes*, 52(6), 1355–1363.
45. Westphal, S., Borucki, K., Taneva, E., Makarova, R., & Luley, C. (2006). Adipokines and treatment with niacin. *Metabolism: Clinical and Experimental*, 55(10), 1283–1285.
46. Schoonjans, K., Staels, B., & Auwerx, J. (1996). Role of the peroxisome proliferator-activated receptor (PPAR) in mediating the effects of fibrates and fatty acids on gene expression. *Journal of Lipid Research*, 37(5), 907–925.
47. Li, P., Zhu, Z., Lu, Y., & Granneman, J. G. (2005). Metabolic and cellular plasticity in white adipose tissue II: Role of peroxisome proliferator-activated receptor-alpha. *American Journal of Physiology. Endocrinology and Metabolism*, 289(4), E617–626.
48. Yki-Jarvinen, H. (2004). Thiazolidinediones. *The New England Journal of Medicine*, 351(11), 1106–1118.
49. Combs, T. P., Pajvani, U. B., Berg, A. H., et al. (2004). A transgenic mouse with a deletion in the collagenous domain of adiponectin displays elevated circulating adiponectin and improved insulin sensitivity. *Endocrinology*, 145(1), 367–383.
50. Trujillo, M. E., & Scherer, P. E. (2005). Adiponectin – journey from an adipocyte secretory protein to biomarker of the metabolic syndrome. *Journal of Internal Medicine*, 257(2), 167–175.
51. Szapary, P. O., Bloedon, L. T., Samaha, F. F., et al. (2006). Effects of pioglitazone on lipoproteins, inflammatory markers, and adipokines in nondiabetic patients with metabolic syndrome. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 26(1), 182–188.

52. Moller, D. E., & Berger, J. P. Role of PPARs in the regulation of obesity-related insulin sensitivity and inflammation. *International Journal of Obesity and Related Metabolic Disorders: Journal of the International Association for the Study of Obesity*, 27(Suppl 3), S17–21.
53. Duffy, D., & Rader, D. (2007). Endocannabinoid antagonism: Blocking the excess in the treatment of high-risk abdominal obesity. *Trends in Cardiovascular Medicine*, 17(2), 35–43.
54. Li, S., Brown, M. S., & Goldstein, J. L. (2010). Bifurcation of insulin signaling pathway in rat liver: mTORC1 required for stimulation of lipogenesis, but not inhibition of gluconeogenesis. *Proceedings of the National Academy of Sciences of the United States of America*, 107(8), 3441–3446.
55. Biddinger, S. B., Hernandez-Ono, A., Rask-Madsen, C., et al. (2008). Hepatic insulin resistance is sufficient to produce dyslipidemia and susceptibility to atherosclerosis. *Cell Metabolism*, 7(2), 125–134.
56. Semple, R. K., Sleight, A., Murgatroyd, P. R., et al. (2009). Postreceptor insulin resistance contributes to human dyslipidemia and hepatic steatosis. *The Journal of Clinical Investigation*, 119(2), 315–322.
57. Taniguchi, C. M., Kondo, T., Sajan, M., et al. (2006). Divergent regulation of hepatic glucose and lipid metabolism by phosphoinositide 3-kinase via Akt and PKC $\lambda$ /zeta. *Cell Metabolism*, 3(5), 343–353.
58. Shimomura, I., Matsuda, M., Hammer, R. E., Bashmakov, Y., Brown, M. S., & Goldstein, J. L. (2000). Decreased IRS-2 and increased SREBP-1c lead to mixed insulin resistance and sensitivity in livers of lipodystrophic and ob/ob mice. *Molecular Cell*, 6(1), 77–86.
59. Leavens, K. F., Easton, R. M., Shulman, G. I., Previs, S. F., & Birnbaum, M. J. (2009). Akt2 is required for hepatic lipid accumulation in models of insulin resistance. *Cell Metabolism*, 10(5), 405–418.
60. Sniderman, A. D., & Cianflone, K. (1993). Substrate delivery as a determinant of hepatic apoB secretion. *Arteriosclerosis and Thrombosis: A Journal of Vascular Biology/American Heart Association*, 13(5), 629–636.
61. Yao, Z., & McLeod, R. S. (1994). Synthesis and secretion of hepatic apolipoprotein B-containing lipoproteins. *Biochimica et Biophysica Acta*, 1212(2), 152–166.
62. Pan, M., Maitin, V., Parathath, S., et al. (2008). Presecretory oxidation, aggregation, and autophagic destruction of apoprotein-B: A pathway for late-stage quality control. *Proceedings of the National Academy of Sciences*, 105(15), 5862–5867.
63. Ota, T., Gayet, C., & Ginsberg, H. N. (2008). Inhibition of apolipoprotein B100 secretion by lipid-induced hepatic endoplasmic reticulum stress in rodents. *The Journal of Clinical Investigation*, 118(1), 316–332.
64. Leung, G. K., Veniant, M. M., Kim, S. K., et al. (2000). A deficiency of microsomal triglyceride transfer protein reduces apolipoprotein B secretion. *Journal of Biological Chemistry*, 275(11), 7515–7520.
65. Tietge, U. J., Bakillah, A., Maugeais, C., Tsukamoto, K., Hussain, M., & Rader, D. J. (1999). Hepatic overexpression of microsomal triglyceride transfer protein (MTP) results in increased in vivo secretion of VLDL triglycerides and apolipoprotein B. *Journal of Lipid Research*, 40(11), 2134–2139.
66. Lin, M. C., Gordon, D., & Wetterau, J. R. (1995). Microsomal triglyceride transfer protein (MTP) regulation in HepG2 cells: Insulin negatively regulates MTP gene expression. *Journal of Lipid Research*, 36(5), 1073–1081.
67. Gordon, D. A., & Jamil, H. (2000). Progress towards understanding the role of microsomal triglyceride transfer protein in apolipoprotein-B lipoprotein assembly. *Biochimica et Biophysica Acta*, 1486(1), 72–83.
68. Sparks, J. D., & Sparks, C. E. (1994). Insulin regulation of triacylglycerol-rich lipoprotein synthesis and secretion. *Biochimica et Biophysica Acta*, 1215(1–2), 9–32.
69. Au, W. S., Kung, H. F., & Lin, M. C. (2003). Regulation of microsomal triglyceride transfer protein gene by insulin in HepG2 cells: Roles of MAPK $\epsilon$  and MAPK $\delta$ . *Diabetes*, 52(5), 1073–1080.



70. Fredenrich, A. (1998). Role of apolipoprotein CIII in triglyceride-rich lipoprotein metabolism. *Diabetes & Metabolism*, 24(6), 490–495.
71. Chen, M., Breslow, J. L., Li, W., & Leff, T. (1994). Transcriptional regulation of the apoC-III gene by insulin in diabetic mice: Correlation with changes in plasma triglyceride levels. *Journal of Lipid Research*, 35(11), 1918–1924.
72. Chan, D. C., Nguyen, M. N., Watts, G. F., & Barrett, P. H. (2008). Plasma apolipoprotein C-III transport in centrally obese men: Associations with very low-density lipoprotein apolipoprotein B and high-density lipoprotein apolipoprotein A-I metabolism. *The Journal of Clinical Endocrinology and Metabolism*, 93(2), 557–564.
73. Duvillard, L., Pont, F., Florentin, E., Galland-Jos, C., Gambert, P., & Verges, B. (2000). Metabolic abnormalities of apolipoprotein B-containing lipoproteins in non-insulin-dependent diabetes: A stable isotope kinetic study. *European Journal of Clinical Investigation*, 30(8), 685–694.
74. Despres, J. P., Couillard, C., Gagnon, J., et al. (2000). Race, visceral adipose tissue, plasma lipids, and lipoprotein lipase activity in men and women: The Health, Risk Factors, Exercise Training, and Genetics (HERITAGE) family study. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 20(8), 1932–1938.
75. Carr, M. C., Ayyobi, A. F., Murdoch, S. J., Deeb, S. S., & Brunzell, J. D. (2002). Contribution of hepatic lipase, lipoprotein lipase, and cholesteryl ester transfer protein to LDL and HDL heterogeneity in healthy women. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 22(4), 667–673.
76. Rashid, S., Uffelman, K. D., & Lewis, G. F. (2002). The mechanism of HDL lowering in hypertriglyceridemic, insulin-resistant states. *Journal of Diabetes and Its Complications*, 16(1), 24–28.
77. Lewis, G. F., Murdoch, S., Uffelman, K., et al. (2004). Hepatic lipase mRNA, protein, and plasma enzyme activity is increased in the insulin-resistant, fructose-fed Syrian golden hamster and is partially normalized by the insulin sensitizer rosiglitazone. *Diabetes*, 53(11), 2893–2900.
78. Rosenson, R. S. (2004). Statins in atherosclerosis: Lipid-lowering agents with antioxidant capabilities. *Atherosclerosis*, 173(1), 1–12.
79. Goldberg, I. J. (1996). Lipoprotein lipase and lipolysis: Central roles in lipoprotein metabolism and atherogenesis. *Journal of Lipid Research*, 37(4), 693–707.
80. Lewis, G. F., & Rader, D. J. (2005). New insights into the regulation of HDL metabolism and reverse cholesterol transport. *Circulation Research*, 96(12), 1221–1232.
81. Dullaart, R. P., Sluiter, W. J., Dikkeschei, L. D., Hoogenberg, K., & Van Tol, A. (1994). Effect of adiposity on plasma lipid transfer protein activities: A possible link between insulin resistance and high density lipoprotein metabolism. *European Journal of Clinical Investigation*, 24(3), 188–194.
82. MacLean, P. S., Vadlamudi, S., MacDonald, K. G., Pories, W. J., & Barakat, H. A. (2005). Suppression of hepatic cholesteryl ester transfer protein expression in obese humans with the development of type 2 diabetes mellitus. *The Journal of Clinical Endocrinology and Metabolism*, 90(4), 2250–2258.
83. Remillard, P., Shen, G., Milne, R., & Maheux, P. (2001). Induction of cholesteryl ester transfer protein in adipose tissue and plasma of the fructose-fed hamster. *Life Sciences*, 69(6), 677–687.
84. Blades, B., Vega, G. L., & Grundy, S. M. (1993). Activities of lipoprotein lipase and hepatic triglyceride lipase in postheparin plasma of patients with low concentrations of HDL cholesterol. *Arterioscler Thromb*, 13(8), 1227–1235.
85. Despres, J. P., Ferland, M., Moorjani, S., et al. (1989). Role of hepatic-triglyceride lipase activity in the association between intra-abdominal fat and plasma HDL cholesterol in obese women. *Arteriosclerosis (Dallas, Tex.)*, 9(4), 485–492.
86. Rashid, S., Barrett, P. H., Uffelman, K. D., Watanabe, T., Adeli, K., & Lewis, G. F. (2002). Lipolytically modified triglyceride-enriched HDLs are rapidly cleared from the circulation. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 22(3), 483–487.
87. Lewis, G. F., Lamarche, B., Uffelman, K. D., et al. (1997). Clearance of postprandial and lipolytically modified human HDL in rabbits and rats. *Journal of Lipid Research*, 38(9), 1771–1778.
88. Lewis, G. F., Murdoch, S., Uffelman, K., et al. (2004). Hepatic lipase mRNA, protein, and plasma enzyme activity is increased in the insulin-resistant, fructose-fed syrian golden hamster and is partially normalized by the insulin sensitizer rosiglitazone. *Diabetes*, 53(11), 2893–2900.



89. Badellino, K. O., Wolfe, M. L., Reilly, M. P., & Rader, D. J. (2006). Endothelial lipase concentrations are increased in metabolic syndrome and associated with coronary atherosclerosis. *PLoS Medicine*, 3(2), e22.
90. Ishida, T., Choi, S., Kundu, R. K., et al. (2003). Endothelial lipase is a major determinant of HDL level. *The Journal of Clinical Investigation*, 111(3), 347–355.
91. Jaye, M., Lynch, K. J., Krawiec, J., et al. (1999). A novel endothelial-derived lipase that modulates HDL metabolism. *Nature Genetics*, 21(4), 424–428.
92. Maugeais, C., Tietge, U. J., Broedl, U. C., et al. (2003). Dose-dependent acceleration of high-density lipoprotein catabolism by endothelial lipase. *Circulation*, 108(17), 2121–2126.
93. Jin, W., Millar, J. S., Broedl, U., Glick, J. M., & Rader, D. J. (2003). Inhibition of endothelial lipase causes increased HDL cholesterol levels in vivo. *The Journal of Clinical Investigation*, 111(3), 357–362.
94. Ma, K., Cilingiroglu, M., Otvos, J. D., Ballantyne, C. M., Marian, A. J., & Chan, L. (2003). Endothelial lipase is a major genetic determinant for high-density lipoprotein concentration, structure, and metabolism. *Proceedings of the National Academy of Sciences of the United States of America*, 100(5), 2748–2753.
95. deLemos, A. S., Wolfe, M. L., Long, C. J., Sivapackianathan, R., & Rader, D. J. (2002). Identification of genetic variants in endothelial lipase in persons with elevated high-density lipoprotein cholesterol. *Circulation*, 106(11), 1321–1326.
96. Zhang, Y., McGillicuddy, F. C., Hinkle, C. C., et al. Adipocyte modulation of high-density lipoprotein cholesterol. *Circulation*, 121(11), 1347–1355.



# Chapter 16

## Gut Microbes, Immunity, and Metabolism

Evelyn Hsu and Gary Wu

### Introduction

The human gut contains a vast number of bacteria, collectively characterized as the “gut microbiome.” An estimated  $10^{13}$  individual bacteria of anywhere between 500 and 1,000 species reside in the mammalian gut, making it the most densely populated microbial communities on Earth [1]. Acting upon the assumption that there are 1,000 bacterial species, the aggregate size of all intestinal microbial genomes may exceed the size of the human genome by more than 100-fold [2]. The larger view of the mammalian physiology should take into account that, together with our microbiome, we are a biologic “supraorganism” that is dynamic and carries out functions in parallel or cooperatively.

The microbial communities associated with the gut are profoundly different from other free-living microbial communities from across the biosphere [3]. When viewed as a whole, the “supraorganism” of the gut can carry out enzymatic reactions distinct from those of the human genome and harvest energy that would otherwise be lost to the host. The consequences of these enzymatic reactions suggest that over the millennia, mammalian metabolism, physiology, and disease have shaped and been shaped by the gut microbiome. In general, we as hosts coexist in either a commensal or symbiotic relationship with our gut microbiome [4]. While we provide members of the gut microbiome a unique niche to inhabit, in turn, the gut microbiome performs critical physiologic functions that benefit the host, including education of the mucosal immune system, extraction of nutrients from undigested carbohydrates through the production of short-chain fatty acids, salvaging nitrogen through the hydrolysis of urea, production of certain vitamins, and the metabolism of bile salts, to name a few.

---

G. Wu (✉)

Department of Medicine, Division of Gastroenterology, University of Pennsylvania  
School of Medicine, Center for Molecular Studies in Digestive and Liver Diseases,  
Philadelphia, PA, USA  
e-mail: gdwu@mail.med.upenn.edu

Although most gut bacterial organisms are refractory to cultivation, new DNA sequencing methods and technologies now permit robust analyses of complex bacterial communities offering not only valuable information about the composition of the gut microbiome, but also a window into the upregulation and downregulation of bacterial gene representation in the face of health and disease.

Numerous diseases in both adults and children have been linked to changes in the gut microbiota, such as *Helicobacter pylori* in the development of stomach cancer [5], inflammatory bowel disease [6, 7], nonalcoholic fatty liver disease [8], irritable bowel syndrome [9], necrotizing enterocolitis in infants [10], and diet-induced obesity (DIO) [11]. The known associations with human disease, coupled with the advanced technology that is now available, make this an extremely compelling area of investigation. In this chapter, we provide a brief overview of the gut microbiome and its impact upon host metabolism with a focus on the pathogenesis of human disease.

## Gut Microecology

Since the initial description of germ-free mammals over a century ago, it has become increasingly obvious that the host-associated microbiome plays a major role in a diverse set of metabolic physiologic responses [12]. With the advent of gnotobiotics, where germ-free animals are colonized with defined bacterial populations, and more sophisticated molecular techniques to characterize patterns of gene expression and metabolic function, new insights into the mechanisms by which the microbiome influences host metabolism have been revealed. Given the essential importance of the intestinal tract on nutrient absorption, it seems intuitively obvious that bacterial communities in the intestinal tract, collectively known as the gut microbiome, would likely play the most important role in this regard. These studies have revealed an important role for a single gut commensal organism, *Bacteroides thetaiotaomicron*, in regulating the expression of genes involved in multiple intestinal functions including nutrient absorption, epithelial barrier function, and xenobiotic metabolism [13]. In addition, more recent studies have revealed that the gut microbiome plays a critical role in the maturation of the mucosal immune system such as the induction of intestinal Th17 cells by the gut communal organism, *segmented filamentous bacteria* [14]. As a result, the coevolution of the mammalian host with its gut microbiome over the millennia has led to the development of complex and robust immunologic mechanisms to maintain homeostasis fostering a commensal relationship with the microbial ecosystem in the gut [15]. Alterations in these homeostatic mechanisms, imparted by host genotype, may be the pathophysiologic basis for the development of a chronic inflammatory disorder of the intestinal tract known as inflammatory bowel disease.

From the standpoint of nutrition, the gut microbiome also plays a critical role in host metabolism. Members of the *Bacteroides* genus, which are Gram-negative anaerobes, comprise a significant proportion of the bacteria in the gut [16].

*B. thetaiotaomicron* is a dominant member of this genus, and the first one to undergo full sequencing of its genome, revealing that this organism possesses a variety of the enzymes necessary for hydrolysis of plant polysaccharides that are otherwise indigestible by the host [17]. Humans and other mammals are able to absorb simple sugars in the proximal small intestine. Certain disaccharides are hydrolyzed to monosaccharides and then absorbed, but mammals are largely unable to digest many other complex polysaccharides, and subsequently, this underutilized dietary carbohydrate source passes into the distal gut and is lost to the host. By adopting a microbiota that has the ability to degrade these carbohydrates, mammals are able to continue harvesting energy from these molecules. *B. thetaiotaomicron* has eight identified genes that participate in starch metabolism and cleave polysaccharides to glucose and other monosaccharides in the distal intestine. The ultimate end result of this fermentative process is the production of short-chain fatty acids such as propionate, butyrate, and acetate [18]. Short-chain fatty acids account for up to 70% of the caloric requirements of ruminant animals that feed upon cellulose-rich plants [19], but in humans and rodents, they account for much less, on the order of roughly 10% of total caloric needs [20]. In the nonruminant mammals such as human and rodents, the short-chain fatty acids are used as substrate for different organs. For example, butyrate is metabolized by the colonic epithelium [21]. Propionate is transported to the liver, where it is used as a substrate for gluconeogenesis [22], and acetate is largely shunted to adipocytes for lipogenesis [19].

Short-chain fatty acids have also been implicated in the overall health of the gut. In addition to being an energy source for colonic epithelium, it is clear that butyrate is associated with the stimulation of intestinal blood flow as well as colonic epithelial proliferation.

Vitamin synthesis by the gut flora is well-known. Vitamin K is synthesized by several taxa of bacteria, including Bacteroidetes, Eubacterium, Propionibacterium, and Fusobacterium [23]. Bacterial flora has also been implicated in the formation and absorption of certain B vitamins.

## **Current Technology and Characterization of the Gut Microbiota/Microbiome**

To fully understand the impact of the gut microbiome on host physiology, it is essential to document its composition in both health and disease. Until this decade, characterization of the gut microbiota has been limited by the methods of detection. Approximately,  $10^{12}$  bacterial cells exist per gram of feces and anaerobic culture-based techniques have characterized roughly 400–500 different species in the intestinal tract of humans [24]. About 75% of human bacterial flora cannot be cultured by conventional microbiologic analyses that are derived by growing colonies of organisms on the polysaccharide-based agar [25]. Recent technological advances have permitted the unprecedented examination of complex microbial communities using techniques that are culture-independent. These studies have revealed the presence

of approximately 1,000 bacterial species and over 7,000 strains of bacteria in the gut microbiome of mammals.

The use of genomic fingerprinting techniques, such as T-RFLP, DGGE, and TGGE, dependent upon polymerase chain reaction (PCR) amplification of a specific gene product such as 16S rRNA, followed by separation by gel electrophoresis [26]. Clustering of band patterns can then be analyzed using statistical techniques such as principal coordinate analysis (PCoA). Although these studies can be used to determine the stability in the dominant members of a community across as large number of samples, the dynamic range is limited and no information can be obtained that relate banding patterns to changes in particular bacterial taxa.

The use of DNA microarrays to monitor the presence of previously known genes was first described in 1995 [27]. This allowed up to 20,000 genes to be monitored on a single array, expanding the approach of biological research from the study of individual genes to genome-wide study [28]. In the study of microbiota, it has been used to identify pathogens and determine host susceptibility, as well as to profile pathogen gene expression in response to antimicrobial drugs or vaccines. A lack of gold standard for microarray data analysis and a lack of consistency between laboratories or experimental conditions have remained criticisms of this technology [29].

The most robust method utilizes high throughput sequencing technology, such as pyrosequencing, which allows investigators to efficiently obtain large amounts of DNA sequence information efficiently in a cost-effective manner. Sequences of small-subunit ribosome RNA genes, in which 16S rRNA gene sequences (for archaea or bacteria) or 18S rRNA (for eukaryotes), can be used as phylogenetic markers to determine the relative abundance of bacterial taxa in a sample. Various methods can be used to isolate bacterial DNA from samples, followed by amplification of bacterial 16S rDNA using the PCR. The 16S rRNA gene possesses both conserved and hypervariable segments that contain robust taxonomic information. Resulting sequences are grouped into Operational Taxonomic Units (OTUs), which are groups of sequence with identity equal to or greater than a predetermined threshold. For example, 97% identified is often used – reconstruction studies suggest this yields a number similar to the number of different bacterial genera present. OTUs are then aligned and introduced into predetermined phylogenetic trees made with full-length 16S rDNA samples using databases such as Greengenes and NAST [30]. The output trees are used for analysis in UniFrac where pairs of communities are marked on a common phylogenetic tree, and then the fraction of the branch length unique to each community determined. This provides a measure of the distance between communities in terms of their shared evolutionary history. This distance matrix can be used to generate clustering maps using dimensionality reduction by PCoA, a geometric technique that converts a matrix of distances between points in multivariate space into a projection that maximizes the amount of variation along a series of orthogonal axes. In this method, the variance in the data is used to generate axes of maximum variation, then the data are plotted in this coordinate system. In a successful analysis of this type, the axes may be attributable to specific biological phenomena [31]. Such studies have revealed that, of approximately 50 bacterial phyla on Earth, only four are associated with humans with the major bacterial phyla in the human gut being Bacteroidetes, Firmicutes, Proteobacteria, and Actinobacteria [32, 33].

It must be noted, however, that 16S rRNA sequencing is limited by the fact that existing nucleotide sequence databases are biased and incomplete. Differences in detection of species could be due to differences between individuals, but also could be attributed to the biases that exist in PCR-based analysis such as DNA extraction method, PCR primer used, and preferential PCR cloning [34]. Both microarray and 16S rRNA high throughput sequencing are poor for quantification and are limited to relative comparisons unless coupled with extremely carefully controlled experimental conditions [35].

## Metagenomics and Metabolomics

While 16S rDNA phylotyping can be used to characterize the composition of a microbial community, it provides little information as to the functional properties of the microbiome under investigation. To obtain this type of information, metagenomics studies where shotgun sequencing of DNA isolated from a specific sample, can be used to determine the relative abundance of genes represented in a given bacterial community. Here DNA sequences are aligned to databases such as MEGAN to identify the taxonomic origin of the sequences (e.g., bacterial vs. mammalian host) followed by alignment with preexisting known pathways such as Kyoto Encyclopedia of Genes and Genomes (KEGG) and clusters of orthologous groups (COG) to map enzymes onto known gene ontologies [36].

As an example of this analytic approach from the standpoint of metabolic function, a metagenomic analysis of the distal gut microbiota of two human subjects showed similar patterns of enrichment for genes involved in the metabolism of starch, sucrose, glucose, galactose, fructose, arabinose, mannose, and xylose [36]. Eighty-one different glycoside hydrolases were described in the microbiome, most of which are not present in the “glycobiome” of the human genome [36]. The end products of the bacterial “glycobiome” consist of short-chain fatty acids that are then absorbed by the host. Bacterial fermentation leads to the accumulation of  $H_2$ , which can reduce the efficiency of dietary polysaccharide digestion [33], unless a methanogenic archaeon is present to shunt the  $H_2$  towards the production of methane. *Methanobrevibacter brevii* is one of the only members of Archaea that has been cultured from the gut. Genes in the methanogenic pathway were also enriched in the same distal human colon.

A larger-scale comparative metagenomic analysis of fecal samples from 13 healthy Japanese individuals (comprised of adults, weaned children, and unweaned infants) revealed a clear structural difference between the microbiota of unweaned infants and that of adults and weaned children, with the notion that at 1 year of age, the microbiota adopted a more complex but functionally more uniform structure regardless of age or sex [37]. Metagenomic analysis showed striking enrichment of carbohydrate metabolism genes and depletion of genes for the biosynthesis of flagella and chemotaxis. There was a predominance of mobile genetic elements that implicated the distal human gut as a setting for horizontal gene transfer [37]. There were



significant differences between Japanese and American samples, suggesting a genetic or environmental influence upon the microbiota composition. In the future, analytic techniques that directly quantify gene expression through metatranscriptomics (analysis of RNA transcripts within a defined community) or metaproteomics (analysis of proteins) may lead to an additional level of information that can be used to validate the concepts derived from observations obtained through metagenomics. Although currently being used to characterize less complex bacterial communities, they have just begun to be applied to studies involving mammalian-associated bacterial communities [38].

A powerful analytic approach to examine the functional impact of the gut microbiome, inferred through metagenomics, is to correlate such data with those quantifying the presence of a large array of metabolites found in host tissues and/or fluids. The use of proton NMR or mass spectroscopy to obtain this information is known as metabolomics. In this regard, there is strong evidence demonstrating that the gut microbiome influences the metabolic phenotype of the mammalian host and participates in microbial–host cometabolic responses [39]. One example of biochemical interactions between the host and its gut microbiome is the synthesis of bile salts in the liver, their excretion into the gut where they are modified by the gut microbiome, and their subsequent return to the host by enterohepatic circulation or reabsorption in the colon [40]. Not only are these processes important for the homeostasis of lipid metabolism in the host, but bile salt hydrolases also mediate bile tolerance *in vitro* and enhances survival of gut microbial symbionts *in vivo* [40]. Another example is the production of short-chain fatty acids by bacterial fermentation of carbohydrates in the gut and their subsequent absorption by the host where they can be used for lipogenesis and play a role in the development of DIO [41,42]. By performing broad scale untargeted profiling by either mass spectrometry [43] or  $^1\text{H}$  NMR [44] on biofluids collected from conventionally housed and germ-free mice, investigators have identified large numbers of metabolites that are produced by the gut microbiome that then influence the metabolome of the mammalian host. Consistently, amino acid metabolites are among that are most greatly impacted. Interestingly, alterations in diet can have an impact on some of these same metabolites in humans suggesting that dietary alteration of the gut microbiome can alter the host metabolome [45].

As metabolic reactions in nature generally occur in the context of the communities, this approach allows the analysis of microbiota metabolism that is occurring above the organization of a single organism and instead looks at the “superorganism” that is made up of the host and the entire microbial community. As we determine the degree to which the microbiome can harvest additional energy from nutrients and supply them to the host, the value of a food becomes relative and not absolute. Further metagenomic studies, coupled with metabolomics, which characterizes metabolites generated in different physiologic conditions, can be used for further understanding of the origins of obesity and malnutrition, making specific therapeutic recommendations that are dictated by the composition of the gut microbiota and its own energy harvesting capabilities.

Further understanding of metabolomics in the context of the gut microbiome will require the construction of new modeling paradigms that incorporate the different genetic, microbial, metagenomic, and metabolomic data to fully understand the different levels of function and influence [46].

## The Gut Microbiota and Obesity

The prevalence of DIO is reaching epidemic proportions in industrialized nations. In parallel, there has been a dramatic increase in type 2 diabetes mellitus (T2DM). Together, these two related disease processes are an enormous health and financial concern to the U.S. population. There are an estimated 143 million people worldwide with diabetes mellitus, 90% of which have T2DM.

The first law of thermodynamics, which states that the amount of energy stored must equal the difference between energy input and work, is highly relevant to DIO where energy is stored in fat deposits. Finely regulated mechanisms are responsible for maintaining energy balance in mammals. To maintain body mass, energy input (food intake) must match energy expenditure (a combination of physical activity, basal metabolism and adaptive thermogenesis). Thus:

Energy input (feeding) = energy output (physical activity + basal metabolism + adaptive thermogenesis).

Physical activity includes all voluntary movement, basal metabolism is this energy required to maintain biochemical processes necessary to sustain life. By contrast, adaptive thermogenesis refers to the amount of energy expended in response to environmental factors such as cold and alterations in diet. Since triglycerides, stored as fat in white adipose tissue, is the most efficient means of energy storage, alterations in energy balance favoring “energy input” can lead to obesity.

Over the past few years, a research group at Washington University in St. Louis, led by Dr. Jeffrey Gordon, has published a series of seminal reports demonstrating the role of the gut microbiome in the development of obesity in murine systems. These investigators discovered that germ-free mice were comparatively lean with 42% less body fat in comparison to conventionally housed mice despite a 29% increase in food intake [36]. The colonization of germ-free mice with a normal colonic microbiome harvested from conventionally-housed mice led to a dramatic increase in body fat within 10–14 days. This effect of the microbiome on host adipose deposition involved an interplay between an increase in short-chain fatty acid production, intestinal absorption of monosaccharides, and enhanced hepatic lipogenesis.

Using a different model of DIO, the *ob/ob* mouse, the authors show that obesity was associated with a significant alteration in the proportion of the two major phyla in the gut microbiome, with a decrease in Bacteroidetes and a proportional increase in Firmicutes [47]. From a mechanistic standpoint, metagenomic studies demonstrate that this phylotypic alteration enhances the representation of genes involved

in the breakdown of indigestible dietary polysaccharides consistent with an increase in short-chain fatty acid concentrations in the fecal pellets of ob/ob mice. Interestingly, using microbiota transplantation, the authors showed that the obese phenotype was transmissible where germ-free mice that received an “obese microbiome” had significantly greater fat mass than those that received a “lean microbiome.” Similar findings were observed in experiments where DIO was induced through a feeding of a “westernized” diet high in fat and simple sugars [48].

Several experiments have investigated the mechanism by which the microbial environment alters physiology. Short-chain fatty acids act as ligands for a G-protein coupled receptor known as Gpr41. Mice that are null for the Gpr41 gene behave similarly to germ-free mice after conventionalization, gaining less weight and adiposity in comparison to germ-free wild-type mice that have been conventionalized [49]. Another mechanism may involve fasting-induced adipose factor (*Fiaf*), a circulating lipoprotein lipase inhibitor that is inhibited by the presence of gut microbiota. [36] Germ-free mice that lack the gene for *Fiaf* are protected from DIO, demonstrating increased weight gain and intraabdominal adiposity despite similar quantities of food intake [50]. In total, these studies clearly demonstrate the ability of the gut microbiome to augment the development of DIO by enhancing the extraction of energy from the feces through the increased fermentation of indigestible carbohydrates leading to the production of short-chain fatty acids (SCFAs). The subsequent increase in short-chain fatty acid absorption in the colon augments caloric intake favoring an increase in fat deposition in the setting of unchanged energy expenditure.

Although clearly important in murine models, is there evidence for a role of the gut microbiome in the development of DIO in humans? Some intriguing clues exist. Similar to the phylotypic alterations observed in mice, an observational study of the gut composition of 12 obese humans placed upon restricted diets over 1 year showed a relative increase in the abundance of Bacteroidetes and a relative decrease in Firmicutes [41]. In a more recent study, investigators analyzed the gut microbiome composition of obese and lean twins (31 monozygotic twin pairs, 23 dizygotic twin pairs, and 46 mothers). The twins were either concordant for obesity or leanness. The results revealed that each subject’s microbiome varies significantly in composition with a comparable degree of covariation between adult monozygotic and dizygotic twin pairs. Remarkably, there was not a single abundant bacterial species that was shared among all of the subjects in the study, suggesting that there may not be a “Core” gut microbiome in humans [51]. Similar to ob/ob mice, however, obese individuals were found to have a relative decreased proportion of Bacteroidetes species and an increased proportion of Actinobacteria, although there was no significant difference in Firmicutes [51]. Interestingly, metagenomic “shotgun sequencing” and analysis revealed that obesity was associated with altered representation of bacterial genes and metabolic pathways, including those involved with nutrient extraction demonstrating that, in humans, obesity is associated with a core microbiome at a gene/functional and not an organismal level [51].

Despite these important findings, future studies may help to further define the mechanisms by which the composition of the gut microbiota is regulated and its relationship to the obese phenotype. What is the evolutionary advantage of

enhanced energy extraction by the gut microbiome in an obese host? What is the mechanism by which obesity alters the composition of the gut microbiome? What is the stability of the obese-associated gut microbiome throughout time? What is the influence of diet on gut microbiome composition? Preliminary studies with respect to this last question are described in the following section.

## Gut Microbiota and Diet

Early studies examining the effect of broadly-defined diets suggested that the alterations on the gut microbiome were modest involving few genera [52]. However, more recent studies using more sophisticated technology to characterize the composition of the gut microbiome provide clear evidence that this initial impression is not correct. Studies in infants have demonstrated that dietary factors such as breast milk, formula, and solid foods have a significant impact on the composition of the gut microbiome [1, 53, 54]. Furthermore, through the use of a reductionist model system, it has been shown that a single gut commensal (*B. thetaiotaomicron*) in gnotobiotic mice adjusts its pattern of gene expression in order to adapt to alterations in host diet [55]. Importantly, a prototypic high-fat/high-sugar Western diet can reproduce some of these same alterations in C57Bl/6J mice as in the *ob/ob* model of obesity [41], with a significant shift of Bacteroidetes to Firmicutes [48]. This alteration was reversible, but unlike the shifts observed in *ob/ob* mice, the augmentation of Firmicutes induced by a Western diet was not division-wide, but was due to a bloom in a single class of Mollicutes [48]. Since the Western diet in this study also led to an increase in fat mass, the contribution of the host obese phenotype, much like that observed in *ob/ob* mice, to the diet-induced microbiome changes are unknown.

Recent studies provide more compelling evidence for the important role that diet plays in the regulation of gut microbiome composition. First, Ley et al. reported a study in which 16S rRNA sequencing was used to determine the gut microbiome composition in humans and 59 other mammalian species. Their results demonstrate that host diet has a strong influence in bacterial diversity that increases from carnivores to herbivores [56]. In this analysis, clustering by diet was highly significant, eclipsing that of order, fiber index, or gut type, supporting a strong association between gut microbiota composition and diet. Second, fasting for 24 h leads to a significant alteration in the composition of the murine gut microbiome with an increase in Bacteroidetes and a corresponding decrease in the Firmicute phylum [57]. Finally, in gnotobiotic mice colonized with a human gut microbiome, a high-fat/high-sugar “Westernized” diet leads to significant alterations in the composition of the microbiome within 18–20 h before any alteration in host phenotype, namely DIO, occurs [58]. Together with evidence from a murine KO model that reduces fat mass on a high-fat diet [30], these studies demonstrate the importance of diet in the regulation of gut microbiome composition. Although definitive data for the importance of diet in the composition of the human microbiome is currently lacking,

there is some evidence to support this notion. Analysis of major groups of fecal microbes using nine 16S rRNA FISH probes in 19 obese subjects after 4 weeks on different diets (maintenance, medium carbohydrate, and low carbohydrate) revealed significant differences in butyrate-producing bacteria (*Roseburia* and *Eubacterium rectale*) as well as *Bifidobacteria* species, although no significant difference was seen in relative counts of Bacteroidetes [59]. Total amounts of fecal short-chain fatty acids and fecal butyrate decreased as carbohydrate intake decreased [59]. These observations provide a rationale for the hypothesis that dietary interventions used to treat disease can influence the microbiome, and that the changes in the microbiome have, in turn, consequences for host metabolism.

## Connecting the Gut Microbiome, Innate Immunity and Obesity

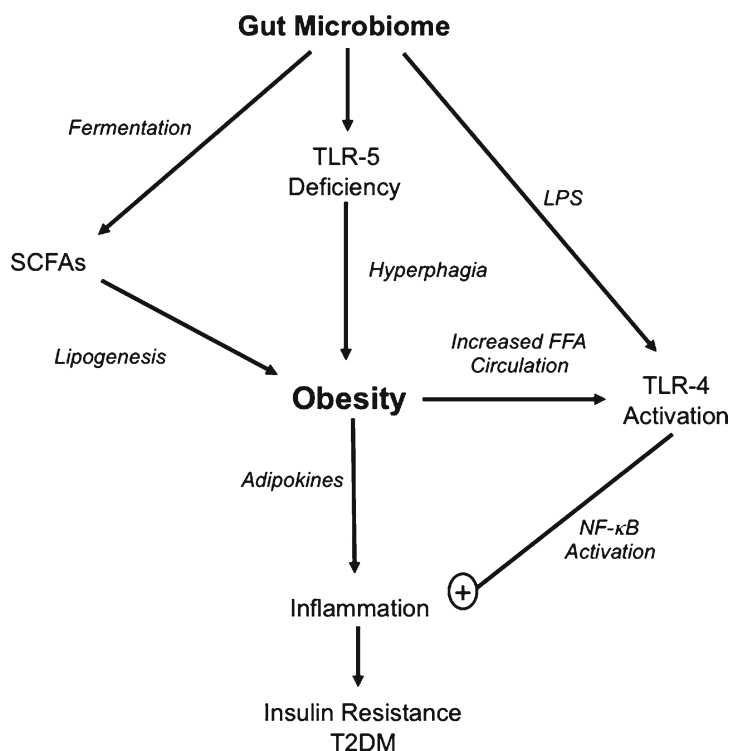
Obesity is the most important factor in the development of insulin resistance, the hallmark of T2DM. There is now compelling evidence that obesity leads to the chronic activation of inflammatory pathways leading to signaling mechanisms that directly inhibit insulin signaling [60, 61]. Indeed, adipose tissue is not only a highly active metabolic tissue, but is also a dynamic endocrine organ capable of producing a wide range of proteins that regulate both metabolism and inflammation. Collectively, these secreted factors are called adipokines. Examples include: leptin, TNF- $\alpha$ , IL-6, resistin, adiponectin, plasminogen activator inhibitor-1, and angiotensinogen, to name a few [62]. A number of these genes are targets of the NF- $\kappa$ B in the development of insulin resistance. For example, heterozygous IKK $\beta^{+/-}$  mice are protected from insulin resistance when fed a high-fat diet or crossed to ob/ob mice, [63] and pharmacologic inhibition with the treatment of salicylates improves insulin resistance in several model systems [63, 64].

Activation of the innate immune system, through ubiquitously-expressed toll-like receptors (TLRs) leads to NF- $\kappa$ B signaling ultimately resulting in an acute inflammatory response. TLRs are a family of type I transmembrane receptors with an extracellular leucine-rich repeat domain and an intracellular Toll/IL-2 receptor (TIR) domain [65]. At least ten TLRs have now been identified, and each has a distinct role in the activation of the innate immune system. Ligands for these receptors number in the dozens, and are extremely diverse in structure and origins. For example, TLR-4 was the first characterized TLR in mammals, and is a signal-transducing receptor for bacterial lipopolysaccharide (LPS) [66] as well as saturated fatty acids [67]. Binding of TLR-4 in conjunction with coreceptors CD14 and MD-2 triggers a downstream signaling cascade that eventually leads to the transcription of proinflammatory genes that encode proinflammatory molecules in a NF- $\kappa$ B dependent manner [68].

Growing evidence demonstrates that the gut microbiome plays a role in the development of insulin resistance by augmenting fat mass, through its observed functional alteration in the setting of a westernized high calorie diet, and via its role in the activation of the innate immune response via TLRs and NF- $\kappa$ B signaling.

These effects may be either direct or indirect (Fig. 1). As described earlier, obesity-associated alterations in the composition of the gut microbiome lead to enhanced energy harvest of the luminal gut contents, resulting in the increased production of short-chain fatty acids that are utilized by the host for lipogenesis. In this manner, the gut microbiome can induce a state of insulin resistance indirectly through its ability to enhance the development of obesity. A second mechanism involves microbiome-dependent activation of TLR-5 [69]. In this recent study, Vijay-Kumar et al. showed that TLR-5<sup>-/-</sup> mice exhibited hyperphagia leading to the development of obesity as well as many features of metabolic syndrome including insulin resistance, hypertension, and hyperlipidemia. Although food restriction prevented the development of obesity in TLR-5<sup>-/-</sup> mice, there was no effect upon insulin resistance. These data suggest that the effect of TLR-5 may modulate insulin resistance by both direct and indirect pathways (Fig. 1). Remarkably, the authors also showed that the transfer of the gut microbiome from TLR-5<sup>-/-</sup> to wild-type germ-free mice was sufficient to establish many features of the metabolic syndrome to the recipients.

TLR-4 has also been implicated in the development of insulin resistance. One possible mechanism, independent of the gut microbiome, involves the direct activation



**Fig. 1** Relationship between the gut microbiome and glucose homeostasis

of TLR-4 by free fatty acids (FFAs), which are often elevated in DIO and have been previously shown to mediate insulin resistance [70]. In vitro, FFAs induce TLR-4 signaling, resulting in activation of NF- $\kappa$ B and the production of proinflammatory cytokines such as TNF- $\alpha$  and IL-6, not only in macrophages, but also in adipocytes. This response occurs both in vitro and in vivo. Importantly, TLR-4 deficiency prevents lipid-induced insulin resistance. Finally, the authors show that TLR-4<sup>-/-</sup> female C57/B6 mice are partially protected from high-fat diet-induced insulin resistance despite an increase in obesity. By contrast, interestingly, two different studies demonstrate that mice with TLR-4 deficiency are actually protected from the development of obesity as well as insulin resistance on a high saturated fat diet [71, 72]. When placed on a high-fat diet, C3H/HeJ (loss-of-function mutation in TLR-4) mice showed decrease weight gain, decreased adiposity, increased metabolic rate, improved glucose tolerance and decreased serum TNF- $\alpha$ , IL-6 and adiponectin levels [71]. 10ScN mice (deletion precluding expression and production of TLR 4) were protected from high-fat diet induced obesity, despite the similar caloric intake as control mice [72]. Ultimately, additional studies will be required to determine whether, like TLR-5, TLR-4 has an indirect effect on insulin resistance by either inducing or inhibiting the development of DIO. Nevertheless, through its activation by FFAs, it seems quite clear that TLR-4 has a direct effect on the development of insulin resistance.

The gut microbiome may also directly augment the development of insulin resistance through the activation of TLR-4 signaling by releasing LPS, a major membrane constituent of gram-negative bacteria, into the systemic circulation [73, 74]. High-fat diets have been reported to chronically increase serum levels of LPS by two or three-fold, possibly by increasing intestinal permeability through the reduced expression of epithelial tight junction proteins [75]. High-fat diet fed mice treated with oral antibiotics reduced body weight gain, fat mass development, expression of systemic inflammatory markers, and glucose intolerance [76]. Similar findings were observed in ob/ob mice treated with oral antibiotics. These effects may be mediated through alterations in TLR-4 signaling, since the authors also demonstrated that CD 14<sup>-/-</sup> (a critical component of the TLR-4 complex) mice bred onto an ob/ob background mimicked the metabolic and antiinflammatory effects of oral antibiotics. Although the effects of antibiotics on glucose homeostasis may be due to an indirect effect mediated by a reduction in obesity through lower levels of short-chain fatty acid production by the gut microbiome, the results in ob/ob CD14<sup>-/-</sup> mice suggest that the activation of TLR-4 by LPS may have a direct impact on insulin resistance.

## Gut Microbiota and Type 1 diabetes

Type I diabetes (T1D) is an autoimmune disease resulting from the destruction of insulin-producing  $\beta$ -cells of the pancreas. Increased incidence over the past decades suggests that environmental and possibly microbial mediated changes may influence



disease development. Wen et al., showed that the incidence of spontaneous T1D in nonobese diabetic (NOD) mice is affected by microbial environment and exposure [77]. Specifically, the effect of MyD88, an adaptor protein that facilitates the ability of ligands to stimulate the inflammatory cascade via a number of TLRs, on the development of T1D in NOD mice was determined in both germ-free and conventionally-housed conditions. NOD mice lacking MyD88 (MyD88<sup>KO</sup> NOD), housed under SPF conditions, fail to develop T1D. Remarkably, germ-free as well as antibiotic treated MyD88<sup>KO</sup> NOD mice developed T1D at higher rates than the same MyD88<sup>KO</sup> mice colonized with specific pathogen free bacteria, suggesting that the presence of normal gut microbiota protects against the development of diabetes [78]. Finally, examination of lymphocytes localized specifically to pancreatic lymph nodes revealed that MyD88 deficiency led to a local tolerance to pancreatic antigens. Thus, in this model system, the gut microbiome induces a state of local tolerance in the pancreas, thereby preventing the development of T1D. Together, these findings provide new insights into mechanisms by which the gut microbiome helps to shape the immunologic response in tissues distinct from those associated with mucosal surfaces. Further characterization of these mechanisms and, perhaps the components of the gut microbiome responsible for these effects, may lead to significant advances in the field of autoimmune disease processes such as T1D.

## Gut Microbiota and Nitrogen Balance

Nitrogen is a crucial constituent of the diet. When body composition is constant, nitrogen intake should be equivalent to losses. In normal growth, recovery of illness, or pregnancy, a positive nitrogen balance is required. Intake of protein, amino acids, and other nitrogenous substances can be calculated from dietary intake. Between 75 and 90% of nitrogen loss from the body are through the urine and feces, primarily in the form of urea, which is produced exclusively in the liver through the urea cycle, entering as ammonia and exiting as urea, which is transported primarily to the kidney for excretion. Urea is also transported into the intestinal lumen, and stool nitrogen accounts for about 9–12% of the total loss [79].

For nearly 60 years, there has been evidence that urinary excretion does not account for all the urea that is produced by the body. Indeed, through either luminal delivery or intestinal secretion, substantial amounts of urea can be found in the colonic lumen. Once in the colonic environment, evidence from germ-free rats and animals treated with antibiotics demonstrate unequivocally that the process of urea hydrolysis, and subsequent nitrogen absorption, is exclusively a function of the gut microbiome [80, 81], with the exception of urea hydrolysis by *H. pylori* in the upper GI tract [82]. Urea hydrolysis in health is primarily a function of the colonic, and perhaps the distal ileal microflora. Indeed, using stable isotope methods, it has been estimated that approximately 15–30% of urea produced by the liver is not excreted in the urine and is hydrolyzed by bacterial urease to ammonia [83]. Previous studies have shown that the concentration of ammonia in the blood draining

the colon was 10 times than that in the inferior vena cava, and that the colonic venous concentration could be reduced by 65% through the administration of oral antibiotics [84]. The ammonia produced in the colon can either be: (1) Absorbed by the host, where it is utilized in the liver for either protein or urea synthesis; (2) Used by the gut bacteria for amino acid and protein synthesis; or (3) Excreted from the body in the feces. Based upon isotopic-labeling studies with  $^{15}\text{N}$ -urea, it has been estimated that 18% of the urea nitrogen enters the urea cycle and is excreted in the urine, approximately 74% enters the metabolic pool of the host, and only 4% is excreted in the stool [85]. Thus, it appears that the vast majority of the nitrogen obtained through urea hydrolysis in the colon is recycled where it is of benefit primarily to the host.

Interestingly, the proportion of colonic nitrogen scavenging through bacterial hydrolysis of urea appears to be proportional to the intake of dietary protein. In humans, when the intake of dietary proteins exceeds 70 grams per day, urea production is 100–120% of intake with about 70% of the urea excreted in the urine and 30% of the nitrogen being salvaged in the colon [86]. The physiologic minimal intake of dietary protein to maintain nitrogen balance in adults is approximately 35 grams. As the intake of protein falls from 70 to 35 grams per day, there is a small but insignificant decrease in urea production of approximately 10% [87]. By contrast, on a 35 grams per day protein diet, 30% of urea produced is excreted with 70% of nitrogen being salvaged. This increase in colonic nitrogen salvage approximately matches the decrease in protein intake. This adaptive metabolic response may be of particular importance in the setting of severe malnutrition [88]. On the other hand, an increase in colonic ammonia absorption may be detrimental to patients with hepatic encephalopathy, chronic renal failure, and inborn errors of the urea cycle.

Urea cycle disorders (UCD) are a group of rare inborn errors of metabolism that commonly present in childhood with episodes of vomiting, lethargy, and coma [89]. Symptoms result from the untoward accumulation of ammonia, a potentially toxic product of protein degradation, which is not adequately metabolized in the liver of affected individuals due to an enzyme deficiency present from birth. Deficiencies in each of the eight enzymes and transporters that comprise the urea cycle have been identified. All are inherited as recessive traits except for the most common disorder, ornithine transcarbamylase deficiency, which is X-linked. The mainstay of treatment is a low-protein diet in order to minimize ammonia production. In recent years, novel approaches to treatment, such as acylation therapy with benzoate and phenylbutyrate, have become commonplace [90]. Unfortunately, even with scrupulous dietary control and diligent therapeutic intervention, the risk of death or severe disability is lamentably high, probably as great as 50% [91]. A major cause of this disappointing outcome is that acute stress, usually a concurrent infection, causes the sudden release of cytokines and adrenal stress hormones that evoke catabolism of body protein and concomitant formation of ammonia in an amount that exceeds the capacity of the congenitally defective urea cycle to detoxify this potentially noxious metabolite. The result is severe hyperammonemia with resultant ataxia, seizures, and coma. Irreparable brain damage frequently ensues, as evidenced by mental retardation, epilepsy, and severe spasticity. Oral antibiotic

therapy, to reduce bacterial hydrolysis of urea and colonic ammonia absorption, has proved very useful as a therapeutic adjunct, especially during a hyperammonemic crisis. This same approach has proven to also be beneficial in patients suffering from hepatic encephalopathy [92].

Although clinically effective, the use of oral antibiotics as an adjunct to a low protein diet in the treatment of patients with UCD and hepatic encephalopathy is currently deployed in a “shotgun” manner. Indeed, given our current lack of knowledge of the gut microbiome, we are unable to “target” those organisms that most robustly hydrolyze urea to ammonia. Future studies that characterize the effect of dietary protein on the taxonomic composition of the gut microbiome as well as metagenomic studies to determine its effects on urease gene representation may provide valuable new insights that may help to more effectively modify populations of gut bacteria that will be of greater benefit in the treatment of patients that are unable to metabolize amino acids appropriately.

## Conclusions and Future Directions

The coevolution between the mammalian host and its microbiome has led to the development of a largely symbiotic relationship. Nowhere is this more clearly demonstrated than the importance of the gut microbiome and its role in host immunologic and metabolic homeostasis. Nevertheless, perhaps due to recent alterations in human society such as dietary intake and other environmental conditions, this symbiotic relationship can become dysfunctional with the gut microbiome playing a role in the pathogenesis diseases such as obesity, diabetes, hepatic encephalopathy, and inflammatory bowel disease. The study of gnotobiotic mice along with recent advances in DNA sequencing technology have provided investigators with an unprecedented opportunity to explore the composition of the gut microbiome and how it may play a role in disease pathogenesis.

Despite these advances, significant questions remain to be addressed. Among these include: What is the relevance of the observations and mechanisms associated with disease pathogenesis, defined in animal models, to human biology? Current evidence suggests that there is no “Core” microbiome in humans. What, then, are the most important determinants of gut microbiome composition in humans? If a dysbiotic gut microbiome plays a role in the pathogenesis of a human disease, are there mechanisms by which its composition can be permanently altered to reduce pathogenicity? What is the effect of diet on the composition of the human gut microbiome and does this have relevance to the pathogenesis of human disease? Are the functional properties of the gut microbiome accurately reflected in the results obtained by studies examining alterations in gene abundance through metagenomics?

A key starting point in addressing some of these issues will be the expanded investigation of the microbiome in humans. This is the major focus of an international research effort known as the Human Microbiome Project [93]. Ultimately, as newer technologies in DNA sequencing technology, metatranscriptomics, metaproteomics,

and metabolomics are developed, together with advances in biocomputational techniques able to extract meaningful relationships from massive amounts of raw data, significant advances in our understanding of the gut microbiome and its role human disease pathogenesis are on the horizon.

## References

1. Savage, D. C. (1977). Microbial ecology of the gastrointestinal tract. *Annual Review of Microbiology*, 31, 107–133.
2. Xu, J., Gordon, J. I. (2003). Inaugural article: Honor thy symbionts. *Proceedings of the National Academy of Sciences of the United States of America*, 100, 10452–10459.
3. Ley, R. E., Lozupone, C. A., Hamady, M., Knight, R., & Gordon, J. I. (2008). Worlds within worlds: evolution of the vertebrate gut microbiota. *Nature Reviews. Microbiology*, 6, 776–788.
4. Hooper, L. V., & Gordon, J. I. (2001). Commensal host-bacterial relationships in the gut. *Science*, 292, 1115–1118.
5. Parsonnet, J., Vandersteen, D., Goates, J., Sibley, R. K., Pritikin, J., Chang, Y. (1991). *Helicobacter pylori* infection in intestinal- and diffuse-type gastric adenocarcinomas. *Journal of the National Cancer Institute*, 83, 640–643.
6. Ott, S. J., Musfeldt, M., Wenderoth, D. F., et al. (2004). Reduction in diversity of the colonic mucosa associated bacterial microflora in patients with active inflammatory bowel disease. *Gut*, 53, 685–693.
7. Seksik, P., Rigottier-Gois, L., Gramet, G., et al. (2003). Alterations of the dominant faecal bacterial groups in patients with Crohn's disease of the colon. *Gut*, 52, 237–242.
8. Dumas, M. E., Barton, R. H., Toye, A., et al. (2006). Metabolic profiling reveals a contribution of gut microbiota to fatty liver phenotype in insulin-resistant mice. *Proceedings of the National Academy of Sciences of the United States of America*, 103, 12511–12516.
9. Shanahan, F. (2007). Irritable bowel syndrome: shifting the focus toward the gut microbiota. *Gastroenterology*, 133, 340–342.
10. Fell, J. M. (2005). Neonatal inflammatory intestinal diseases: necrotising enterocolitis and allergic colitis. *Early Human Development*, 81, 117–122.
11. Cani, P. D., Possemiers, S., Van de Wiele, T., et al. (2009). Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. *Gut*, 58:1091–1103.
12. Wostmann, B. S. (1981). The germfree animal in nutritional studies. *Annual Review of Nutrition*, 1, 257–279.
13. Hooper, L. V., Wong, M. H., Thelin, A., Hansson, L., Falk, P. G., Gordon, J. I. (2001). Molecular analysis of commensal host-microbial relationships in the intestine. *Science*, 291, 881–884.
14. Ivanov, I. I., Atarashi, K., Manel, N., et al. (2009). Induction of intestinal Th17 cells by segmented filamentous bacteria. *Cell*, 139, 485–498.
15. Neish, A. S. (2009). Microbes in gastrointestinal health and disease. *Gastroenterology*, 136, 65–80.
16. Moore, W. E., Cato, E. P., & Holdeman, L. V. (1978). Some current concepts in intestinal bacteriology. *The American Journal of Clinical Nutrition*, 31, S33–42.
17. Xu, J., Bjursell, M. K., Himrod, J., et al. (2003). A genomic view of the human – *Bacteroides thetaiotaomicron* symbiosis. *Science*, 299, 2074–2076.
18. Hooper, L. V., Midtvedt, T., & Gordon, J. I. (2002). How host-microbial interactions shape the nutrient environment of the mammalian intestine. *Annual Review of Nutrition*, 22, 283–307.
19. Bergman, E. N. (1990). Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. *Physiological Reviews*, 70, 567–590.

20. McNeil, N. I. (1984). The contribution of the large intestine to energy supplies in man. *The American Journal of Clinical Nutrition*, 39, 338–342.
21. Roediger, W. E. (1980). Role of anaerobic bacteria in the metabolic welfare of the colonic mucosa in man. *Gut*, 21, 793–798.
22. Bergman, E. N., Roe, W. E., & Kon, K. (1966). Quantitative aspects of propionate metabolism and gluconeogenesis in sheep. *The American Journal of Physiology*, 211, 793–799.
23. Hill, M. J. (1997). Intestinal flora and endogenous vitamin synthesis. *European Journal of Cancer Prevention: the Official Journal of the European Cancer Prevention Organisation (ECP)*, 6, Suppl 1:S43–S45.
24. Moore, W. E., & Holdeman, L. V. (1974). Human fecal flora: the normal flora of 20 Japanese-Hawaiians. *Applied Microbiology*, 27, 961–979.
25. Hayashi, H., Sakamoto, M., & Benno, Y. (2002). Phylogenetic analysis of the human gut microbiota using 16S rDNA clone libraries and strictly anaerobic culture-based methods. *Microbiology and Immunology*, 46, 535–548.
26. Anderson, I. C., & Cairney, J. W. (2004). Diversity and ecology of soil fungal communities: increased understanding through the application of molecular techniques. *Environmental Microbiology*, 6, 769–779.
27. Schena, M., Shalon, D., Davis, R. W., & Brown, P. O. (1995). Quantitative monitoring of gene expression patterns with a complementary DNA microarray. *Science*, 270, 467–470.
28. Coppee, J. Y. (2008). Do DNA microarrays have their future behind them? *Microbes and Infection/Institut Pasteur*, 10, 1067–1071.
29. Tan, P. K., Downey, T. J., Spitznagel, E. L., Jr., et al. (2003). Evaluation of gene expression measurements from commercial microarray platforms. *Nucleic Acids Research*, 31, 5676–5684.
30. Hildebrandt, M. A., Hoffmann, C., Sherrill-Mix, S. A., et al. (2009). High-fat diet determines the composition of the murine gut microbiome independently of obesity. *Gastroenterology*, 137, 1716–1724 e1–e2.
31. McKenna, P., Hoffmann, C., Minkah, N., et al. (2008). The macaque gut microbiome in health, lentiviral infection, and chronic enterocolitis. *PLoS Pathogens*, e20, 4.
32. Backhed, F., Ley, R. E., Sonnenburg, J. L., Peterson, D. A., & Gordon, J. I. (2005). Host-bacterial mutualism in the human intestine. *Science*, 307, 1915–1920.
33. Eckburg, P. B., Bik, E. M., Bernstein, C. N., et al. (2005). Diversity of the human intestinal microbial flora. *Science*, 308, 1635–1638.
34. von Wintzingerode, F., Gobel, U. B., & Stackebrandt, E. (1997). Determination of microbial diversity in environmental samples: pitfalls of PCR-based rRNA analysis. *FEMS Microbiology Reviews*, 21, 213–229.
35. Call, D. R., Borucki, M. K., & Loge, F. J. (2003). Detection of bacterial pathogens in environmental samples using DNA microarrays. *Journal of Microbiological Methods*, 53, 235–243.
36. Backhed, F., Ding, H., Wang, T., et al. (2004). The gut microbiota as an environmental factor that regulates fat storage. *Proceedings of the National Academy of Sciences of the United States of America*, 101, 15718–15723.
37. Kurokawa, K., Itoh, T., Kuwahara, T., et al. (2007). Comparative metagenomics revealed commonly enriched gene sets in human gut microbiomes. *DNA Research: An International Journal for Rapid Publication of Reports on Genes and Genomes*, 14, 169–181.
38. Verberkmoes, N. C., Russell, A. L., Shah, M., et al. (2009). Shotgun metaproteomics of the human distal gut microbiota. *ISME Journal*, 3, 179–189.
39. Martin, F. P., Dumas, M. E., Wang, Y., et al. (2007). A top-down systems biology view of microbiome-mammalian metabolic interactions in a mouse model. *Molecular Systems Biology*, 3, 112.
40. Jones, B. V., Begley, M., Hill, C., Gahan, C. G., & Marchesi, J. R. (2008). Functional and comparative metagenomic analysis of bile salt hydrolase activity in the human gut microbiome. *Proceedings of the National Academy of Sciences of the United States of America*, 105, 13580–13585.
41. Ley, R. E., Turnbaugh, P. J., Klein, S., & Gordon, J. I. (2006). Microbial ecology: human gut microbes associated with obesity. *Nature*, 444, 1022–1023.

42. Turnbaugh, P. J., Ley, R. E., Mahowald, M. A., Magrini, V., Mardis, E. R., & Gordon, J. I. (2006). An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature*, 444, 1027–1031.
43. Wikoff, W. R., Anfora, A. T., Liu, J., et al. (2009). Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites. *Proceedings of the National Academy of Sciences of the United States of America*, 106, 3698–3703.
44. Claus, S. P., Tsang, T. M., Wang, Y., et al. (2008). Systemic multicompartmental effects of the gut microbiome on mouse metabolic phenotypes. *Molecular Systems Biology*, 4, 219.
45. Stella, C., Beckwith-Hall, B., Cloarec, O., et al. (2006). Susceptibility of human metabolic phenotypes to dietary modulation. *Journal of Proteome Research*, 5, 2780–2788.
46. Nicholson, J. K., & Wilson, I. D. (2003). Opinion: understanding ‘global’ systems biology: metabonomics and the continuum of metabolism. *Nature reviews. Drug discovery*, 2, 668–676.
47. Ley, R. E., Backhed, F., Turnbaugh, P., Lozupone, C. A., Knight, R. D., & Gordon, J. I. (2005). Obesity alters gut microbial ecology. *Proceedings of the National Academy of Sciences of the United States of America*, 102, 11070–11075.
48. Turnbaugh, P. J., Backhed, F., Fulton, L., & Gordon, J. I. (2008). Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome. *Cell Host Microbe*, 3, 213–223.
49. Samuel, B. S., Shaito, A., Motoike, T., et al. (2008). Effects of the gut microbiota on host adiposity are modulated by the short-chain fatty-acid binding G protein-coupled receptor, Gpr41. *Proceedings of the National Academy of Sciences of the United States of America*, 105, 16767–16772.
50. Backhed, F., Manchester, J. K., Semenkovich, C. F., & Gordon, J. I. (2007). Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. *Proceedings of the National Academy of Sciences of the United States of America*, 104, 979–984.
51. Turnbaugh, P. J., Hamady, M., Yatsunenko, T., et al. (2009). A core gut microbiome in obese and lean twins. *Nature*, 457, 480–484.
52. Dethlefsen, L., Eckburg, P. B., Bik, E. M., & Relman, D. A. (2006). Assembly of the human intestinal microbiota. *Trends in Ecology & Evolution (Personal Edition)*, 21, 517–523.
53. Harmsen, H. J., Wildeboer-Veloo, A. C., Raangs, G. C., et al. (2000). Analysis of intestinal flora development in breast-fed and formula-fed infants by using molecular identification and detection methods. *Journal of Pediatric Gastroenterology and Nutrition*, 30, 61–67.
54. Favier, C. F., Vaughan, E. E., De Vos, W. M., & Akkermans, A. D. (2002). Molecular monitoring of succession of bacterial communities in human neonates. *Applied and Environmental Microbiology*, 68, 219–226.
55. Sonnenburg, J. L., Xu, J., Leip, D. D., et al. (2005). Glycan foraging in vivo by an intestine-adapted bacterial symbiont. *Science*, 307, 1955–1959.
56. Ley, R. E., Hamady, M., Lozupone, C., et al. (2008). Evolution of mammals and their gut microbes. *Science*, 320, 1647–1651.
57. Crawford, P. A., Crowley, J. R., Sambandam, N., et al. (2009). Regulation of myocardial ketone body metabolism by the gut microbiota during nutrient deprivation. *Proceedings of the National Academy of Sciences of the United States of America*, 106, 11276–11281.
58. Turnbaugh, P. J., Ridaura, V. K., Faith, J. J., Rey, F. E., Knight, R., & Gordon, J. I. (2009). The effect of diet on the human gut microbiome: a metagenomic analysis in humanized gnotobiotic mice. *Science Translational Medicine*, 1, 6ra14.
59. Duncan, S. H., Belenguer, A., Holtrop, G., Johnstone, A. M., Flint, H. J., & Lobley, G. E. (2007). Reduced dietary intake of carbohydrates by obese subjects results in decreased concentrations of butyrate and butyrate-producing bacteria in feces. *Applied and Environmental Microbiology*, 73, 1073–1078.
60. Ahima, R. S., & Flier, J. S. (2000). Adipose tissue as an endocrine organ. *Trends in Endocrinology and Metabolism: TEM*, 11, 327–332.
61. Yudkin, J. S., Stehouwer, C. D., Emeis, J. J., & Coppack, S. W. (1999). C-reactive protein in healthy subjects: associations with obesity, insulin resistance, and endothelial dysfunction: a



- potential role for cytokines originating from adipose tissue? *Arteriosclerosis, Thrombosis, and Vascular Biology*, 19, 972–978.
62. Lyon, C. J., Law, R. E., & Hsueh, W. A. (2003). Minireview: adiposity, inflammation, and atherogenesis. *Endocrinology*, 144, 2195–2200.
63. Yuan, M., Konstantopoulos, N., Lee, J., et al. (2001). Reversal of obesity- and diet-induced insulin resistance with salicylates or targeted disruption of Ikkbeta. *Science*, 293, 1673–1677.
64. Hundal, R. S., Petersen, K. F., Mayerson, A. B., et al. (2002). Mechanism by which high-dose aspirin improves glucose metabolism in type 2 diabetes. *The Journal of Clinical Investigation*, 109, 1321–1326.
65. Medzhitov, R. (2001). Toll-like receptors and innate immunity. *Nature Reviews. Immunology*, 1, 135–145.
66. Poltorak, A., He, X., Smirnova, I., et al. (1998). Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. *Science*, 282, 2085–2088.
67. Lee, J. Y., Ye, J., Gao, Z., et al. (2003). Reciprocal modulation of Toll-like receptor-4 signaling pathways involving MyD88 and phosphatidylinositol 3-kinase/AKT by saturated and polyunsaturated fatty acids. *The Journal of Biological Chemistry*, 278, 37041–37051.
68. Zuany-Amorim, C., Hastewell, J., & Walker, C. (2002). Toll-like receptors as potential therapeutic targets for multiple diseases. *Nature Reviews. Drug discovery*, 1, 797–807.
69. Vijay-Kumar, M., Aitken, J. D., Carvalho, F. A., et al. As per style specification (APA), Please provide six author names and et al. in the reference list.. Metabolic syndrome and altered gut microbiota in mice lacking Toll-like receptor 5. *Science*, 328, 228–231.
70. Shi, H., Kokoeva, M. V., Inouye, K., Tzameli, I., Yin, H., & Flier, J. S. (2006). TLR4 links innate immunity and fatty acid-induced insulin resistance. *The Journal of Clinical Investigation*, 116, 3015–3025.
71. Tsukumo, D. M., Carvalho-Filho, M. A., Carvalheira, J. B., et al. (2007). Loss-of-function mutation in Toll-like receptor 4 prevents diet-induced obesity and insulin resistance. *Diabetes*, 56, 1986–1998.
72. Davis, J. E., Gabler, N. K., Walker-Daniels, J., & Spurlock, M. E. (2008). Tlr-4 deficiency selectively protects against obesity induced by diets high in saturated fat. *Obesity (Silver Spring)*, 16, 1248–1255.
73. Wright, S. D., Ramos, R. A., Tobias, P. S., Ulevitch, R. J., & Mathison, J. C. (1990). CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein. *Science*, 249, 1431–1433.
74. Neal, M. D., Leaphart, C., Levy, R., et al. (2006). Enterocyte TLR4 mediates phagocytosis and translocation of bacteria across the intestinal barrier. *Journal of Immunology (Baltimore, Md.: 1950)*, 176, 3070–3079.
75. Cani, P. D., Amar, J., Iglesias, M. A., et al. (2007). Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes*, 56, 1761–1772.
76. Cani, P. D., Bibiloni, R., Knauf, C., et al. (2008). Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. *Diabetes*, 57, 1470–1481.
77. Pozzilli, P., Signore, A., Williams, A. J., & Beales, P. E. (1993). NOD mouse colonies around the world—recent facts and figures. *Immunology Today*, 14, 193–196.
78. Wen, L., Ley, R. E., Volchkov, P. Y., et al. (2008). Innate immunity and intestinal microbiota in the development of Type 1 diabetes. *Nature*, 455, 1109–1113.
79. McGilvery, R. (1970). *Biochemistry: A functional approach*. Philadelphia: Saunders.
80. Dintzis, R. Z., & Hastings, A. B. (1953). The effect of antibiotics on urea breakdown in mice. *Proceedings of the National Academy of Sciences of the United States of America*, 39, 571–578.
81. Levenson, S. M., Crowley, L. V., Horowitz, R. E., & Malm, O. J. (1959). The metabolism of carbon-labeled urea in the germ free rat. *The Journal of Biological Chemistry*, 234, 2061–2062.



82. Graham, D. Y., Klein, P. D., Evans, D. J., Jr., et al. (1987). *Campylobacter pylori* detected noninvasively by the  $^{13}\text{C}$ -urea breath test. *Lancet*, *1*, 1174–1177.
83. Walser, M., & Bodenlos, L. J. (1959). Urea metabolism in man. *The Journal of Clinical Investigation*, *38*, 1617–1626.
84. Silen, W., Harper, H. A., Mawdsley, D. L., & Weirich, W. L. (1955). Effect of antibacterial agents on ammonia production within the intestine. *Proceedings of the Society for Experimental Biology and Medicine. Society for Experimental Biology and Medicine*(New York, N.Y.), *88*, 138–140.
85. Moran, B. J., & Jackson, A. A. (1990).  $^{15}\text{N}$ -urea metabolism in the functioning human colon: luminal hydrolysis and mucosal permeability. *Gut*, *31*, 454–457.
86. Jackson, A. A., Danielsen, M. S., & Boyes, S. (1993). A noninvasive method for measuring urea kinetics with a single dose of  $^{15}\text{N}$ -urea in free-living humans. *The Journal of Nutrition*, *123*, 2129–2136.
87. Langran, M., Moran, B. J., Murphy, J. L., & Jackson, A. A. (1992). Adaptation to a diet low in protein: effect of complex carbohydrate upon urea kinetics in normal man. *Clinical Science (Lond)*, *82*, 191–198.
88. Picou, D., & Phillips, M. (1972). A study with  $^{15}\text{N}$ -urea on the effects of a low protein diet and malnutrition on urea metabolism in children. *Clinical Science*, *43*, 17.
89. Brusilow, S. W., & Maestri, N. E. (1996). Urea cycle disorders: diagnosis, pathophysiology, and therapy. *Advances in Pediatrics*, *43*, 127–170.
90. Batshaw, M. L., MacArthur, R. B., & Tuchman, M. (2001). Alternative pathway therapy for urea cycle disorders: twenty years later. *The Journal of Pediatrics*, *138*, S46–S54; discussion S-5.
91. Maestri, N. E., Clissold, D., & Brusilow, S. W. (1999). Neonatal onset ornithine transcarbamylase deficiency: A retrospective analysis. *The Journal of Pediatrics*, *134*, 268–272.
92. MacLayton, D. O., & Eaton-Maxwell, A. (2009). Rifaximin for treatment of hepatic encephalopathy. *The Annals of Pharmacotherapy*, *43*, 77–84.
93. Turnbaugh, P. J., Ley, R. E., Hamady, M., Fraser-Liggett, C. M., Knight, R., & Gordon, J. I. (2007). The human microbiome project. *Nature*, *449*, 804–810.

# Chapter 17

## Impact of Obesity on Female Reproductive Health

Moshood O. Olatinwo, Djana Harp, Winston Thompson,  
Hyeong-Kyu Park, and Roland Mathews

### Introduction

In the USA, more than 70% of the adult population is either overweight (BMI >25) or obese (BMI >30). An estimated 73% of the adult population were overweight or obese in 2008, reflecting the continued and steady increase in body weight over time. Indeed, nearly 50% of women in the USA aged 15–49 are overweight or obese [1]. Several well known medical complications are associated with obesity, including the risk of atherosclerosis, cardiovascular disease, hypertension, diabetes, and cancer. Obesity is caused by an imbalance between energy intake and energy expenditure. The proximate cause of obesity is the excessive accumulation of white adipose tissue; the major form of energy reserve in the body.

Obesity poses unique reproductive health risks in women. Obesity and excessive weight gain in pregnancy are associated with maternal and fetal complications of pregnancy, including preterm labor, gestational diabetes, preeclampsia, operative delivery, fetal macrosomia, and birth defects [2–7]. Additionally, childhood obesity in girls is associated with early onset of puberty, menstrual irregularities during adolescence and polycystic ovary syndrome (PCOS) [8, 9]. Obesity can increase the risk of anovulation and may also decrease ovulatory response to fertility treatment [10]. Moreover, the efficacy and safety of hormonal contraceptives can be severely compromised by increased body weight [11]. Conversely, weight reduction enhances reproductive outcomes and diminishes obesity-related maternal, perinatal, cardiovascular, and cancer risks [12–15]. The fact that most of the obesity-associated deleterious effect on female fertility is ameliorated by bariatric surgery strongly suggests a causative role for obesity in the development of these adverse effects on reproduction. Although several published reports have shown that maternal obesity is associated with increased morbidity and mortality for both mother and offspring, the mechanisms underlying the

---

M.O. Olatinwo(✉)

Department of Obstetrics and Gynecology, Morehouse School of Medicine, Atlanta, GA, USA  
and

Summit ObGyn, 655 Bienville Circle, Natchitoches, LA 71457, USA

e-mail: molatinwo@msn.com

increased reproductive health risk associated with maternal obesity are not well understood. In this chapter, we aim to discuss the impact of obesity on female reproduction with a focus on the effect of leptin on fertility problems in obese women. Finally, we comment on the possible cross talk in the molecular and cellular pathways utilized by leptin and insulin to alter reproductive processes in obese women with PCOS.

## Energy Homeostasis and Reproduction

In humans, reproductive function declines at both extremes of energy balance. The possibility of a factor secreted by white adipose tissue that reflects the level of total body energy stores and possibly regulates hypothalamic control of feeding was first proposed by Kennedy [16, 17] and is supported by classic cross-circulation (parabiosis) experiments in rodents [18, 19]. This was later confirmed by the discovery of leptin by positional cloning in 1994 [20]. In humans, the amount of body fat is the principal determinant of circulating levels of leptin [21]. Leptin is secreted in pulses that are positively correlated to gonadotropins, estradiol, and thyrotropin [22]. Leptin secretion in women has a diurnal pattern characterized by a nocturnal rise that synchronizes with a similar rise in gonadotropins and estradiol [23], suggesting a functional link between leptin and the hypothalamic–pituitary–ovarian axis.

*Leptin and reproduction.* Leptin is a major regulator of food intake and energy homeostasis. In addition, leptin relays information to the brain about the adequacy of peripheral energy stores to sustain reproduction. When energy stores are adequate, leptin suppresses feeding behavior and permits neuroendocrine functions that facilitate energy expenditure [24–26]. Conversely, a lower level of serum leptin reflecting inadequate energy stores elicits adaptive responses by diverting energy resources away from energy demanding processes, such as reproduction to those functions that are essential for the survival of the organism [27, 28]. This is reflected in clinical conditions associated with hypoleptinemia. For instance, a congenital absence of leptin as seen in individuals with homozygous mutations in the gene coding for leptin is associated with infertility in humans and in mice [29–32]. Infertility also characterizes acquired conditions associated with low levels of circulating leptin such as exercise-induced amenorrhea, functional hypothalamic amenorrhea, and anorexia nervosa [33, 34]. Leptin treatment has been shown to restore fertility in adult humans with congenital or acquired leptin deficiency [33, 34]. However, the precise mechanisms by which leptin acts to normalize reproductive function have not been fully defined.

*Leptin and the hypothalamic–pituitary–gonadal axis.* Previous studies suggest that leptin acts at the hypothalamic level to stimulate the release of gonadotropin releasing hormone (GnRH) and consequently the release of luteinizing hormone (LH) and follicle stimulating hormone (FSH) from the pituitary [35, 36]. More recently, studies from our laboratory [37, 38] and others [39–42] have suggested that leptin has distinct local effects on the ovary independent of its action on the hypothalamic–pituitary axis. These effects appear to be bimodal in nature. At physiological

concentrations, leptin increased estrogen production from human luteinized granulosa cells [42], and both estradiol and progesterone production from in vitro cultures of preantral mouse follicles [40]. Leptin has also been shown to enhance nuclear and cytoplasmic maturation of the oocyte and to induce ovulation via an LH-independent pathway in the mouse [39]. In contrast to supraphysiological concentrations, leptin inhibited ovarian steroidogenesis. For example, leptin at a concentration of 10 ng/ml increased the in vitro production of progesterone from porcine granulosa cells in vitro, but inhibited progesterone production at 1,000 ng/ml [43]. Further evidence that leptin may have a direct effect on the ovary was provided by a recent clinical study in which recombinant human leptin was used to treat hypoleptinemic lipodystrophic patients for a period of 10 months. Regular menses were restored in eight of ten women with primary amenorrhea or irregular menses [33], but this treatment was without any significant effect on serum levels of LH or FSH. This suggests that the therapeutic effect of leptin in women with lipodystrophic atrophy may be mediated directly at the level of the ovary or at least via a mechanism that does not alter circulating levels of gonadotropins. We have previously shown that the impaired folliculogenesis in leptin-deficient mice is associated with a subnormal number of follicles, a higher level of granulosa cell apoptosis, and an increased number of atretic follicles [37]. Subsequently, we compared the effect of gonadotropin treatment on ovarian follicle development in prepubertal leptin-deficient mice to age-matched controls [38]. The ovarian responsiveness to gonadotropin administration appeared to be subnormal in the leptin-deficient mice (fewer follicles developed to the late antral stage, and animals failed to ovulate), suggesting that leptin may directly alter the process of folliculogenesis independent of its action in hypothalamic-pituitary axis.

These data and others further suggest that leptin may be necessary to maintain a normal ovarian responsiveness to gonadotropins. Perhaps leptin signaling in the ovary may be required to maintain a normal expression levels of FSH and LH receptors, or in the absence of leptin signaling, the affinity of these receptors for their ligands may be reduced. One of the commonest reproductive disorders associated with obesity in women is PCOS. PCOS is characterized by excessive ovarian androgen production, anovulation, and polycystic ovaries (PCO). Obesity may exacerbate PCOS and conversely, PCOS may worsen the degree of obesity. In the next few sections of this chapter, we will use PCOS as our disease entity to explore the complex relationship between obesity and reproductive health in women.

## Pathogenesis of Polycystic Ovary Syndrome

A large proportion, 60–80%, of women with PCOS have high concentrations of circulating testosterone, and about 25% have high concentrations of dehydroepiandrosterone sulfate (DHEAS), leading some investigators to surmise that uncontrolled steroidogenesis might be the primary abnormality in this disorder [44, 45]. PCO have a thickened thecal layer, and thecal cells derived from these ovaries

secrete excessive androgens in vitro under basal conditions or in response to LH stimulation [44, 45]. The excessive androgen production that characterizes the polycystic ovary may result from the very high ratio of LH to FSH levels or it may be due to an intrinsic abnormality of the ovary in PCOS.

LH and FSH are synthesized and secreted by gonadotropes in the anterior pituitary gland. Each of these hormones is comprised of a heterodimer of a common  $\alpha$ -subunit non-covalently bound to a unique  $\beta$ -subunit. The  $\alpha$ -subunit is encoded by a single gene, whereas the unique  $\beta$ -subunits arise from separate genes and confer biological specificity. The pulsatile secretion of GnRH from the hypothalamus is essential for determining the relative proportion of LH and FSH synthesized within the gonadotrope. Consequently, an increased pulse frequency of hypothalamic GnRH favors transcription of the  $\beta$ -subunit of LH over the  $\beta$ -subunit of FSH; conversely, decreased pulse frequency of GnRH favors transcription of the  $\beta$ -subunit of FSH, which decreases the ratio of LH to FSH. Several previous studies have shown that women with PCOS display unusual patterns of gonadotropin secretion characterized by excessive secretion of LH but normal secretion of FSH. This pattern of secretion gives rise to an abnormally high ratio of circulating LH to FSH in the serum of some patients with PCOS [46–49]. Whereas LH regulates the androgenic synthesis of theca cells, FSH is responsible for regulating the aromatase activity of granulosa cells, thereby determining how much estrogen is synthesized from androgenic precursors. When the concentration of LH increases relative to that of FSH, the ovaries preferentially synthesize androgens.

Because women with PCOS appear to have an increased LH pulse frequency, it has been inferred that the pulse frequency of GnRH must be accelerated in the syndrome. It is not clear whether this accelerated pulse frequency is due to an intrinsic abnormality in the GnRH pulse generator or caused by the relatively low levels of progesterone resulting from infrequent ovulatory events. Since progestins decrease the GnRH pulse frequency, low circulating progestin levels in women with PCOS may lead to acceleration in the pulsatility of GnRH, increased levels of LH, and overproduction of ovarian androgens. The relative increase in pituitary secretion of LH leads to an increase in androgen production by ovarian theca cells. Increased efficiency in the conversion of androgenic precursors in theca cells leads to enhanced production of androstenedione, which is then converted by 17 $\beta$ -hydroxysteroid dehydrogenase (17 $\beta$ -HSD) to form testosterone or aromatized by the aromatase enzyme to form estrone. Within the granulosa cell, estrone is then converted into estradiol by 17 $\beta$ -HSD. The adipose tissue is a major endocrine organ that secretes adipocytokines with autocrine, paracrine, and endocrine actions that may modulate the effects of both LH and insulin on ovarian androgen production.

*PCOS and obesity.* PCOS affects 5–10% of women in their reproductive years and a substantial proportion of these patients are obese, have profound peripheral insulin resistance with consequent hyperinsulinemia from enhanced basal insulin secretion and decreased hepatic insulin extraction [50, 51]. Although anovulatory infertility is common among women with PCOS, the cause of ovulatory dysfunction in these women is poorly understood. Insulin resistance and pancreatic  $\beta$ -cell dysfunction, both independent of obesity and unrelated to the actions of androgens

on insulin dynamics, are strongly associated with PCOS [52–57]. Insulin interacts synergistically with LH within the theca cells of PCO (in which theca cell hyperplasia is usually present) to cause activation of the enzyme, P450c17 $\alpha$ , the key enzyme in the biosynthesis of ovarian androgens such as testosterone. Hyperinsulinemia may also have adverse effects in women with PCOS through its action at non-ovarian sites. These include the pituitary through enhancement of pituitary LH pulse amplitude, the liver through suppression of hepatic synthesis of steroid hormone binding-globulin (SHBG) and the adrenal gland through stimulation of P450c17 $\alpha$  activity, thereby increasing adrenal androgen production.

Metformin, an insulin-sensitizing agent, has been used for the treatment of PCOS in adult women. Metformin acts by decreasing hepatic glucose production and increasing peripheral insulin sensitivity. Studies in lean and overweight adult women have demonstrated beneficial effects of Metformin, particularly in the prevention of type 2 diabetes. Metformin lowers androgen levels, improves lipid profiles, and increases insulin sensitivity. It may therefore delay or prevent metabolic syndrome and cardiovascular morbidity in the long-term. Metformin appears to be safe in the short term and is generally well tolerated. In addition, several small, short term studies have demonstrated safety and beneficial effects of metformin in girls with PCOS [58, 59]. Although metformin is effective in treating the insulin resistance and the consequent metabolic complications of PCOS, it is less effective in the treatment of anovulation in women with PCOS [58]. This suggests that other factors probably in conjunction with insulin may be involved in the pathogenesis of the ovulatory defect in PCOS.

Currently, the mechanism by which hyperinsulinemia is related to the altered ovarian steroidogenesis in the presence of peripheral insulin resistance that is characteristic of PCOS is unknown. However, the insulin resistance associated with PCOS appears to be tissue selective and has been reported in muscle, adipose tissue and the liver, but not in the ovary [52, 53]. Perhaps, in women with PCOS, the ovary maintains normal sensitivity to insulin raising the possibility that the hyperinsulinemia associated with this disorder directly augments ovarian response to the gonadotropins. Previous studies have shown that insulin acting through its cognate receptors promotes hyperandrogenemia and exacerbates the severity of PCOS by facilitating ovarian and adrenal steroidogenesis and decreasing hepatic production of SHBG thereby increasing free androgen levels [47, 50]. Insulin also inhibits hepatic synthesis of SHBG and thereby increases free or bio-available testosterone levels [60]. Furthermore, insulin decreases hepatic and ovarian synthesis of insulin-like binding protein-1 (IGFBP-1), which promotes insulin-like growth factor 1 (IGF-1) actions, such as stimulation of ovarian androgen production [60]. Therefore, hyper-insulinemia contributes to hyperandrogenism and ovarian dysfunction in women with PCOS. A further adverse effect of hyperinsulinemia on the ovary in women with PCOS includes the arrest of ovarian follicle development at 5–10 mm, well before a mature follicle would be expected to ovulate [60] thereby contributing towards anovulation. The arrest of follicular maturation in these women is reminiscent of the follicular response that we recently described in female leptin-deficient mice following gonadotropin stimulation [38]. These findings suggest a functional link

between insulin and leptin-signaling pathways that may be operational in the ovaries of women with PCOS.

*Cross talk between leptin and insulin-signaling pathways in PCOS.* The JAK-2/STAT-3 pathway is the major signaling mechanism activated by leptin in the hypothalamus to regulate energy and metabolic homeostasis. This pathway is not essential for regulating reproductive function [61–64]. This notion is supported by the findings from a recent study showing that although leptin activation of the STAT-3 signaling pathway is critical in the regulation of body weight and some neuroendocrine functions (thyroid, adrenal, and lactation), hypothalamic STAT-3 signaling is not essential for maintaining reproductive function [65]. This suggests that leptin may regulate reproduction through a STAT-3-independent signaling pathway. A possible alternative to STAT-3 system for leptin signaling is the mitogen-activated protein kinase (MAPK) signaling cascade. The extracellular regulated kinase (ERK) members of the MAPK family are serine/threonine kinases that are activated by a wide range of stimuli and are components of the well-defined Ras/MAPK signaling cascade [66].

Previous studies have shown that when insulin levels are reduced by hypocaloric diet or treatment with insulin sensitizers, the reproductive and metabolic outcomes in obese women with PCOS remarkably improves even before significant weight loss is observed [12]. Although the mechanism underlying this improvement in reproductive profile is unclear, it may involve the MAPK signaling pathway [67]. The MAPKs are mediators of signal transduction from the cytosol to the nucleus. Previous studies have shown that alternative signaling pathways, including the MAPK and protein kinase B (Akt) pathways are associated with LH-induced changes in steroid biosynthesis. This has been corroborated by a more recent study that compared androgen biosynthesis in propagated normal and PCOS theca cells via MAPK kinase (MEK1/2) and ERK1/2 phosphorylation. That study revealed that MEK1/2 phosphorylation was decreased more than 70%, and ERK1/2 phosphorylation was reduced 50% in PCOS cells as compared with normal cells. This suggests a causative role for alterations in the MAPK pathway in the pathogenesis of excessive ovarian androgen production in PCOS. This pathway may be particularly relevant to the reproductive consequences of obesity because an alteration in the MAPK pathway has also been implicated in excessive ovarian androgen production associated with leptin in oocytes [67].

The MAPK signaling pathway is a possible point of cross-talk between leptin and insulin signaling. MAPK signaling can be activated via the long or the short leptin receptor isoforms. The short form of the leptin receptor which is the dominant form expressed in the oocyte is capable of activating MAPK signaling in white adipose tissue, monocytes, and osteoblast precursor cells [68–70]. In a recent study, leptin treatment increased phosphorylated MAPK content and enhanced oocyte maturation at all stages of follicular development [40]. Significantly, this leptin-stimulated oocyte maturation was inhibited by MAPK inhibitor, suggesting that leptin enhances oocyte maturation via activation of the MAPK pathway.



Another potential point of cross talk between leptin and insulin involves insulin signaling. Leptin has been reported to activate some components of insulin signaling, including insulin receptor substrate-1 (IRS-1) and insulin receptor substrate-2 (IRS-2) [71]. One of the major downstream pathways activated by IRS proteins involves recruitment of the regulatory subunit of PI3K to tyrosine-phosphorylated IRS proteins, leading to subsequent activation of the lipid kinase and the phosphorylation of phosphatidylinositol 4,5-bisphosphate to phosphatidylinositol 3,4,5-trisphosphate (PIP<sub>3</sub>) [72–76]. Compelling evidence suggests that members of PI3K family control cell cycle progression, differentiation, and survival [77]. Several biological effects of PI3K are mediated through the activation of the downstream target Akt [78]. Akt, a serine/threonine protein kinase, also known as protein kinase B (PKB), is the mammalian homolog of the transforming viral oncogene *v-Akt*. Akt is recruited to the plasma membrane by the products of PI3K in response to a variety of stimuli (hormones, growth factors, and cytokines). Then, Akt is phosphorylated at threonine 308 and at serine 473 by a still undefined kinase [79, 80]. This double phosphorylation activates Akt. The significant role of this system in granulosa cell differentiation was recently demonstrated by Zeleznik et al. [81]. They found that when adenovirus vectors were employed to modulate specific intracellular signaling systems in undifferentiated granulosa cells, the dominant active PKB vector amplified FSH-induced aromatase and LH receptor mRNA levels. Conversely, the dominant negative PKB vector completely abolished the actions of FSH suggesting that PKB is an essential component of the FSH-mediated granulosa cell differentiation.

## Conclusions

Clinical and experimental data support the concept that leptin plays a major role in regulating reproductive function. Although previous studies suggest that one target site for leptin's action on reproductive processes is the hypothalamus (via the regulation of gonadotropin secretion) studies from our laboratory and others suggest that leptin may also act directly on the ovary as well. Based on these findings, we hypothesize that leptin is essential for maintaining the normal response of ovarian follicles to FSH and LH signaling. In the absence of leptin, folliculogenesis is compromised. Obese patients have elevated serum leptin levels but may exhibit leptin resistance so that some biological effects of leptin are compromised. These individuals are predisposed to PCOS and metabolic syndrome. A systematic study of the molecular pathways involved in leptin signaling in the ovary is essential to define the role of leptin as a causative factor in the altered sex hormone profiles and amenorrhea seen in obese women. Specifically, studies designed to unravel the complex interactions between hyperleptinemia, hyperinsulinemia, and hyperandrogenemia in PCOS not only elucidates the intricate pathophysiology, but may also potentially lead to the development of novel therapeutic approaches for the treatment of obesity-related reproductive disorders.

## References

1. U.S. obesity trends by 1985–2008. <http://www.cdc.gov/obesity/data/trends> Accessed April 10, 2010.
2. Biggio, J. R., Jr., Chapman, V., Neely, C., Cliver, S. P., Rouse, D. J., et al. (2010). Fetal anomalies in obese women: The contribution of diabetes. *Obstetrics and Gynecology*, 115(2 Pt 1), 290–296.
3. Brown, K., Apuzzio, J., & Weiss, G. (2010). Maternal obesity and associated reproductive consequences. *Women's Health (London, England)*, 6(2), 197–203.
4. Madan, J., Chen, M., Goodman, E., Davis, J., Allan, W., & Dammann, O. (2010). Maternal obesity, gestational hypertension, and preterm delivery. *The Journal of Maternal-Fetal & Neonatal Medicine*, 23(1), 82–88.
5. Nohr, E. A., Timpson, N. J., Andersen, C. S., Davey Smith, G., Olsen, J., & Sorensen, T. I. (2009). Severe obesity in young women and reproductive health: The Danish National Birth Cohort. *PLoS One*, 4(12), e8444.
6. Pandey, S., & Bhattacharya, S. (2010). Impact of obesity on gynecology. *Women's Health (London, England)*, 6(1), 107–117.
7. Wax, J. R. (2009). Risks and management of obesity in pregnancy: Current controversies. *Current Opinion in Obstetrics & Gynecology*, 21(2), 117–123.
8. Burt Solorzano, C. M., McCartney, C. R., Blank, S. K., Knudsen, K. L., & Marshall, J. C. (2010). Hyperandrogenaemia in adolescent girls: Origins of abnormal gonadotropin-releasing hormone secretion. *BJOG*, 117(2), 143–149.
9. Shayya, R., & Chang, R. J. (2010). Reproductive endocrinology of adolescent polycystic ovary syndrome. *BJOG*, 117(2), 150–155.
10. Nestler, J. E. (2000). Obesity, insulin, sex steroids and ovulation. *International Journal of Obesity and Related Metabolic Disorders*, 24(Suppl 2), S71–S73.
11. Higginbotham, S. (2009). Contraceptive considerations in obese women: Release date 1 September 2009, SFP Guideline 20091. *Contraception*, 80(6), 583–590.
12. Huber-Buchholz, M. M., Carey, D. G., & Norman, R. J. (1999). Restoration of reproductive potential by lifestyle modification in obese polycystic ovary syndrome: Role of insulin sensitivity and luteinizing hormone. *The Journal of Clinical Endocrinology and Metabolism*, 84(4), 1470–1474.
13. Merhi, Z. O. (2009). Impact of bariatric surgery on female reproduction. *Fertility and Sterility*, 92(5), 1501–1508.
14. Sheiner, E., Balaban, E., Dr'isher, J., Levi, I., & Levy, A. (2009). Pregnancy outcome in patients following different types of bariatric surgeries. *Obesity Surgery*, 19(9), 1286–1292.
15. Guelinckx, I., Devlieger, R., & Vansant, G. (2009). Reproductive outcome after bariatric surgery: A critical review. *Human Reproduction Update*, 15(2), 189–201.
16. Kennedy, G. C. (1951). Experimental hypothalamic obesity. *Proceedings of the Royal Society of Medicine*, 44(10), 899–902.
17. Kennedy, G. C. (1966). The hypothalamus and obesity. *Proceedings of the Royal Society of Medicine*, 59(12), 1276–1277.
18. Hummel, K. P., Dickie, M. M., & Coleman, D. L. (1966). Diabetes, a new mutation in the mouse. *Science*, 153(740), 1127–1128.
19. Lane, P. W. (1959). The pituitary-gonad response of genetically obese mice in parabiosis with thin and obese siblings. *Endocrinology*, 65, 863–868.
20. Zhang, Y., Proenca, R., Maffei, M., Barone, M., Leopold, L., & Friedman, J. M. (1994). Positional cloning of the mouse obese gene and its human homologue. *Nature*, 372(6505), 425–432.
21. Maffei, M., Halaas, J., Ravussin, E., et al. (1995). Leptin levels in human and rodent: Measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. *Nature Medicine*, 1(11), 1155–1161.
22. Sinha, M. K., Sturis, J., Ohannesian, J., et al. (1996). Ultradian oscillations of leptin secretion in humans. *Biochemical and Biophysical Research Communications*, 228(3), 733–738.

23. Teirmaa, T., Luukkaa, V., Rouru, J., Koulu, M., & Huupponen, R. (1998). Correlation between circulating leptin and luteinizing hormone during the menstrual cycle in normal-weight women. *European Journal of Endocrinology*, 139(2), 190–194.
24. ESHRE Capri Workshop group (2006). Nutrition and reproduction in women. *Human Reproduction Update*, 12(3), 193–207.
25. Chan, J. L., & Mantzoros, C. S. (2005). Role of leptin in energy-deprivation states: Normal human physiology and clinical implications for hypothalamic amenorrhoea and anorexia nervosa. *Lancet*, 366(9479), 74–85.
26. Judd, S. J. (1998). Disturbance of the reproductive axis induced by negative energy balance. *Reproduction, Fertility, and Development*, 10(1), 65–72.
27. Ahima, R. S., Dushay, J., Flier, S. N., Prabakaran, D., & Flier, J. S. (1997). Leptin accelerates the onset of puberty in normal female mice. *The Journal of Clinical Investigation*, 99(3), 391–395.
28. Barash, I. A., Cheung, C. C., Weigle, D. S., et al. (1996). Leptin is a metabolic signal to the reproductive system. *Endocrinology*, 137(7), 3144–3147.
29. Farooqi, I. S., Keogh, J. M., Kamath, S., et al. (2001). Partial leptin deficiency and human adiposity. *Nature*, 414(6859), 34–35.
30. Montague, C. T., Farooqi, I. S., Whitehead, J. P., et al. (1997). Congenital leptin deficiency is associated with severe early-onset obesity in humans. *Nature*, 387(6636), 903–908.
31. Strobel, A., Issad, T., Camoin, L., Ozata, M., & Strosberg, A. D. (1998). A leptin missense mutation associated with hypogonadism and morbid obesity. *Nature Genetics*, 18(3), 213–215.
32. Clement, K., Vaisse, C., Lahlou, N., et al. (1998). A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. *Nature*, 392(6674), 398–401.
33. Musso, C., Cochran, E., Javor, E., Young, J., Depaoli, A. M., & Gorden, P. (2005). The long-term effect of recombinant methionyl human leptin therapy on hyperandrogenism and menstrual function in female and pituitary function in male and female hypoleptinemic lipodystrophic patients. *Metabolism*, 54(2), 255–263.
34. Welt, C. K., Chan, J. L., Bullen, J., et al. (2004). Recombinant human leptin in women with hypothalamic amenorrhea. *The New England Journal of Medicine*, 351(10), 987–997.
35. Watanobe, H. (2002). Leptin directly acts within the hypothalamus to stimulate gonadotropin-releasing hormone secretion in vivo in rats. *The Journal of Physiology*, 545(Pt 1), 255–268.
36. Yu, W. H., Kimura, M., Walczewska, A., Karanth, S., & McCann, S. M. (1997). Role of leptin in hypothalamic-pituitary function. *Proceedings of the National Academy of Sciences of the United States of America*, 94(3), 1023–1028.
37. Hamm, M. L., Bhat, G. K., Thompson, W. E., & Mann, D. R. (2004). Folliculogenesis is impaired and granulosa cell apoptosis is increased in leptin-deficient mice. *Biology of Reproduction*, 71(1), 66–72.
38. Olatinwo, M. O., Bhat, G. K., Stah, C. D., & Mann, D. R. (2005). Impact of gonadotropin administration on folliculogenesis in prepubertal ob/ob mice. *Molecular and Cellular Endocrinology*, 245(1–2), 121–127.
39. Barkan, D., Hurgin, V., Dekel, N., Amsterdam, A., & Rubinstein, M. (2005). Leptin induces ovulation in GnRH-deficient mice. *The FASEB Journal*, 19(1), 133–135.
40. Craig, J., Zhu, H., Dyce, P. W., Petrik, J., & Li, J. (2004). Leptin enhances oocyte nuclear and cytoplasmic maturation via the mitogen-activated protein kinase pathway. *Endocrinology*, 145(11), 5355–5363.
41. Kitawaki, J., Kusuki, I., Koshiba, H., Tsukamoto, K., & Honjo, H. (1999). Leptin directly stimulates aromatase activity in human luteinized granulosa cells. *Molecular Human Reproduction*, 5(8), 708–713.
42. Swain, J. E., Dunn, R. L., McConnell, D., Gonzalez-Martinez, J., & Smith, G. D. (2004). Direct effects of leptin on mouse reproductive function: Regulation of follicular, oocyte, and embryo development. *Biology of Reproduction*, 71(5), 1446–1452.
43. Ruiz-Cortes, Z. T., Martel-Kennes, Y., Gevry, N. Y., Downey, B. R., Palin, M. F., & Murphy, B. D. (2003). Biphasic effects of leptin in porcine granulosa cells. *Biology of Reproduction*, 68(3), 789–796.

44. Ehrmann, D. A. (2005). Polycystic ovary syndrome. *The New England Journal of Medicine*, 352(12), 1223–1236.
45. Norman, R. J., Dewailly, D., Legro, R. S., & Hickey, T. E. (2007). Polycystic ovary syndrome. *Lancet*, 370(9588), 685–697.
46. Anttila, L., Ding, Y. Q., Ruutiainen, K., Erkkola, R., Irjala, K., & Huhtaniemi, I. (1991). Clinical features and circulating gonadotropin, insulin, and androgen interactions in women with polycystic ovarian disease. *Fertility and Sterility*, 55(6), 1057–1061.
47. Apter, D., Butzow, T., Laughlin, G. A., & Yen, S. S. (1994). Accelerated 24-hour luteinizing hormone pulsatile activity in adolescent girls with ovarian hyperandrogenism: Relevance to the developmental phase of polycystic ovarian syndrome. *The Journal of Clinical Endocrinology and Metabolism*, 79(1), 119–125.
48. Fauser, B. C., Pache, T. D., Lamberts, S. W., Hop, W. C., de Jong, F. H., & Dahl, K. D. (1991). Serum bioactive and immunoreactive luteinizing hormone and follicle-stimulating hormone levels in women with cycle abnormalities, with or without polycystic ovarian disease. *The Journal of Clinical Endocrinology and Metabolism*, 73(4), 811–817.
49. Molloy, B. G., El Sheikh, M. A., Chapman, C., Oakey, R. E., Hancock, K. W., & Glass, M. R. (1984). Pathological mechanisms in polycystic ovary syndrome: Modulation of LH pulsatility by progesterone. *British Journal of Obstetrics and Gynaecology*, 91(5), 457–465.
50. Barber, T. M., McCarthy, M. I., Wass, J. A., & Franks, S. (2006). Obesity and polycystic ovary syndrome. *Clinical Endocrinology*, 65(2), 137–145.
51. Escobar-Morreale, H. F., & San Millan, J. L. (2007). Abdominal adiposity and the polycystic ovary syndrome. *Trends in Endocrinology and Metabolism*, 18(7), 266–272.
52. Dunaif, A., Segal, K. R., Futterweit, W., & Dobrjansky, A. (1989). Profound peripheral insulin resistance, independent of obesity, in polycystic ovary syndrome. *Diabetes*, 38(9), 1165–1174.
53. Dunaif, A., Segal, K. R., Shelley, D. R., Green, G., Dobrjansky, A., & Licholai, T. (1992). Evidence for distinctive and intrinsic defects in insulin action in polycystic ovary syndrome. *Diabetes*, 41(10), 1257–1266.
54. Echiburu, B., Perez-Bravo, F., Maliqueo, M., Sanchez, F., Crisosto, N., & Sir-Petermann, T. (2008). Polymorphism T C (-34 base pairs) of gene CYP17 promoter in women with polycystic ovary syndrome is associated with increased body weight and insulin resistance: A preliminary study. *Metabolism*, 57(12), 1765–1771.
55. Kulshreshtha, B., Ganie, M. A., Praveen, E. P., et al. (2008). Insulin response to oral glucose in healthy, lean young women and patients with polycystic ovary syndrome. *Gynecological Endocrinology*, 24(11), 637–643.
56. Michelmores, K., Ong, K., Mason, S., et al. (2001). Clinical features in women with polycystic ovaries: Relationships to insulin sensitivity, insulin gene VNTR and birth weight. *Clinical Endocrinology*, 55(4), 439–446.
57. Michelmores, K. F., Balen, A. H., Dunger, D. B., & Vessey, M. P. (1999). Polycystic ovaries and associated clinical and biochemical features in young women. *Clinical Endocrinology*, 51(6), 779–786.
58. The Thessaloniki ESHRE/ASRM sponsored PCOS consensus group (2008). Consensus on infertility treatment related to polycystic ovary syndrome. *Human Reproduction (Oxford, England)*, 23(3), 462–477.
59. Cataldo, N. A., Barnhart, H. X., Legro, R. S., et al. (2008). Extended-release metformin does not reduce the clomiphene citrate dose required to induce ovulation in polycystic ovary syndrome. *The Journal of Clinical Endocrinology and Metabolism*, 93(8), 3124–3127.
60. ACOG. (2009). Polycystic ovary syndrome. *Clinical Updates in Women's Health Care*, VIII(1), 11–20.
61. Fruhbeck, G. (2006). Intracellular signalling pathways activated by leptin. *The Biochemical Journal*, 393(Pt 1), 7–20.
62. Hegyi, K., Fulop, K., Kovacs, K., Toth, S., & Falus, A. (2004). Leptin-induced signal transduction pathways. *Cell Biology International*, 28(3), 159–169.
63. Sweeney, G. (2002). Leptin signalling. *Cellular Signalling*, 14(8), 655–663.

64. Banks, A. S., Davis, S. M., Bates, S. H., & Myers, M. G., Jr. (2000). Activation of downstream signals by the long form of the leptin receptor. *The Journal of Biological Chemistry*, 275(19), 14563–14572.
65. Bates, S. H., Stearns, W. H., Dundon, T. A., et al. (2003). STAT3 signalling is required for leptin regulation of energy balance but not reproduction. *Nature*, 421(6925), 856–859.
66. Bjorbaek, C., Buchholz, R. M., Davis, S. M., et al. (2001). Divergent roles of SHP-2 in ERK activation by leptin receptors. *The Journal of Biological Chemistry*, 276(7), 4747–4755.
67. Nelson-Degrave, V. L., Wickenheisser, J. K., Hendricks, K. L., et al. (2005). Alterations in mitogen-activated protein kinase kinase and extracellular regulated kinase signaling in theca cells contribute to excessive androgen production in polycystic ovary syndrome. *Molecular Endocrinology (Baltimore, Md.)*, 19(2), 379–390.
68. Chan, J. L., Moschos, S. J., Bullen, J., et al. (2005). Recombinant methionyl human leptin administration activates signal transducer and activator of transcription 3 signaling in peripheral blood mononuclear cells in vivo and regulates soluble tumor necrosis factor- $\alpha$  receptor levels in humans with relative leptin deficiency. *The Journal of Clinical Endocrinology and Metabolism*, 90(3), 1625–1631.
69. Dreyer, M. G., Juge-Aubry, C. E., Gabay, C., et al. (2003). Leptin activates the promoter of the interleukin-1 receptor antagonist through p42/44 mitogen-activated protein kinase and a composite nuclear factor kappa B/PU.1 binding site. *The Biochemical Journal*, 370(Pt 2), 591–599.
70. Mehebiek, N., Jaubert, A. M., Sabourault, D., Giudicelli, Y., & Ribiere, C. (2005). Leptin-induced nitric oxide production in white adipocytes is mediated through PKA and MAP kinase activation. *American Journal of Physiology. Cell Physiology*, 289(2), C379–C387.
71. Burks, D. J., Font de Mora, J., Schubert, M., et al. (2000). IRS-2 pathways integrate female reproduction and energy homeostasis. *Nature*, 407(6802), 377–382.
72. Benomar, Y., Roy, A. F., Aubourg, A., Djiane, J., & Taouis, M. (2005). Cross down-regulation of leptin and insulin receptor expression and signalling in a human neuronal cell line. *The Biochemical Journal*, 388(Pt 3), 929–939.
73. Morton, G. J., Gelling, R. W., Niswender, K. D., Morrison, C. D., Rhodes, C. J., & Schwartz, M. W. (2005). Leptin regulates insulin sensitivity via phosphatidylinositol-3-OH kinase signaling in mediobasal hypothalamic neurons. *Cell Metabolism*, 2(6), 411–420.
74. Sahu, A. (2003). Leptin signaling in the hypothalamus: Emphasis on energy homeostasis and leptin resistance. *Frontiers in Neuroendocrinology*, 24(4), 225–253.
75. Xu, A. W., Kaelin, C. B., Takeda, K., Akira, S., Schwartz, M. W., & Barsh, G. S. (2005). PI3K integrates the action of insulin and leptin on hypothalamic neurons. *The Journal of Clinical Investigation*, 115(4), 951–958.
76. Zhao, A. Z., Huan, J. N., Gupta, S., Pal, R., & Sahu, A. (2002). A phosphatidylinositol 3-kinase phosphodiesterase 3B-cyclic AMP pathway in hypothalamic action of leptin on feeding. *Nature Neuroscience*, 5(8), 727–728.
77. Goswami, A., Ranganathan, P., & Rangnekar, V. M. (2006). The phosphoinositide 3-kinase/Akt1/Par-4 axis: A cancer-selective therapeutic target. *Cancer Research*, 66(6), 2889–2892.
78. Bellacosa, A., Testa, J. R., Staal, S. P., & Tsichlis, P. N. (1991). A retroviral oncogene, Akt, encoding a serine-threonine kinase containing an SH2-like region. *Science*, 254(5029), 274–277.
79. Brodbeck, D., Cron, P., & Hemmings, B. A. (1999). A human protein kinase Bgamma with regulatory phosphorylation sites in the activation loop and in the C-terminal hydrophobic domain. *The Journal of Biological Chemistry*, 274(14), 9133–9136.
80. Burgering, B. M., & Coffey, P. J. (1995). Protein kinase B (c-Akt) in phosphatidylinositol-3-OH kinase signal transduction. *Nature*, 376(6541), 599–602.
81. Zeleznik, A. J., Saxena, D., & Little-Ihrig, L. (2003). Protein kinase B is obligatory for follicle-stimulating hormone-induced granulosa cell differentiation. *Endocrinology*, 144(9), 3985–3994.



# Chapter 18

## Lessons from HIV Lipodystrophy and Drug-Induced Metabolic Dysfunction

Steven Grinspoon

### Introduction

With the introduction of highly active antiretroviral therapy (HAART), metabolic and body composition changes are increasingly observed among HIV-infected patients. These changes are characterized by lipoatrophy of subcutaneous abdominal, extremity and facial fat, increased visceral fat, as well as dyslipidemia and insulin resistance, and have been viewed by many to constitute an acquired lipodystrophy syndrome. The etiology of this syndrome is clearly multifactorial and it is not a homogenous syndrome, but rather exhibits significant heterogeneity depending on environmental, genetic, and treatment factors.

The mechanisms of specific antiretroviral effects on lipids and glucose, as well as fat, are becoming increasingly clear. Certain protease inhibitors (PIs) affect GLUT-4 and glucose trafficking. In addition, there may be effects of specific PIs on PPAR-gamma expression in adipocytes. In addition, specific nucleoside reverse transcriptase inhibitors (NRTIs) have been shown to impair mitochondrial function via effects on DNA polymerase gamma, contributing to fat atrophy and insulin resistance. The HIV virus itself may contribute to changes in lipid metabolism, which may interact with effects of specific PIs and other agents on lipids. In addition, acute and chronic effects of the HIV virus and related infections, including hepatitis C, may increase inflammatory indices. Moreover, changes related to excess visceral fat and/or subcutaneous fat loss and cytokine signaling may further contribute to abnormal glucose, lipid, and inflammatory changes seen among HIV-infected patients. Importantly, it has become increasingly recognized that HIV-infected patients are at increased cardiovascular risk and demonstrate higher myocardial infarction rates. Traditional and inflammatory risk markers related to antiretroviral drugs, changes in fat distribution, and metabolic abnormalities may all contribute to this increased risk.

---

S. Grinspoon (✉)

Massachusetts General Hospital, Program in Nutritional Metabolism,  
Harvard Medical School, Boston, MA 02114, USA  
e-mail: sgrinspoon@partners.org



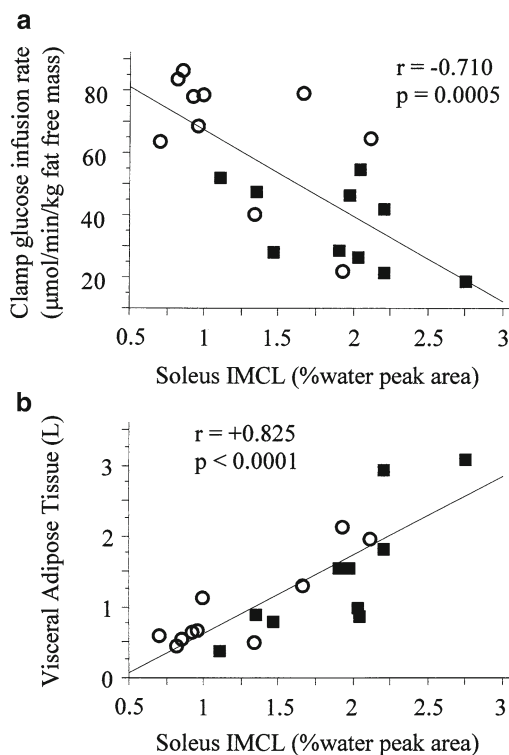
Treatment strategies to reduce cardiovascular risk and address the numerous metabolic changes seen among HIV-infected patients are being developed and can be broadly classified into strategies that address the specific effects of antiretroviral medications, strategies to improve glucose homeostasis and dyslipidemia, and strategies to improve fat distribution, including strategies to potentially increase subcutaneous fat and other strategies to reduce excess visceral fat. In this chapter, the lessons learned from the HIV lipodystrophy syndrome will be explored, particularly as they relate to the metabolic consequences and potentially useful strategies for changes in fat distribution. Novel treatment strategies are now being developed to address these metabolic changes in HIV patients, which may also be useful for other genetic or acquired lipodystrophy syndrome, as well as metabolic deregulation associated with obesity.

## **Metabolic and Anthropometric Changes Among HIV-Infected Patients**

Prior to the era of HAART, it was recognized that HIV-infected patients demonstrated hypertriglyceridemia, and this was associated with increased very low density lipoprotein (VLDL) synthesis and reduced clearance [1, 2]. With the development of HAART, PIs, NRTIs, and non-NNRTIs were developed, and often used in conjunction, for a multidrug regimen to prevent the development of HIV resistance. In antiretroviral naïve patients initiating combination therapy containing a PI and an NRTI, both extremity and truncal fat increase with initiation of therapy, likely consistent with generalized weight gain in the context of improving cachexia and wasting, but extremity fat subsequently decreases, below its starting level, whereas truncal fat continues to increase [3, 4]. Thus, patients develop a significantly increased trunk to extremity fat ratio. Detailed anthropometric studies have demonstrated that the trunk fat accumulation is visceral, with simultaneous loss of abdominal subcutaneous fat [5].

The metabolic abnormalities associated with these anthropometric changes include insulin resistance and dyslipidemia, increased inflammation, reduced adiponectin, impaired fibrinolysis, and endothelial dysfunction [5–7]. In terms of lipid concentration, total cholesterol and LDL levels are low in patients with untreated HIV and return toward normal with treatment [8]. HDL levels are low and remain low with treatment, whereas triglyceride levels are high and may increase further with specific antiretroviral agents. Insulin resistance has been commonly reported, and recently, the development of diabetes has been demonstrated. The prevalence of DM was estimated at 14% among HIV-infected men using HAART compared with 5% of non-HIV-infected men in a large cohort study. Over 4 years of follow-up, the incident rate ratio was 4.11; 95% CI, 1.85–9.16, adjusted for age and body mass index [9]. Insulin resistance is likely multifactorial, in part due to changes in fat distribution itself and the effects of specific antiretroviral medications.

In addition to loss of subcutaneous fat and increased visceral and upper trunk fat, HIV-infected patients on HAART demonstrate significant ectopic fat accumulation in the liver and muscle, which may independently contribute to further increases in insulin resistance. Among patients with HIV-related lipodystrophy, Gan et al. demonstrated increased intramyocellular lipid (IMCL) and a strong inverse correlation between IMCL and total body insulin-stimulated glucose disposal [10] (Fig. 1). Increased hepatic lipid content has also been shown and strongly correlates with increased omental mesenteric fat and insulin resistance in regression modeling [11]. Moreover, increased visceral fat itself, as well as being a predictor of increased hepatic lipid accumulation [11], has been shown to correlate most strongly with glucose disposal [12]. In other studies, reduced subcutaneous fat also has been shown to be highly associated with insulin resistance and soluble TNFR2 concentrations [13].



**Fig. 1** Relationships between IMCL and insulin-stimulated glucose disposal (a) and between IMCL and visceral fat (b) in lipodystrophic subjects (filled square) and control subjects (open circle). Simple regression coefficients ( $r$ ) and the relevant  $P$  values are presented for analyses with both groups combined as there was no difference in relationships between the two groups as assessed by ANCOVA. Reprinted with permission requested from the American Diabetes Association, Copyright 2002

Increased lipolytic rates have been demonstrated among HIV-infected patients [14] with central fat accumulation and may result in substrate flux toward muscle and liver, with subsequent reduction in insulin signaling through effects on PI-3 kinase. Behrens et al. demonstrated that glucose uptake into the muscle was reduced 66% in patients receiving HAART and postulated that increased free fatty acids (FFA) and lipolytic rates may impair hexokinase activity and insulin signaling, as well as increasing hepatic glucose production [15].

Taken together, the studies suggest that HIV-infected patients with fat redistribution demonstrate relative increases in omental and visceral fat, reduction in peripheral and abdominal subcutaneous fat, and ectopic accumulation of hepatic and muscle fat, in association with increased lipolytic rates. These factors may all contribute to severe insulin resistance which is in part related to changes in fat distribution, but as well to specific effects of antiretroviral drugs.

## **Effects of Specific Antiretroviral Medications on Body Composition and Metabolic Changes**

Specific antiretroviral therapies have recently been shown to affect critical metabolic functions in HIV-infected patients, highlighting the potential effects of dysfunction in lipid and glucose metabolism, adipogenesis, and energy metabolism. Specific PIs were shown to be associated with reduced GLUT-4-mediated insulin signaling in adipocytes [16], and this observation was confirmed in vivo, in experiments in which non-HIV-infected patients demonstrated reduced glucose disposal even after one dose of indinavir [17]. A number of additional mechanisms have been suggested by which PIs might contribute to insulin resistance, including effects on SOCS-1 [18].

PIs have been associated with hypertriglyceridemia. Purnell et al. investigated the effects of the PI ritonavir over 2 weeks in healthy non-HIV-infected control subjects and demonstrated increased plasma concentrations of triglyceride, VLDL, apoB, and reduced HDL. These changes were not associated with changes in body composition over the short treatment period and were not shown to result from impairment of peripheral lipoprotein lipase or uptake of remnant lipoproteins. However, hepatic lipase was significantly reduced and increased VLDL formation was postulated to be the mechanism of increased triglyceride levels with ritonavir [19].

In addition, PIs have been shown to be associated with smaller adipocytes and reduction in expression of PPAR- $\gamma$  expression, C/EBP- $\alpha$  and  $\beta$ , and SREBP-1c in subcutaneous fat of HIV-infected patients, suggesting a potential role of PIs to inhibit and alter adipose tissue generation. Of note, increased TNF- $\alpha$  correlated with expression of SREBP-1c and related transcription factors [20]. In addition, Caron et al. demonstrated that reduced Lamin A maturation might limit nuclear translocation of SREBP1C [21]. Using microarray analysis,

Adler-Wailes et al. demonstrated that ritonavir altered gene expression of inflammatory cytokines, stress response, and oxidative stress genes [22]. These direct effects may alter intermediary metabolism and fat deposition and contribute to insulin resistance.

In addition to specific effects of PIs, NRTIs may also contribute to insulin resistance through effects on mitochondrial function and critical metabolic pathways involved in energy regulation, glucose trafficking, and adipogenesis. Among non-HIV-infected patients, Fleischman et al. demonstrated that 4 weeks of stavudine resulted in a significant 52% reduction in mtDNA/nuclear DNA in the muscle. Furthermore, reduced mitochondrial function in muscle assessed in vivo using P31 spectroscopy was significantly associated with development of insulin resistance using euglycemic clamp [23]. Specific cellular effects of certain NRTIs include alteration of lipid storage, presumably due to impairment of oxidative phosphorylation. Use of NRTIs clinically has been associated with reduced extremity fat, particularly for stavudine and to a lesser extent for other NRTIs.

## **Genetic Predictors of Metabolic Risk in Acquired HIV Lipodystrophy Syndrome**

A limited number of studies have been performed to date, primarily in small groups of subjects, to determine if genetic polymorphisms predispose to the development of lipid, glucose, and body composition changes in HIV-infected patients. Ranade et al. demonstrated that a single polymorphism in the resistin gene was associated with more significant development of limb fat loss and insulin resistance on HAART [24]. Hulgán et al. demonstrated that patients with HFE187C/G, a polymorphism in the hemochromatosis gene, were significantly less likely to develop lipoatrophy with initiation of HAART [25]. APOC3 455 CT was protective against fat loss [26]. APOC3 alleles have also been associated with the development of higher triglyceride levels in HIV-infected patients receiving PI containing HAART [27]. A number of studies have focused on mitochondrial haplotype groups. In a relatively large study of 346 patients, Nasi et al. did not demonstrate a significant association between mtDNA haplotypes and lipodystrophic changes in fat or metabolic parameters [28]. In contrast, Hulgán et al. demonstrated that mitochondrial haplotype J was associated with differential degrees of limb fat change [25]. Other polymorphisms that have been preliminarily associated with lipodystrophy include TNF- $\alpha$  238 [29] and IL-1  $\beta$ , which was associated with greater incidence of lipodystrophy on stavudine therapy [30].

Although preliminary studies suggest a potential impact of genetic polymorphisms on the development of lipodystrophy and related metabolic consequences, additional, large studies are needed to determine more definitively these relationships.

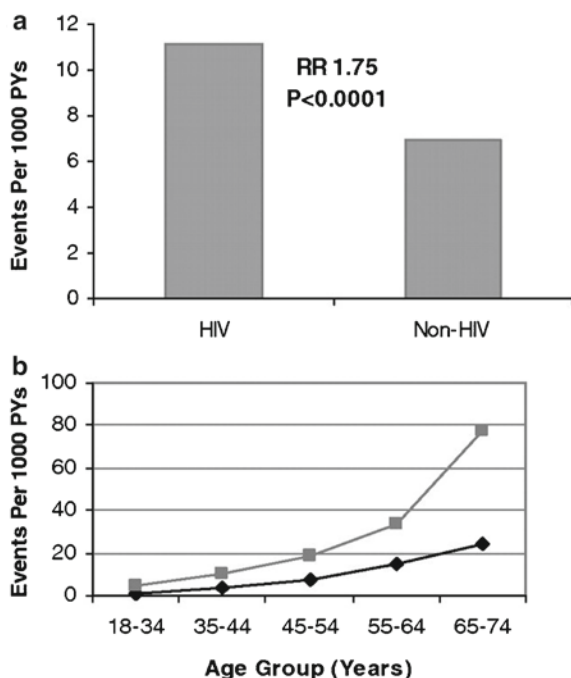
## Cardiovascular Risk Among HIV-Infected Patients and Potential Mechanisms

Early studies suggested increased cardiovascular risk among HIV-infected patients with fat redistribution. In a case control study, Hadigan et al. investigated cardiac risk in HIV-infected patients with evidence of fat redistribution, either of subcutaneous fat loss, abdominal hypertrophy, or both, and manifested by a marked increase in WHR. HIV-infected subjects were matched on BMI and age, with randomly selected subjects in the Framingham Offspring Cohort and demonstrated an increased risk of myocardial infarction using the Framingham Risk Calculator (29.1 vs. 12.8% with >10% 10 year risk, HIV vs. Framingham control subjects ( $P = 0.001$ ) [31, 32]), whereas HIV-infected patients without evidence of fat redistribution did not demonstrate increased risk of myocardial infarction. Moreover, those with lipoatrophy demonstrated the highest predicted 10-year risk, suggesting loss of fat was an equally great or greater risk for myocardial infarction.

Whether the Framingham risk equation developed for a predominantly Caucasian population would be valid for HIV-infected patients remains unknown, but Law et al. used the Framingham risk equation to compare predicted MI rates vs. observed in the Data Collection on Adverse Events of anti-HIV drugs (DAD) study, a prospective Cohort study of HIV-infected patients [33]. The utility of the Framingham risk equation was reviewed at a recent American Heart Association (AHA) State of the Science Meeting on cardiovascular risk in HIV-infected patients, which concluded that it performed reasonably well, ranking CHD risk effectively in HIV-infected patients. However, experts at the AHA meeting concluded that the Framingham equation would require some recalibration in prospective studies among HIV-infected patients, as it may have systematically underpredicted events compared to those observed, particularly among smokers [34]. Development of HIV-specific prediction equations that utilize more specific anthropometric information, for example on waist circumference or total fat, is needed.

In the DAD study, the relative risk of AMI was shown to be 1.26 [95% confidence interval, 1.12–1.41];  $P < 0.001$ ) for each additional year of highly active antiretroviral use [35]. In the DAD study, age, but not weight, was related significantly to increased risk of myocardial infarction. However, median BMI was 23.0 kg/m<sup>2</sup> among the group and thus the subjects were not overweight per se. Moreover, lipodystrophy status was not assessed specifically with anthropometric measurements. Increased cholesterol and triglyceride did attenuate the relationship between antiretroviral therapy and myocardial infarction, suggesting potentially that ARVs contributed to increased CVD risk through effects on lipid concentrations.

In a large health center data registry, Triant et al. compared the relative risk of developing an MI between HIV and non-HIV-infected patients by ICD-9 coding [36]. This study of almost 4,000 HIV-infected patients, covering a period from 1996 to 2004, demonstrated an increased relative risk of MI of 1.75 (95% CI 1.51–2.02;  $P < 0.0001$ ) for HIV-infected vs. non-HIV-infected patients. This increased relative risk was seen in gender-stratified models and was greater with increasing age in the HIV vs. non-HIV-infected patients (Fig. 2). A higher percentage of HIV-infected



**Fig. 2** (a) Myocardial infarction rates and corresponding adjusted RR. Bars indicate crude rates of AMI events per 1,000 PY as determined by ICD coding. RR and associated  $P$  value are shown above the bars. RR was determined from Poisson regression analysis adjusting for age, gender, race, hypertension, diabetes, and dyslipidemia. Associated 95% CIs for RR shown are 1.51–2.02. (b) Myocardial infarction rates by age group. Light line indicates patients diagnosed with HIV disease. Dark line indicates patients not diagnosed with HIV disease. Data shown include both genders. Rates represent number of events per 1,000 PY as determined by ICD coding. Reprinted with permission granted from The Endocrine Society, Copyright 2007

patients demonstrated hypertension (21.2 vs. 15.9%), diabetes (11.5 vs. 6.6%), and dyslipidemia (23.3 vs. 17.6%) compared to non-HIV-infected patients, ( $P < 0.0001$  for each comparison). Adjustment for traditional risk factors including hypertension, diabetes, and dyslipidemia attenuated the risk approximately 25%, suggesting that other risk factors which could not be fully assessed, including smoking, as well as inflammation and body composition changes might be contributing significantly to increased cardiovascular risk in the HIV group. Indeed, in a follow-up study, increased CRP was seen more frequently in the HIV group. Both HIV infection and increased CRP were independently associated with increased myocardial infarction rates. Indeed, being HIV positive and having an increased CRP together increased the risk of AMI by approximately fourfold [37], regardless of the reason for drawing a CRP, which was more often to characterize degree of inflammation or infection than to assess CVD risk, among the HIV group.

The hypothesis that inflammation may be contributing to increased CVD risk among HIV-infected patients was recently investigated by the Strategies for Management of Antiretroviral Therapy (SMART) study, in which HIV-infected

patients were randomized to receive ongoing continuous “suppressive” antiretroviral therapy to maintain steady improvement in immunological function or to episodic treatment based on specific immune parameters, to spare exposure to antiretroviral drugs to the greatest degree possible. The *a priori* hypothesis of SMART was that continuous ARV treatment would be associated with more metabolic dysregulation, based on the mechanisms discussed above, and thus more cardiovascular disease. In fact, the opposite occurred and CVD event rates were higher in the ARV sparing arm (RR 1.6 95% CI 1.0–2.6,  $P = 0.05$ ) [38]. These data suggested that inflammation or other factors, related to poor antiretroviral control, may indeed contribute to increased cardiovascular disease in the HIV population.

One interesting hypothesis, not yet tested directly, is that severe abnormalities in fat distribution may further contribute to increased inflammation, and thus contribute to increased CVD in patients with HIV lipodystrophy. Indeed, increased WHR ratio, and not HIV status, predicted increased CRP and other inflammatory risk markers in a study comparing HIV-infected women and age and BMI-matched non-HIV-infected control subjects [5]. In modeling using more detailed measures of body composition, increased visceral fat was shown to be the most highly significant predictor of increased CRP and IL-6 [5].

Additional studies have investigated surrogate markers, such as IMT in HIV-infected patients. Increased IMT has been demonstrated in some but not all studies comparing HIV vs. non-HIV-infected patients [39, 40]. These differences relate largely to the choice of control group and differences in study design. Importantly, from the perspective of risk assessment, traditional risk factors, including age, HDL, TGL, and BMI, have been shown to be significant predictors of IMT in the HIV group [40]. In a recent study that included detailed information on fat distribution, hypertension, trunk/limb fat ratio by DEXA, and insulin resistance were shown to be the most significant predictors of carotid intima media thickness (cIMT) among a cohort of HIV-infected patients receiving long-term antiretroviral therapy [41]. The relationship of ARV use *per se* to increased cIMT remains controversial.

Taken together, the studies to date do suggest increased cardiovascular risk, mediated only in part by increased traditional risk factors (diabetes, dyslipidemia, hypertension, and smoking) in HIV-infected patients. Increased inflammation due to the HIV virus or related to abnormal fat distribution may also contribute. Further studies are needed to distinguish the specific effects of fat redistribution, inflammation, traditional risk factors, and specific ARV effects on long-term cardiovascular risk in the HIV population.

## **Novel Pharmacological Strategies to Improve Metabolic Abnormalities and Fat Redistribution Among HIV-Infected Patients**

Treatment of dyslipidemia among HIV-infected patients has centered largely around the use of fibric acid derivatives, which are PPAR- $\alpha$  agonists, and largely useful for hypertriglyceridemia. These agents are moderately effective among HIV-infected



patients, but do not normalize dyslipidemia in most patients because lipid levels may be severely increased at baseline and HIV-infected patients are often simultaneously taking agents that increase triglycerides, including PIs. For example, Gerber et al. demonstrated a reduction of 58% in triglyceride levels, from a baseline of 694 mg/dL, with only 16.7% achieving a normalization of triglyceride levels [42]. In addition, HIV itself is associated with increased triglyceride levels through effects to increase VLDL secretion and decrease clearance [1, 2]. Niacin has also been studied and shown to be reasonably effective to lower triglyceride levels, increase HDL, and improve LDL particle size, without significantly aggravating glucose levels [43]. In addition, fish oil has been shown to improve hypertriglyceridemia by 25.5% among HIV-infected patients [44]. Use of HMGCoA reductase inhibitors has also been investigated in HIV-infected patients, but may be less useful as such agents primarily target LDL, which may not be elevated among HIV-infected patients. Though some HMGCoA reductase inhibitors do decrease triglyceride, including atorvastatin, it is important to consider potential interactions between specific lipid-lowering agents and antiviral medications. For example, Fichtenbaum et al. demonstrated an increase of >3,000% in simvastatin AUC in patients receiving ritonavir/saquinavir [45] and efavirenz treatment significantly reduced simvastatin, atorvastatin, and pravastatin AUC through effects on CYP3A4 [46].

A novel treatment of dyslipidemia in HIV-infected patients with hypertriglyceridemia and fat redistribution is acipimox. Acipimox is a nicotinic acid inhibitor and potent inhibitor of lipolysis. HIV-infected patients with lipodystrophic changes in fat redistribution demonstrate increased FFA and lipolytic rates [14, 47]. In a randomized, placebo-controlled trial, acipimox significantly improved triglyceride and FFA concentrations, reduced lipolytic rates, and improved insulin sensitivity as determined by euglycemic clamp. Moreover, the improvement in insulin sensitivity was highly correlated with the reduction in lipolysis [48]. This novel therapeutic strategy suggested that reduction in lipolysis may improve not only dyslipidemia, but also insulin sensitivity, perhaps via reduction in the FFA pool and intramyocellular lipid [48]. Accumulation of fat in the muscle may lead to reduced insulin sensitivity through effects on PI-3 kinase and insulin signaling.

Strategies to improve insulin sensitivity and simultaneously improve altered fat distribution have also been employed among HIV-infected patients. The most rationale of these approaches has targeted those with insulin resistance and investigated metformin for patients with overweight and increased abdominal obesity. In contrast, drugs of the thiazolidinedione class have been investigated to improve in insulin sensitivity. Simultaneously, these drugs were investigated for potential effects on adipogenesis to improve the loss of subcutaneous fat.

Metformin, a biguanide insulin-sensitizing agent, has been thought to act primarily at the liver to affect endogenous glucose production. Recent data suggest an effect of metformin to stimulate AMP kinase, thereby inducing fatty acid oxidation and suppression of lipogenic pathways [49]. These potential actions of metformin on AMP kinase may help to explain the effects of metformin on weight, fat loss, and glucose homeostasis. In an early randomized, placebo-controlled study of metformin among HIV-infected patients with insulin resistance and central fat accumulation,

Hadigan et al. demonstrated significant effects on insulin sensitivity, weight, waist circumference, blood pressure, and indices of impaired thrombolysis, which improved in parallel with insulin sensitivity [7, 50].

In contrast to metformin, the thiazolidinediones are PPAR- $\gamma$  agonists that stimulate glucose uptake into muscle, increase adipogenesis, and decrease lipolysis. In studies of patients with congenital lipodystrophy, troglitazone was shown to reduce triglyceride and FFA concentrations, improve insulin sensitivity, and increase subcutaneous fat, without reducing visceral fat [51]. Use of the glitazones is an attractive potential therapeutic option for HIV-infected patients, in whom reduced PPAR- $\gamma$ , C/EBP- $\alpha$ , and SREBP-1c expression has been shown in subcutaneous fat [20], and adiponectin levels are reduced in association with severe insulin resistance and reduced extremity fat [6]. Moreover, in an animal model of lipoatrophy, surgical implantation of fat improved insulin resistance, suggesting the potential clinical importance of pharmacological strategies to improve subcutaneous fat [52].

Recent studies have investigated use of thiazolidinediones among HIV-infected patients with subcutaneous fat loss. In a number of studies, rosiglitazone increased subcutaneous fat, while simultaneously improving insulin sensitivity, glucose tolerance, and adiponectin [53], as well as reducing liver fat [54] and improving PPAR- $\gamma$  and PGC-1 gene expression [55]. In contrast, studies that did not select patients on the basis of insulin resistance more often did not show significant improvements in subcutaneous fat [56]. Moreover, the majority of studies investigating rosiglitazone among HIV-infected patients with fat redistribution demonstrated adverse effects on lipids, including increased LDL, decreased HDL, and increased small dense LDL particle concentrations and reduced HDL particle size [53, 57, 58]. There are a number of potential explanations for these results, the most likely of which is that the patients enrolled were often taking nucleoside reverse transcriptase agents, which simultaneously contributed to impaired mitochondrial function and limited the effects of glitazones to stimulate adipogenesis and PPAR- $\gamma$  expression [59]. In contrast to rosiglitazone, pioglitazone, which has been shown to reduce liver fat in patients with hepatic steatosis [60], was shown to improve subcutaneous extremity fat and insulin resistance and increase HDL among lipodystrophic HIV-infected patients [61]. Taken together, the data on glitazones suggest a potentially important effect to increase PPAR gene expression and subcutaneous adipogenesis, insulin sensitivity, and adiponectin expression. However, these effects may be limited by the very therapies, e.g., the NRTIs that contribute to fat atrophy and insulin resistance in the HIV population. In contrast, treatments with thiazolidinediones may be very useful for HIV patients not receiving or switched off NRTIs or for other non-HIV lipodystrophic populations not receiving NRTIs.

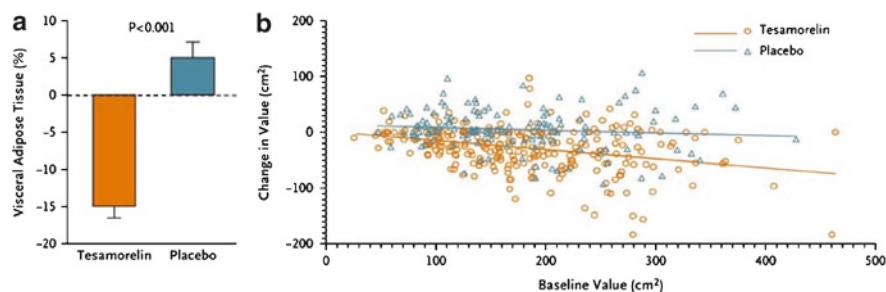
Therapeutic administration of leptin has also been considered in HIV-infected patients with significant lipoatrophy and insulin resistance. In non-HIV-infected patients with severe lipodystrophy, insulin resistance, hypertriglyceridemia, and low serum leptin levels, administration of recombinant human leptin significantly improved insulin sensitivity, dyslipidemia, and liver volume [62]. Among

HIV-infected patients, leptin levels are low among lipoatrophic patients with insulin resistance, and leptin administration improved insulin resistance and HDL [63]. In both HIV and non-HIV patients, leptin reduces caloric intake and weight, which may contribute to its metabolic effects, but may not be a desired effect in the long run for low weight lipoatrophic patients. Further studies are needed to determine the long-term effects, safety, and optimal dosing of leptin among HIV-infected patients with significant fat atrophy.

Recent data suggest that HIV-infected patients with fat redistribution demonstrate relative growth hormone (GH) deficiency, with reduced overnight GH secretion relative to HIV patients of similar BMI without fat redistribution as well as to non-HIV control subjects [64]. Reduced GH is significantly and inversely related to visceral adiposity, even controlling for weight, suggesting that increased visceral adiposity associated with the HIV lipodystrophy syndrome might contribute to reduced GH. In turn, reduced GH is associated with adverse cardiovascular indices such as dyslipidemia. Detailed investigation of the pattern of GH secretion in HIV-infected patients with fat redistribution suggests reduced pulse height and width, but preserved GH pulse frequency [65].

A number of randomized, placebo-controlled studies have been conducted investigating high-dose GH. In one large Phase III study of GH at 4 mg/day over 12 weeks, a significant 20% reduction in visceral fat and significant improvements in non-HDL cholesterol as well as HDL cholesterol were demonstrated, but GH administration resulted in supraphysiological GH concentrations, symptoms, and signs of GH excess, including swelling, edema and joint aches, and increased glucose concentrations [66]. In contrast, Lo et al. investigated physiological GH administration at an average dose of 0.3 mg/day, over 18 months among HIV-infected patients with central fat accumulation and reduced GH response to GHRH/arginine stimulation and demonstrated a significant 9% reduction in visceral fat, with improvements in triglyceride and blood pressure. However, even at the low physiological dose of GH, 2-h glucose increased significantly in the GH group compared to placebo [67]. Taken together, these data show a significant effect of GH to reduce visceral fat and improve dyslipidemia in HIV-infected patients with central fat accumulation. However, the therapeutic window may be narrow, as even low-dose GH may increase glucose levels, particularly in those patients with baseline impaired glucose tolerance.

Another approach to the treatment of central fat accumulation among HIV-infected patients has been the use of growth hormone-releasing hormone (GHRH). In a pilot study, GHRH [1–29] was shown to decrease trunk fat and increase lean mass in HIV-infected patients with fat accumulation, without decreasing extremity fat, thus improving the trunk to extremity fat ratio [68]. Additional Phase III studies using GHRH<sub>1–40</sub> were also completed [69]. These studies demonstrated a 20% reduction in visceral fat relative to placebo (Fig. 3), with significant improvements in total cholesterol, triglyceride, HDL, and cholesterol to HDL ratio. Adiponectin also improved and glucose was not adversely affected, in contrast to the effects seen in response to GH administration. Importantly, patients and physicians alike noted significant improvement in the shape of the abdomen, which reduced the stress associated with abdominal enlargement in this population.



**Fig. 3** Changes in visceral adipose tissue from baseline to 26 weeks. (a) shows the mean difference in visceral adipose tissue according to study group; T bars denote the standard error. The *P* value for the between-group comparison was calculated by analysis of covariance. (b) shows the relationship of the changes from baseline to 26 weeks in visceral adipose tissue. In the regression equations, the changes from baseline equal 1.24 minus 0.163 times the baseline value in the tesamorelin group and 13.2 minus 0.048 times the baseline value in the placebo group. Reprinted with permission granted from the Massachusetts Medical Society, Copyright 2007

## Conclusions

HIV-infected patients treated with antiretroviral agents often develop a unique acquired lipodystrophy syndrome characterized by accumulation of excess visceral fat, severe loss of subcutaneous fat, dyslipidemia, hypoadiponectinemia, insulin resistance, and impaired endothelial function. These metabolic changes contribute to an increased CVD rate among HIV-infected patients. Novel strategies to reduce lipolysis with acipimox, redistribute fat using PPAR-gamma agonists, and to selectively reduce visceral fat, using GH stimulating analogues, have been developed, which may improve CVD risk in this population and could have broader applicability to congenital and acquired lipodystrophy syndromes, as well as to obesity and the metabolic syndrome.

## References

1. Hellestein, M. K., Grunfeld, C., Wu, K., Christiansen, M., Kaempfer, S., Kletke, C., et al. (1993). Increased de novo hepatic lipogenesis in human immunodeficiency virus infection. *J Clin Endocrinol Metab*, 76(3), 559–565.
2. Grunfeld, C., Pang, M., Doerrler, W., Shigenaga, J. K., Jensen, P., Feingold, K. R., (1992). Lipids, lipoproteins, triglyceride clearance, and cytokines in human immunodeficiency virus infection and the acquired immunodeficiency syndrome. *J Clin Endocrinol Metab*, 74(5), 1045–1052.
3. Dube, M.P., Parker, R.A., Tebas, P., Grinspoon, S.K., Zackin, R.A., Robbins, G.K., et al. (2005). Glucose metabolism, lipid, and body fat changes in antiretroviral-naïve subjects randomized to nelfinavir or efavirenz plus dual nucleosides. *Aids*, 19(16), 1807–1818.
4. Mallon, P.W., Miller, J., Cooper, D.A., Carr, A. (2003). Prospective evaluation of the effects of antiretroviral therapy on body composition in HIV-1-infected men starting therapy. *AIDS*, 17(7), 971–979.

5. Dolan, S.E., Hadigan, C., Killilea, K.M., Sullivan, M.P., Hemphill, L., Lees, R.S., et al. (2005). Increased cardiovascular disease risk indices in HIV-infected women. *J Acquir Immune Defic Syndr*, 39(1), 44–54.
6. Tong, Q., Sankale, J.L., Hadigan, C.M., Tan, G., Rosenberg, E.S., Kanki, P.J., et al. (2003). Regulation of adiponectin in human immunodeficiency virus-infected patients: relationship to body composition and metabolic indices. *J Clin Endocrinol Metab*, 88(4), 1559–1564.
7. Hadigan, C., Meigs, J. B., Rabe, J., D'Agostino, R. B., Wilson, P. W., Lipinska, I., et al. (2001). Increased PAI-1 and tPA antigen levels are reduced with metformin therapy in HIV-infected patients with fat redistribution and insulin resistance. *J Clin Endocrinol Metab*, 86(2), 939–943.
8. Riddler, S. A., Smit, E., Cole, S. R., Li, R., Chmiel, J. S., Dobs, A., et al. (2003). Impact of HIV Infection and HAART on Serum Lipids in Men, *JAMA* 289(22), 2978–2982.
9. Brown, T. T., Cole, S. R., Li, X., Kingsley, L. A., Palella, F. J., Riddler, S. A., et al. (2005). Antiretroviral therapy and the prevalence and incidence of diabetes mellitus in the multicenter AIDS cohort study. *Arch Intern Med* 165(10), 1179–1184.
10. Gan, S. K., Samaras, K., Thompson, C. H., Kraegen, E. W., Carr, A., Cooper, D. A., et al. (2002). Altered myocellular and abdominal fat partitioning predict disturbance in insulin action in HIV protease inhibitor-related lipodystrophy. *Diabetes* 51(11), 3163–3169.
11. He, Q., Engelson, E. S., Ionescu, G., Glesby, M. J., Albu, J. B., Kotler, D. P. (2008). Insulin resistance, hepatic lipid and adipose tissue distribution in HIV-infected men, *Antivir Ther*. 13(3), 423–428.
12. Hadigan, C., Kamin, D., Liebau, J., Mazza, S., Barrow, S., Torriani, M., et al. (2006). Depot-specific regulation of glucose uptake and insulin sensitivity in HIV-lipodystrophy. *Am J Physiol Endocrinol Metab*, 290(2), E289–298.
13. Mynarcik, D.C., McNurlan, M.A., Steigbigel, R.T., Fuhrer, J., Gelato, M.C. (2000). Association of severe insulin resistance with both loss of limb fat and elevated serum tumor necrosis factor receptor levels in HIV lipodystrophy. *J Acquir Immune Defic Syndr*, 25(4), 312–321.
14. Hadigan, C., Borgonha, S., Rabe, J., Young, V., Grinspoon, S. (2002). Increased rates of lipolysis among human immunodeficiency virus-infected men receiving highly active antiretroviral therapy. *Metabolism*, 51(9), 1143–1147.
15. Behrens, G. M., Boerner, A. R., Weber, K., van den Hoff, J., Ockenga, J., Brabant, G., et al. (2002). Impaired glucose phosphorylation and transport in skeletal muscle cause insulin resistance in HIV-1-infected patients with lipodystrophy. *Journal of Clinical Investigation*, 110(9), 1319–1327.
16. Murata, H., Hruz, P. W., Mueckler, M. (2000). The mechanism of insulin resistance caused by HIV protease inhibitor therapy. *J Biol Chem*, 275(27), 20251–20254.
17. Noor, M. A., Seneviratne, T., Aweeka, F. T., Lo, J. C., Schwarz, J. M., Mulligan, K., et al. (2002). Indinavir acutely inhibits insulin-stimulated glucose disposal in humans: a randomized, placebo-controlled study. *AIDS*, 16(5), F1–8.
18. Carper, M. J., Cade, W. T., Cam, M., Zhang, S., Shalev, A., Yarasheski, K. E., et al. (2008). HIV-protease inhibitors induce expression of suppressor of cytokine signaling-1 in insulin-sensitive tissues and promote insulin resistance and type 2 diabetes mellitus. *Am J Physiol Endocrinol Metab*, 294(3), E558–567.
19. Purnell, J. Q., Zambon, A., Knopp, R. H., Pizzuti, D. J., Achari, R., Leonard J. M., et al. (2000). Effect of zidovudine on lipids and post-heparin lipase activities in normal subjects. *AIDS*, 14(1), 51–57.
20. Bastard, J. P., Caron, M., Vidal, H., Auclair, M., Vigouroux, C., Lubinski, J., et al. (2002). Association between altered expression of adipogenic factor SREBP1 in lipotrophic adipose tissue from HIV-1-infected patients and abnormal adipocyte differentiation and insulin resistance. *Lancet*, 359(9311), 1026–1031.
21. Caron, M., Vigouroux, C., Bastard, J. P., Capeau, J., (2009). Antiretroviral-Related Adipocyte Dysfunction and Lipodystrophy in HIV-Infected Patients: Alteration of the PPARgamma-Dependent Pathways. *PPAR Res*, 2009:507141. Epub 2008 Dec 30. PMID: 19125203 [PubMed - in process].

22. Adler-Wailes, D. C., Guiney, E. L., Koo, J., Yanovski, J. A. (2008). Effects of ritonavir on adipocyte gene expression: evidence for a stress-related response. *Obesity (Silver Spring)*. 16 (10), 2379–2387.
23. Fleischman, A., Johnsen, S., Systrom, D. M., Hrovat, M., Farrar, C. T., Frontera W., et al. (2007). Effects of a nucleoside reverse transcriptase inhibitor, stavudine, on glucose disposal and mitochondrial function in muscle of healthy adults. *Am J Physiol Endocrinol Metab*. 292(6), E1666–1673.
24. Ranade, K., Geese, W. J., Noor, M., Flint, O., Tebas, P., Mulligan, K., et al. (2008). Genetic analysis implicates resistin in HIV lipodystrophy. *Aids*. 22(13), 1561–1568.
25. Hulgán, T., Tebas, P., Canter, J. A., Mulligan, K., Haas, D. W., Dubé, M., et al. (2008). Hemochromatosis gene polymorphisms, mitochondrial haplogroups, and peripheral lipodystrophy during antiretroviral therapy. *J Infect Dis*. 197(6), 858–866.
26. Zanone Poma, B., Riva, A., Nasi, M., Zanone Poma, B., Riva, A., Nasi, M., et al. (2008). Genetic polymorphisms differently influencing the emergence of atrophy and fat accumulation in HIV-related lipodystrophy. *Aids*. 22(14), 1769–1778.
27. Bonnet, E., Bernard, J., Fauvel, J., Massip, P., Ruidavets, J. B., Perret, B. (2008). Association of APOC3 polymorphisms with both dyslipidemia and lipodystrophy in HAART-receiving patients. *AIDS Res Hum Retroviruses*. 24(2), 169–171.
28. Nasi, M., Guaraldi, G., Orlando, G., Nasi, M., Guaraldi, G., Orlando, G., et al. (2008). Mitochondrial DNA haplogroups and highly active antiretroviral therapy-related lipodystrophy. *Clin Infect Dis*. 47(7), 962–968.
29. Maher, B., Alfirevic, A., Vilar, F. J., Wilkins, E. G., Park, B. K., Pirmohamed, M. (2002). TNF-alpha promoter region gene polymorphisms in HIV-positive patients with lipodystrophy. *Aids*. 16(15), 2013–2018.
30. Asensi, V., Rego, C., Montes, A. H., Asensi, V., Rego, C., Montes, A. H., et al. (2008). IL-1beta (+3954C/T) polymorphism could protect human immunodeficiency virus (HIV)-infected patients on highly active antiretroviral treatment (HAART) against lipodystrophic syndrome. *Genet Med*. 10(3), 215–223.
31. Wilson, P. W., D'Agostino, R. B., Levy, D., Belanger, A. M., Silbershatz, H., Kannel, W. B. (1998). Prediction of coronary heart disease using risk factor categories. *Circulation*. 97(18), 1837–1847.
32. Hadigan, C., Meigs, J. B., Wilson, P. W., Hadigan, C., Meigs, J. B., Wilson, P. W., et al. (2003). Prediction of coronary heart disease risk in HIV-infected patients with fat redistribution. *Clin Infect Dis*. 36(7), 909–916.
33. Law, M. G., Friis-Moller, N., El-Sadr, W. M., Weber, R., Reiss, P., D'Arminio Monforte, A., et al. (2006). The use of the Framingham equation to predict myocardial infarctions in HIV-infected patients: comparison with observed events in the D:A:D Study. *HIV Medicine*. 7(4), 218–230.
34. Schambelan, M., Wilson, P. W., Yarasheski, K. E., Cade, W. T., Dávila-Román, V. G., D'Agostino, R. B., Sr., et al. (2008). Development of appropriate coronary heart disease risk prediction models in HIV-infected patients. *Circulation*. 118(2), e48–53.
35. Friis-Moller, N., Sabin, C. A., Weber, R., d'Arminio Monforte, A., El-Sadr, W. M., Reiss, P., et al. (2003). Combination antiretroviral therapy and the risk of myocardial infarction. *N Engl J Med*. 349(21), 1993–2003.
36. Triant, V. A., Lee, H., Hadigan, C., Grinspoon, S. K. (2007). Increased Acute Myocardial Infarction Rates and Cardiovascular Risk Factors Among Patients with HIV Disease. *J Clin Endocrinol Metab*. 92, 2506–2512.
37. Triant, V. A., Meigs, J. B., Grinspoon, S. (2008). Association of C-Reactive Protein and HIV Infection with Acute Myocardial Infarction. Paper presented at: 10th International Workshop on Adverse Drug Reactions and Lipodystrophy; November 6–8, London, UK.
38. El-Sadr, W. M., Lundgren, J. D., Neaton, J. D., Gordin, F., Abrams, D., Arduino, R. C., et al. (2006). CD4+ count-guided interruption of antiretroviral treatment. *N Engl J Med*. 355(22), 2283–2296.



39. Hsue, P. Y., Lo, J. C., Franklin, A., Bolger, A. F., Martin, J. N., Deeks, S. G., et al. (2004). Progression of atherosclerosis as assessed by carotid intima-media thickness in patients with HIV infection. *Circulation*. 109, 1603–1608.
40. Currier, J. S., Kendall, M. A., Zackin, R., Henry, W. K., Alston-Smith, B., Torriani, F. J., et al. (2005). Carotid artery intima-media thickness and HIV infection: traditional risk factors overshadow impact of protease inhibitor exposure. *Aids*. 19(9), 927–933.
41. Mondy, K. E., de las Fuentes, L., Waggoner, A., Onen, N. F., Bopp, C. S., Lassa-Claxton, S., et al. (2008). Insulin resistance predicts endothelial dysfunction and cardiovascular risk in HIV-infected persons on long-term highly active antiretroviral therapy. *Aids*. 22(7), 849–856.
42. Gerber, J. G., Kitch, D. W., Fichtenbaum, C. J., Zackin, R. A., Charles, S., Hogg, E., et al. (2008). Fish oil and fenofibrate for the treatment of hypertriglyceridemia in HIV-infected subjects on antiretroviral therapy: results of ACTG A5186. *J Acquir Immune Defic Syndr*. 47(4), 459–466.
43. Dube, M. P., Wu, J. W., Aberg, J. A., Deeg, M. A., Alston-Smith, B. L., McGovern, M. E., et al. (2006). Safety and efficacy of extended-release niacin for the treatment of dyslipidaemia in patients with HIV infection: AIDS Clinical Trials Group Study A5148. *Antivir Ther*. 11(8), 1081–1089.
44. De Truchis, P., Kirstetter, M., Perier, A., Meunier, C., Zucman, D., Force, G., et al. (2007). Reduction in triglyceride level with N-3 polyunsaturated fatty acids in HIV-infected patients taking potent antiretroviral therapy: a randomized prospective study. *J Acquir Immune Defic Syndr*. 44(3), 278–285.
45. Fichtenbaum, C. J., Gerber, J. G., Rosenkranz, S. L., Segal, Y., Aberg, J. A., Blaschke, T., et al. (2002). Pharmacokinetic interactions between protease inhibitors and statins in HIV seronegative volunteers: ACTG Study A5047. *AIDS*. 16(4), 569–577.
46. Gerber, J. G., Rosenkranz, S. L., Fichtenbaum, C. J., Vega, J. M., Yang, A., Alston, B. L., et al. (2005). Effect of efavirenz on the pharmacokinetics of simvastatin, atorvastatin, and pravastatin: results of AIDS Clinical Trials Group 5108 Study. *J Acquir Immune Defic Syndr*. 39(3), 307–312.
47. Meininger, G., Hadigan, C., Laposata, M., Brown, J., Rabe, J., Louca, J., et al. (2002). Elevated concentrations of free fatty acids are associated with increased insulin response to standard glucose challenge in human immunodeficiency virus-infected subjects with fat redistribution. *Metabolism*. 51(2), 260–266.
48. Hadigan, C., Liebau, J., Torriani, M., Andersen, R., Grinspoon, S. (2006). Improved triglycerides and insulin sensitivity with 3 months of acipimox in human immunodeficiency virus-infected patients with hypertriglyceridemia. *J Clin Endocrinol Metab*. 91, 4439–4444.
49. Zhou, G., Myers, R., Li, Y., Chen, Y., Shen, X., Fenyk-Melody, J., et al. (2001). Role of AMP-activated protein kinase in mechanism of metformin action. *J Clin Invest*. 108(8):1167–1174.
50. Hadigan, C., Corcoran, C., Basgoz, N., Davis, B., Sax, P., Grinspoon, S. (2000). Metformin in the treatment of HIV lipodystrophy syndrome: A randomized controlled trial. *JAMA*. 284(4), 472–477.
51. Arioglu, E., Duncan-Morin, J., Sebring, N., Rother, K. I., Gottlieb, N., Lieberman, J., et al. (2000). Efficacy and safety of troglitazone in the treatment of lipodystrophy syndromes. *Ann Intern Med*. 133(4), 263–274.
52. Gavrilova, O., Marcus-Samuels, B., Graham, D., Kim, J. K., Shulman, G. I., Castle, A. L., et al. (2000). Surgical implantation of adipose tissue reverses diabetes in lipoatrophic mice. *J Clin Invest*. 105(3), 271–278.
53. Hadigan, C., Yawetz, S., Thomas, A., Havers, F., Sax, P. E., Grinspoon S. (2004). Metabolic effects of rosiglitazone in HIV lipodystrophy: A randomized controlled trial. *Ann Intern Med*. 140, (10)786–794.
54. Sutinen, J., Hakkinen, A. M., Westerbacka, J., Seppälä-Lindroos, A., Vehkavaara, S., Halavaara, J., et al. (2003). Rosiglitazone in the treatment of HAART-associated lipodystrophy—a randomized double-blind placebo-controlled study. *Antivir Ther*. 8(3), 199–207.



55. Sutinen, J., Kannisto, K., Korshennikova, E., Fisher, R. M., Ehrenborg, E., Nyman, T., et al. (2004). Effects of rosiglitazone on gene expression in subcutaneous adipose tissue in highly active antiretroviral therapy-associated lipodystrophy. *Am J Physiol Endocrinol Metab.* 286(6), E941–949.
56. Carr, A., Workman, C., Carey, D., Rogers, G., Martin, A., Baker, D., et al. (2004). No effect of rosiglitazone for treatment of HIV-1 lipodystrophy: randomized, double-blind, placebo-controlled trial. *Lancet.* 363(9407), 429–438.
57. Mulligan, K., Yang, Y., Wininger, D. A., Koletar, S. L., Parker, R. A., Alston-Smith, B. L., et al. (2007). Effects of Metformin and Rosiglitazone in HIV-Infected Patients with Hyperinsulinemia and Elevated Waist to Hip Ratio. *AIDS.* 21, 47–57.
58. Hadigan, C., Mazza, S., Crum, D., Grinspoon, S. (2007). Rosiglitazone increases small dense low-density lipoprotein concentration and decreases high-density lipoprotein particle size in HIV-infected patients. *AIDS.* 21(18), 2543–2546.
59. Mallon, P. W., Sedwell, R., Rogers, G., Nolan, D., Unemori, P., Hoy, J., et al. (2008). Effect of rosiglitazone on peroxisome proliferator-activated receptor gamma gene expression in human adipose tissue is limited by antiretroviral drug-induced mitochondrial dysfunction. *J Infect Dis.* 198(12), 1794–1803.
60. Belfort, R., Harrison, S. A., Brown, K., Darland, C., Finch, J., Hardies, J., et al. (2006). A placebo-controlled trial of pioglitazone in subjects with nonalcoholic steatohepatitis. *N Engl J Med.* 355(22), 2297–2307.
61. Slama, L., Lanoy, E., Valantin, M. A., Bastard, J. P., Chermak, A., Boutekajirt, A., et al. (2008). Effect of pioglitazone on HIV-1-related lipodystrophy: a randomized double-blind placebo-controlled trial (ANRS 113). *Antivir Ther.* 13(1), 67–76.
62. Oral, E. A., Simha, V., Ruiz, E., Andewelt, A., Premkumar, A., Snell, P., et al. (2002). Leptin-replacement therapy for lipodystrophy. *N Engl J Med.* 346(8), 570–578.
63. Lee, J. H., Chan, J. L., Sourlas, E., Raptopoulos, V., Mantzoros, C. S. (2006). Recombinant methionyl human leptin therapy in replacement doses improves insulin resistance and metabolic profile in patients with lipodystrophy and metabolic syndrome induced by the highly active antiretroviral therapy. *J Clin Endocrinol Metab.* 91(7), 2605–2611.
64. Rietschel, P., Hadigan, C., Corcoran, C., Stanley, T., Neubauer, G., Gertner, J., et al. (2001). Assessment of growth hormone dynamics in human immunodeficiency virus-related lipodystrophy. *J Clin Endocrinol Metab.* 86(2), 504–510.
65. Koutkia, P., Meininger, G., Canavan, B., Breu, J., Grinspoon, S. (2004). Metabolic regulation of growth hormone by free fatty acids, somatostatin, and ghrelin in HIV-lipodystrophy. *Am J Physiol Endocrinol Metab.* 286(2), E296–303.
66. Grunfeld, C., Thompson, M., Brown, S. J., Richmond, G., Lee, D., Muurahainen, N., et al. (2007). Recombinant human growth hormone to treat HIV-associated adipose redistribution syndrome: 12 week induction and 24-week maintenance therapy. *J Acquir Immune Defic Syndr.* 45(3), 286–297.
67. Lo, J., You, S. M., Canavan, B., Liebau, J., Beltrani, G., Koutkia, P., et al. (2008). Low-dose physiological growth hormone in patients with HIV and abdominal fat accumulation: a randomized controlled trial. *JAMA.* 300(5), 509–519.
68. Koutkia, P., Canavan, B., Breu, J., Torriani, M., Kissko, J., Grinspoon, S. (2004). Growth Hormone-Releasing Hormone in HIV-Infected Men With Lipodystrophy: A Randomized Controlled Trial. *JAMA.* 292(2), 210–218.
69. Falutz, J., Allas, S., Blot, K., Potvin, D., Kotler, D., Somero, M., et al. (2007). Metabolic effects of a growth hormone-releasing factor in patients with HIV. *N Engl J Med.* 357(23), 2359–2370.

# Chapter 19

## Principles of Obesity Therapy

Rexford S. Ahima

### Introduction

Obesity may be defined as an excessive accumulation of fat in adipose tissue and other organs, of sufficient magnitude to have an adverse impact on health. The body-mass index (BMI), calculated as the weight (kg) divided by height squared ( $m^2$ ), is often used as a measurement of body fat in clinical and epidemiological studies [1]. The National Heart, Lung, and Blood Institute and the World Health Organization (WHO) define normal weight as a BMI of 18.5–24.9  $kg/m^2$ , overweight as a BMI  $\geq 25$ –29.9  $kg/m^2$ , and obese as a BMI  $\geq 30$   $kg/m^2$ . The BMI criteria for Asia and Oceania are slightly different: normal weight is a BMI of 18.5–22.9  $kg/m^2$ , overweight is a BMI  $\geq 23$ –24.9  $kg/m^2$ , and obesity is a BMI  $\geq 25$   $kg/m^2$ . At a given BMI, body fat is about 12% higher in women than in men [1]. Children with a BMI >97th percentile in regard to age and gender are considered to be obese. Children with BMI >90th percentile but <97th percentile are considered to be overweight.

In 2005, the WHO estimated that approximately 1.6 billion adults (age 15 years and above) were overweight, and at least 400 million were obese. Given the current trends, the WHO projects that by 2015, approximately 2.3 billion adults will be overweight and more than 700 million will be obese. At least 20 million children under the age of 5 years were overweight globally in 2005, and this figure is expected to rise in the coming years. Obesity was once considered a health problem in developed countries, but is now highly prevalent in low and middle income countries, particularly in urban areas. In the United States, approximately 70% of adults are overweight and 30% are obese [1]. Moreover, obesity rates are higher among African American women, Hispanics, and Native Americans [1].

The fundamental cause of overweight and obesity is an imbalance between energy intake and expenditure. Obesity and fat distribution are influenced by genetics, maternal

---

R.S. Ahima (✉)

Division of Endocrinology, Diabetes and Metabolism, and the Institute for Diabetes, Obesity and Metabolism, University of Pennsylvania School of Medicine, 415 Curie, Boulevard, 712A Clinical Research Building, Philadelphia, PA 19104, USA  
e-mail: ahima@mail.med.upenn.edu

and perinatal factors, and gender. However, the global increases in overweight and obesity are attributable mainly to a shift in diet towards energy-dense foods, rich in fat and sugars, but low in vitamins and other micronutrients, as well as sedentary lifestyle in modern societies. “Metabolic syndrome” denotes a connection between central obesity and insulin resistance, hypertension, dyslipidemia, impaired glucose tolerance or diabetes, all of which increase the risk of coronary artery disease. Obesity is also associated with greater risk of sleep apnea, nonalcoholic fatty liver disease, cancer, osteoarthritis, reproductive dysfunction, and other serious medical conditions. Obesity leads to social stigmatization and disability, and is recognized as an independent risk factor for mortality [2, 3]. Among more than 90,000 women studied in the Women’s Health Initiative, the risk of death was strongly associated with increasing BMI from normal levels to more than 40 kg/m<sup>2</sup> [3]. Another study of more than 80,000 men and women revealed that mortality among those younger than 55 years was directly related to BMI [2]. Mortality among those 55 years or older was higher starting at a BMI of 25 kg/m<sup>2</sup> in women and 30 kg/m<sup>2</sup> in men [2].

The Diabetes Prevention Program (DPP) highlighted the importance of a modest (5–10%) weight loss on the development of diabetes [4]. The study participants with impaired glucose tolerance were randomized to intensive lifestyle modification, or treatment with metformin or placebo, respectively. The average duration of treatment was 2.8 years. Those assigned to lifestyle intervention lost 5.6 kg, compared to 2.1 kg in the metformin group and 0.1 kg in the placebo group. The risk of developing diabetes was reduced by 58% in the lifestyle intervention group compared to the placebo group, and by 31% in the lifestyle intervention group compared the metformin-treated group. Lifestyle intervention significantly reduced the risk of developing the “metabolic syndrome” by 17% compared to metformin treatment, and by 41% compared to placebo. Studies in other countries have shown similar effects of weight loss in reducing the risk of diabetes [5, 6].

Weight loss also decreases the risk of cardiovascular disease. In the Framingham cohort, a modest weight loss of 6.8 kg or more reduced the risk of hypertension by 28% in middle age adults and by 37% in older adults [7]. In a clinical trial using lifestyle intervention to decrease blood pressure, the risk for hypertension fell by 65% when weight loss of 4.5 kg was maintained for 30 months [8]. Maintenance of reduced weight in the long-term lowered the incidence of cardiovascular disease, especially in participants with preexisting cardiovascular risk factors [8].

## Clinical Evaluation of Obesity

*History.* The timing and progression of obesity, exacerbating factors, attempts at weight loss, and associated diseases are all important. Rare genetic syndromes associated with obesity include the Prader–Willi syndrome, which results from a paternal chromosomal abnormality, and Bardet–Biedl syndrome, Ahlstrom syndrome and Cohen syndrome, which result from autosomal recessive abnormalities [9]. Melanocortin-4 receptor (MC4R) defects are thought to occur in approximately 4% of morbidly obese individuals, and are associated with early onset obesity,

**Table 1** Drugs associated with weight increase

Class	Drug	Alternatives
Antipsychotic	Clozapine	Ziprasidone
	Risperidone	Aripiprazole
	Olanzapine	
	Quetiapine	
Antidepressant	Tricyclics	Fluoxetine
	Lithium	Sertraline
	MAOIs	Bupropion
	Paroxetine	Nefazodone
	Fluvoxamine	
	Citalopram	
	Mirtazapine	
Anticonvulsant	Venlafaxine	
	Carbamazepine	Topiramate
	Gabapentin	Lamotrigine
	Valproic acid	Zonisamide
Diabetes	Insulin	Metformin
	Thiazolidinedione	Exenatide
	Sulfonylurea	Pramlintide
		Sitagliptin
		Acarbose
Steroid hormone		Miglitol
	Corticosteroid	
	Estrogen	
Hypertension	Progestin	
	$\alpha$ -blocker	ACE inhibitor or ARB
	$\beta$ -blocker	Calcium channel blocker

hyperphagia, insulin resistance, and increased height [9]. Despite the obesity, MC4R null patients are protected from hypertension [10]. Null mutations of leptin or leptin receptor genes cause hyperphagia, early onset obesity, insulin resistance, immunosuppression, and hypothalamic hypogonadism [9]. Obesity resulting from monogenic or chromosomal abnormalities occurs during childhood, progress rapidly, and may be associated with characteristic features e.g., mental retardation or abnormal facies, as in the rare cases of chromosomal abnormalities.

Rapid weight increase can occur in secondary causes of obesity, including endocrinopathies (e.g., hyperinsulinism, hypothyroidism, Cushing's syndrome, oral contraceptives); medications, (e.g., insulin, sulfonylureas or thiazolidinediones, atypical anti-psychotics, antidepressants, and  $\alpha$ - and  $\beta$ -adrenergic blockers (Table 1); hypothalamic injury (e.g., postsurgical resection and radiation therapy for craniopharyngioma and other sellar tumors); or pseudotumor cerebri. The natural history of primary (idiopathic) obesity is influenced by complex genetics, intra-uterine and perinatal factors as well the social environment, particularly the availability of energy-dense foods rich in fat and sugar, and sedentary lifestyle [11–13]. Childhood obesity often starts in the first 4–6 years and is strongly predicted by parental obesity [12]. The history should capture the time-line of

obesity, the timing and quantities foods and drinks consumed, how much vegetables and processed foods are consumed, and whether the food is prepared at home or bought. Aberrant eating behaviors, such as bingeing, bulimia, and night-eating syndrome, point to primary psychiatric disorders [14, 15].

Documentation of time spent on sedentary activities, such as watching television, walking, and other forms of exercise is important [16–18]. A careful drug history, including prescription drugs and over-the-counter supplements, is essential. Systemic review should elicit symptoms suggestive of diabetes or glucose impairment, hypothyroidism, Cushing’s syndrome, dyslipidemia, angina, stroke, congestive heart failure, venous thrombosis, dyspnea, fatigue, sleep apnea, hypoventilation, sexual dysfunction, stress incontinence, gastroesophageal reflux, cholelithiasis, meralgia paresthetica, depression, bingeing, bulimia, night-eating, obsessive compulsive disorder, osteoarthritis, skin infections, and lymphedema.

*Physical examination.* Specific measures in the physical examination include the BMI, waist circumference, neck circumference, blood pressure, and stigmata pointing to endocrinopathies or cardiovascular, pulmonary, neurological or musculoskeletal complications. The BMI is easy to measure and associated with obesity-related diseases [19]. However, the BMI may not be the best predictor of obesity-related health problems in some ethnic groups, such as African Americans and Hispanic-American women, who have more fat-free mass than Caucasians [19]. A high BMI in athletes does not indicate obesity because of increases in fat-free and muscle mass. In contrast, the relationship between BMI and body fat in elderly patients can be affected by loss of height or decreased muscle mass [20].

Waist circumference should be measured in overweight patients because it is among the criteria for metabolic syndrome, and correlates strongly with cardiovascular morbidity [21] (Tables 2 and 3). The waist circumference is determined with a tape measure at a horizontal line above the superior iliac crest. The hip circumference is measured at the highest point of the greater trochanter. Waist-to-hip ratios greater than 0.95 in men and 0.85 in women are considered elevated.

A neck circumference greater than 17 in. in men and 16 in. in women is predictive of sleep apnea [22] (Table 4). Clinicians should look for evidence of

**Table 2** 2005 revised ATP III screening criteria for metabolic syndrome (American Heart Association, and the National Heart Lung and Blood Institute)

Increased waist circumference	≥102 cm in men ≥88 cm in women
Increased serum triglycerides	≥150 mg/dL (1.7 mmol/L) or on drug treatment for elevated serum triglycerides
Reduced HDL cholesterol	<40 mg/dL (0.9 mmol/L) in men <50 mg/dL (1.1 mmol/L) in women Or drug treatment for reduced HDL cholesterol
Increased blood pressure	≥130 mmHg systolic blood pressure or ≥85 mmHg diastolic blood pressure or anti-hypertensive drug treatment in a patient with a history of hypertension
Increased fasting glucose	≥100 mg/dL drug treatment for elevated glucose

**Table 3** International Diabetes Federation waist circumference criteria for metabolic syndrome

European	Male ≥ 94 cm Female ≥ 80 cm
South Asian	Male ≥ 90 cm Female ≥ 80 cm
Chinese	Male ≥ 90 cm Female ≥ 80 cm
Japanese	Male ≥ 90 cm Female ≥ 80 cm

**Table 4** Assessment of wake-time sleepiness

The Epworth Sleepiness Scale is a patient questionnaire that identifies the level of wake-time sleepiness	
Rate the degree of sleepiness during the following activities using this scale: 0 = never doze; 1 = slight chance of dozing; 2 = moderate chance of dozing; 3 = high chance of dozing	
<i>Activity</i>	<i>Sleepiness score (0–3)</i>
Sitting and reading	
Watching TV	
Sitting in public	
Passenger in a car for 1 h	
Lying down in the afternoon	
Sitting and talking to someone	
Sitting quietly after lunch	
Stopped for few minutes in traffic	
<i>Total score</i> (Total score > 10 requires evaluation to determine whether patient is getting adequate sleep or has an underlying sleep disorder)	

hypertension, endocrinopathies (e.g., acanthosis nigricans in hyperinsulinism; proximal myopathy, dorsal cervical adiposity, facial plethora and hyperpigmented striae in hypercortisolism; goiter, myxedematous skin changes, and hyporeflexia in hypothyroidism), and systemic abnormalities.

*Laboratory evaluation.* Laboratory tests are intended to evaluate overweight and obese patients at high risk for diabetes, cardiovascular disease, hypothyroidism and other diseases [21, 23]. Essential tests are fasting plasma glucose and lipid panel, and glycosylated hemoglobin. Thyroid-stimulating hormone (TSH) is useful for excluding hypothyroidism, especially in older women. Screening with urinary free cortisol is needed when there are specific symptoms and signs of hypercortisolism, such neuropsychiatric symptoms, proximal myopathy, hyperpigmented striae or osteoporosis. Based on the clinical assessment, other tests may be needed, e.g., polysomnography for sleep disorders, abdominal ultrasound for cholelithiasis and nonalcoholic fatty liver disease, electrocardiogram, echocardiogram, angiography and other cardiac evaluation, and computed tomography or magnetic resonance imaging of pituitary

disorders. Genetic testing is required for diagnosing monogenic obesity in highly consanguineous families, and rare chromosomal disorders associated with obesity.

Treatment of Obesity

*Goals.* Like any complex disease, the treatment of obesity demands a long-term perspective and multidisciplinary approach. The patient’s interests, desires and ability should be the primary determinants of the weight management plan. A team approach involving the patient, family and social support group, primary care physician, and specialists is needed. The goal of treatment is to improve existing obesity-related diseases, reduce the risk of developing obesity-related diseases, and enhance overall quality of life. Studies have shown that an initial weight loss of 10% of the baseline weight and maintenance at 5–10% of the baseline weight are realistic goals which improve disease outcomes in obesity [4]. Tables 5 and 6 summarize the risk assessment and treatment of obesity.

*Lifestyle modification.* Overweight or obese patients need to make lifestyle changes to achieve and maintain the weight loss goals. Patients often wish they could to lose 30% or more of their initial body weight, but a more realistic goal is 5–10% of initial weight [4]. Importantly, this weight loss target decreases the incidence of diabetes [4]. Patients should be educated about the fundamentals of body weight regulation. Most patients, often based on their own experiences, are aware that after losing an initial amount of weight they hit a plateau and fail to lose more weight. In fact, only few individuals who lose weight are able to sustain that level for 3 years [24]. This phenomenon may be driven by a failure to adhere to behavioral, dietary, and exercise guidelines. Adaptive metabolic and neuroendocrine responses, mediated by leptin and other hormones, suppress energy expenditure and increase appetite in an attempt to restore weight [25].

What are the characteristics of those who are successful in losing weight? They eat less, exercise more, practice positive thinking and other stress-relieving techniques, and rigorously monitor their eating behavior and physical activity [26, 27]. Behavior modification strategies include keeping a diary and recording details of eating behavior, such as what foods are eaten, the timing and setting in which food is eaten, and

Table 5 Risk assessment of obesity

	BMI (kg/m <sup>2</sup> )	Obesity class	Disease risk relative to normal weight and waist	
			Men < 40 in (102 cm)	Men > 40 in (102 cm)
			Women < 35 in (88 cm)	Women > 35 in (88 cm)
Underweight	<18.5			
Normal	18.5–24.9			
Overweight	25–29.9		Increased	High
Obesity	30–34.9	I	High	Very high
	35–39.9	II	Very high	Very high
	≥40	III	Extremely high	Extremely high



**Table 6** Guide to obesity treatment

Treatment	Body Mass Index				
	25–25.9	27–29.9	30–34.9	35–39.9	≥40
Diet, exercise & behavior therapy	With comorbidities	With comorbidities	+	+	+
Pharmacotherapy		With comorbidities	+	+	+
Surgery				With comorbidities	+

factors which trigger eating. This information is valuable to the patient as well as the healthcare provider, because it provides a framework for formulation of a personalized eating plan for the patient. Based on the DPP paradigm, lifestyle modification is provided on a weekly basis for 16–26 weeks in groups of 10–20 patients, for 60–90 min sessions [4, 27, 28]. Group therapy is more effective, at least initially, because it offers an empathic environment, social support and healthy competition among participants. Weekly weigh-ins can motivate some patients. During the weight-loss maintenance phase, a blend of individual and group behavior therapy is more practical than group therapy alone because of specific medical needs, personalized treatment of weight regain, and different work schedules, and other social needs. Interactions via the internet, telephone, or mail can be very helpful between clinic visits [29, 30]. Regardless of the method, it is important that participants keep food and activity diaries, document their weights weekly, and complete other behavioral assignments.

*Diet.* The degree of weight loss depends on adherence to a dietary plan. Unfortunately, there is so much hype surrounding the importance of the macronutrient content to successful weight loss. The fundamental principle of energy homeostasis is that in order to lose weight, a person must ingest fewer calories than is needed to maintain resting metabolic rate and physical activity. Assuming the weight has been stable, the daily caloric intake is equal to total energy expenditure. The total energy expenditure (kilocalories) can be estimated from the product of the resting metabolic rate (i.e.,  $10 \times$  body weight in pounds for women, or  $11 \times$  body weight in pounds for men) and activity index (ranging from 1.2 for sedentary to 2 for very active).

A popular recommendation for a low calorie diet comprises of 500–1,000 kcal deficit per day, carbohydrates  $\leq 55\%$ , protein 15%, total fat  $\leq 30\%$ , saturated fat 8–10%, polyunsaturated fat  $< 10\%$ , monounsaturated fat  $\leq 15\%$ , cholesterol  $\leq 30$  g, fiber 20–30 g [21, 23]. This high-carbohydrate low-fat diet emphasizes an adequate intake of vegetables, whole grains and fruits [21, 23].

Very low calorie diets (VLCDs) provide 400–800 kcal and 70–100 g of protein per day, and are designed for rapid weight loss and preservation of lean body mass [31]. VLCDs, often served as liquid diets can be helpful when extremely obese patients need to lose weight rapidly for medical procedures [31, 32]. VLCDs should be administered under medical supervision because patients may develop electrolyte abnormalities and gall stones [31, 33]. Patients can lose 15–25% of their initial weight within the first 12–16 weeks on VLCDs, but the long-term weight losses are comparable to low calorie diets, because of greater weight regain in VLCD patients [33].

Meal replacements provide a structured approach toward attaining caloric goals [33, 34]. The meal replacements provide 500–1,000 kcal/day as 2 or 3 servings of a liquid diet. This is complemented by a conventional meal. Because obese patients underestimate their daily calorie intake by as much as 40–50% when eating conventional meals, meal replacements have an advantage of providing a known calorie content [33–35]. The meal replacement regimen is simple, involves no food preparation by the patient, and avoids poor food choices. A meta-analysis showed that meal replacements were more effective than isocaloric diets composed of conventional foods [36]. Meal replacements also resulted in significantly greater weight loss and glycemic control in diabetics [37].

Portion-controlled servings of conventional foods can be more effective than conventional meals in reducing weight [38–41]. For example, patients who received a diet of 1,000 kcal/day designated as 5 breakfasts and 5 dinners in a week lost more weight compared to patients who consumed the same total calories as self-selected meals [40]. Portion-controlled meals can be provided as frozen-food entrees as an alternative to liquid diets [41].

Low-carbohydrate high-fat diets, popularized by the Atkins diet, drastically reduce carbohydrate intake and increase fat and protein intake [42]. Carbohydrate intake less than 50 g/day induces ketosis, which in combination with the high protein intake is thought to suppress appetite [42]. Although randomized clinical trials have reported greater weight loss in patients on low-carbohydrate high-fat diets at 6 months, this effect was not sustained after a year [43–46].

Low glycemic index diets are popular because they limit the glucose load during meals, attenuate the rise in insulin levels, and avoid the adverse metabolic consequences of hyperinsulinemia [47, 48]. The glycemic index of a food is determined by measuring the change in blood glucose following ingestion of 50 g of the target food, compared to the same amount of control food (i.e., white bread or glucose). Whole grains, legumes, vegetables, and fruits with low glycemic index may induce satiation while maintaining low insulin levels [48]. However, studies in obese individuals have not consistently shown greater effects of low glycemic index foods on weight loss [47, 49–51]. Nonetheless, low glycemic index diets are associated with greater reductions in glucose and glycated proteins than high glycemic index diets [47, 52].

This review of various diets highlights the fact that in the long-term, total caloric intake is more relevant than the macronutrient composition of foods. The key to a successful diet plan is to emphasize patients' preferences, compliance, and cost considerations.

## Exercise

There is ample evidence that exercise improves cardiopulmonary and overall health [53–55]. However, exercise alone cannot be a primary treatment for weight loss. Contrary to popular belief, it takes a lot of intense exercise to lose weight [56–58]. In order to lose a pound a week, one needs to achieve a 3,500 kcal deficit which

requires walking 5 miles daily for 7 days in a week. Obviously most individuals cannot achieve this feat. The major effect of exercise is to facilitate the maintenance of reduced weight [58]. The typical recommendation is for obese individuals to increase walking to 30–60 min daily for 5 or more days a week. A meta-analysis of weight loss trials found that exercise alone resulted in a 3 kg weight loss, whereas a combination of exercise and diet resulted in a 11 kg weight loss [59, 60]. An objective and practical method for monitoring exercise is to wear a pedometer. A mile is about 2,000 steps and the daily cumulative target is 10,000 steps.

## Pharmacotherapy

Drugs approved by the United States Food and Drug Administration (FDA) for the treatment of obesity are shown in Table 7.

*Sibutramine* is a norepinephrine-serotonin reuptake inhibitor approved for long-term treatment of obesity. Sibutramine has been studied in several clinical trials. In a short-term clinical trial lasting 8 weeks, sibutramine decreased weight in a dose-dependent manner, when administered as 5 vs. 20 mg/day [61]. Over 6 months, 67% of patients treated with sibutramine achieved a 5% weight loss from baseline and 35% lost 10% or more [61]. The patients regained weight when sibutramine was discontinued, indicating that the drug is effective when treatment is maintained [61]. In the Sibutramine Trial of Obesity Reduction and Maintenance (STORM), which lasted 2 years, patients were initially enrolled in a 6-month open-label trial and treated with a sibutramine dose of 10 mg/day [62]. Two thirds of those who lost 8 kg or more were then randomized to sibutramine and one third to placebo. Over 18 months, the placebo-treated patients regained 80% of their weight, while the sibutramine-treated patients maintained their weight for 12 months and then 20% [62].

**Table 7** Drugs approved by the United States Food and Drug Administration for the treatment of obesity

Generic name	Trade name	Drug Enforcement Agency schedule	Approved use	Year approved
Orlistat (120 mg)	Xenical	None	Long-term	1999
Orlistat (60 mg)	Alli	None	Long-term	2007
Sibutramine*	Meridia	IV	Long-term (Withdrawn in 2010)	1997 (Withdrawn in 2010)
Diethylpropion	Tenulate	IV	Short-term	1973
Phentermine	Adipex Ionamin	IV	Short-term	1973
Phendimetrazine	Bontril Prelu-2	III	Short-term	1961
Benzphetamine	Didrex	III	Short-term	1960

\*Sibutramine was withdrawn by the European Medicines Agency's Committee for Medicinal Products for Human Use in January 2010, and the United States Food and Drug Administration in October 2010, due to adverse cardiovascular events.

A common side effect of sibutramine is hypertension, which has raised safety concerns. In a 52-week trial in obese patients with hypertension whose blood pressure was controlled with calcium channel blockers and/or  $\beta$ -blockers or thiazides, weight loss was greater in the sibutramine-treated patients than the placebo-treated patients [63]. Diastolic blood pressure was decreased by 1.3 mmHg in the placebo-treated group and increased by 2 mmHg in the sibutramine-treated group. Systolic blood pressure was increased by 1.5 mmHg in the placebo-treated group and by 2.7 mmHg in the sibutramine-treated group. Sibutramine also increased the heart rate by 4.9 beats/min [63]. These modest changes did not result in cardiovascular morbidity.

Sibutramine is effective in obese diabetic patients [64, 65]. Patients with diabetes treated with sibutramine at 15 mg/day lost 2.8% of body weight compared to 0.12% in the placebo group after 12 weeks [64]. Hemoglobin A1c levels fell by 0.3% in the sibutramine-treated group, but did not change in the placebo group. In a 24-week trial, sibutramine decreased weight by 4.3% compared to 0.3% in placebo-treated patients. Hemoglobin A1c levels fell by 1.67% in the sibutramine-treated group compared to 0.53% in the placebo-treated group [65].

Sibutramine has also been used in obese children [66–68]. In a 12-month multicenter trial, adolescents aged 12–16 years were assigned to placebo or sibutramine, 10 mg/day that was increased to 15 mg/day in patients who lost more than 10% of body weight in 6 months. The mean change in BMI in the sibutramine-treated patients was -8.2% compared to -0.8% in the placebo group. Triglycerides, HDL cholesterol, and insulin sensitivity all improved after sibutramine treatment. Importantly, there was no difference in systolic or diastolic blood pressure between placebo and sibutramine treatment [67].

Sibutramine is more effective when combined with a behavioral program. Weight loss over 12 months was  $5.0 \pm 7.4$  kg in sibutramine-treated patients. Behavior modification alone resulted in weight loss of  $6.7 \pm 7.9$  kg. Addition of minimal behavioral therapy to sibutramine resulted in a slightly larger weight loss of  $7.5 \pm 8.0$  kg. On the other hand, adding an intensive lifestyle intervention program to sibutramine increased the weight loss to  $12.1 \pm 9.8$  kg [68].

To summarize, the usual starting dose of sibutramine is 10 mg/day, which can be titrated up or down depending on the response in 4–6 weeks. Sibutramine decreases triglycerides, total cholesterol, and LDL cholesterol levels, and increases HDL cholesterol levels, depending on the degree of weight loss. Side effects of sibutramine include hypertension, palpitations, pseudoageusia, headaches, insomnia, and asthenia. Sibutramine increases systolic by approximately 0.8 mmHg, diastolic blood pressure by 0.6 mmHg, and pulse rate by 4–5 beats/min. Sibutramine is contraindicated in patients with coronary artery disease, congestive heart failure, arrhythmias, or stroke. Sibutramine should not be used with selective serotonin reuptake inhibitors or monoamine oxidase inhibitors. The latter should be withdrawn for at least 2 weeks before starting sibutramine.

*Sympathomimetic drugs:* Benzphetamine, diethylpropion, phendimetrazine, and phentermine which block norepinephrine reuptake in the brain, suppress appetite and are approved for short-term treatment of obesity, typically 12 weeks (Table 7).

A clinical trial lasting 36 weeks showed that continuous or intermittent phentermine treatment resulted in greater weight loss than placebo [69]. Patients treated intermittently regained weight during the drug-free period and lost weight when phentermine was restarted [69]. Phentermine and diethylpropion are classified by the United States Drug Enforcement Agency as schedule IV drugs, and benzphetamine and phendimetrazine are classified as schedule III drugs, indicating their potential for abuse. The side-effects of sympathomimetic drugs include hypertension, palpitations, insomnia, dry mouth, asthenia, and constipation.

*Orlistat* inhibits pancreatic lipase, reduces the intestinal digestion of triglycerides, and increases fecal fat loss. Orlistat is not absorbed to any significant extent, and has minimal effect when patients eat a low-fat diet. Orlistat has been studied in many clinical trials. In one trial, patients received a 500 kcal/day deficit diet for 1 year and were treated with placebo or orlistat, and were then switched to placebo vs. orlistat or versa in a cross-over design [70]. After the first year, the placebo group lost 6.1% of their initial body weight and the orlistat group lost 10.2%. In the second year, patients switched from orlistat to placebo gained weight from -10 to -6% lower than baseline, whereas patients who were switched from placebo to orlistat lost weight from -6 to -8.1% lower than baseline [70].

In a 4-year, double-blind, randomized, placebo-controlled trial in 3,304 overweight patients, the lowest body weight after 1 year was more than -11% of initial weight in the orlistat-treated group and -6% lower than initial weight in the placebo-treated group [71]. After the fourth year, the weights of orlistat-treated patients were -6.9% lower than baseline, compared to -4.1% for those receiving placebo. The conversion of patients from impaired glucose tolerance to overt diabetes was reduced by 37% in the orlistat group [71].

Orlistat has been tested in diabetic patients. In one study, orlistat treatment decreased body weight by 6.5% after 1 year compared to 4.2% in the placebo group [72]. Hemoglobin A1c levels were significantly reduced by orlistat [72]. Another study involving pooled data from three 2-year studies revealed that 6.6% of the patients taking orlistat converted from a normal to an impaired glucose tolerance test result, whereas 10.8% in the placebo-treated group developed diabetes [73]. Among the normoglycemic patients, 7.6% in the placebo group developed diabetes compared to 3% in the orlistat-treated group [73].

A multicenter trial tested the effects of orlistat in obese adolescents on a hypocaloric diet containing 30% fat [74]. The BMI was decreased by 0.55 kg/m<sup>2</sup> in the orlistat group compared to an increase of 0.31 kg/m<sup>2</sup> in the placebo group. At the end of the study, the weight increased by 0.51 kg in the orlistat group and 3.14 kg in the placebo group.

Orlistat is effective in maintenance of weight loss [75]. In a 1 year study in patients who had lost more than 8% of their body weight over 6 months while eating a 1,000-kcal/day diet, the placebo-treated patients regained 56% of their body weight, compared to a 32.4% in the those treated with orlistat at a dose of 120 mg three times per day. Lower doses of orlistat did not prevent the regain of weight [75].

The usual prescription dose of orlistat is 120 mg three times a day with meals. An over the counter dose of orlistat 60 mg three times daily is also available. Flatulence,

oily stools and related gastrointestinal symptoms are common initially, but tend to subside when patients learn to avoid fatty meals. Orlistat can cause significant decreases in fat-soluble vitamins, hence a multivitamin should be taken before bedtime.

*Rimonabant* is a cannabinoid receptor-1 antagonist that inhibits feeding, and improves glycemia and lipid levels in patients with the metabolic syndrome [76]. Rimonabant was approved by European drug regulatory agencies in 2006 for obesity treatment. The drug was withdrawn in October 2008 because of psychiatric side effects, including depression and anxiety [77].

### ***Drugs Used to Treat Obesity But Are Not Approved by the United States FDA***

Fluoxetine is a selective serotonin-reuptake inhibitor approved by the FDA for treatment of depression. Fluoxetine at a dose of 60 mg/day (that is three times the usual dose for the treatment of depression) reduces body weight in overweight patients, but 50% of the lost weight is regained during the second 6 months of treatment [78]. Thus, fluoxetine is not an appropriate long-term treatment for obesity.

Bupropion is a norepinephrine- and dopamine reuptake inhibitor approved for the treatment of depression and smoking cessation. Bupropion has been shown to decrease body weight [79]. It appears that non-depressed patients respond to better to bupropion with weight loss than depressed patients [80]. Topiramate is approved for the treatment of seizure disorders. The anti-seizure activity involves the modulation of GABA<sub>A</sub> receptor, kainate subtype of the glutamate receptor, and voltage-dependent sodium and calcium channels. Topiramate inhibits food intake through the GABA<sub>A</sub> receptor [81]. Although significant weight loss of 16% was observed after 44 weeks of topiramate treatment vs. 8.9% in the placebo group, the trial has terminated due to associated adverse events [82]. Zonisamide is an anti-seizure drug with serotonergic and dopaminergic activity. Weight loss occurred in patients treated with zonisamide [83].

Some anti-diabetic drugs have been associated with significant weight loss. For example, several studies have shown that patients treated with metformin lose more weight [4, 84]. In the DPP study of individuals with impaired glucose tolerance, patients treated with metformin (850 mg twice a day) lost 2.5% of their initial body weight compared with those treated with placebo [4]. This weight loss is modest, but establishes metformin as a logical choice for diabetes treatment in overweight or obese individuals.

Amylin is co-secreted with insulin and reduces glucose levels. Unlike insulin and many anti-diabetic drugs, pramlintide (amylin) decreases body weight [85]. In a study of patients with type 1 diabetes randomized to placebo or subcutaneous pramlintide at a dosage of 60 µg three or four times a day along with insulin injection, the weight decreased by 1.2 kg relative to placebo, and the hemoglobin A1c decreased from 0.29 to 0.34% [86]. The most common side effect of pramlintide is nausea, which usually abates after 4 weeks. Recent studies have shown that combining amylin and leptin produces significant weight loss in both rodents and humans [86, 87].

Exenatide (exendin-4) is a 39-amino acid peptide with 53% homology with GLP-1. Exenatide is approved for treatment of type 2 diabetes in patients with poorly controlled glycemia, while being treated with metformin or sulfonylureas. Several clinical trials have shown that in addition to reducing the HbA1c levels, exenatide produces a significant weight loss [88–90]. Liraglutide is a GLP-1 agonist with a slower rate of degradation of GLP-1 due to insertion of a fatty acid residue. Studies indicate that liraglutide has beneficial effects on glucose as well as weight [91].

Combination therapy is a logical strategy for enhancing the efficacy of anti-obesity drugs. For example, phentermine and fenfluramine showed a highly significant weight loss when used in combination [92]. However, the drug was withdrawn due to reports of valvular regurgitation associated with its use [93, 94]. Other combinations of existing drugs are under development, including phentermine and topiramate, phentermine and zonisamide, and naltrexone and bupropion. Early results suggest that combined drugs may have additive effects on weight loss, but long-term studies are needed to evaluate the side effects.

## Surgical Treatment of Obesity

Surgical treatment should be considered for obesity patients with BMI > 40 kg/m<sup>2</sup>, or a BMI greater than 35 kg/m<sup>2</sup> with serious comorbid conditions, such as sleep apnea, poorly controlled diabetes mellitus, and joint disease (Tables 5 and 6). Surgical candidates should have tried and failed non-surgical weight loss therapy, understand the benefits and risks of surgery, and adhere to postoperative recommendations. Roux-en-Y gastric bypass involves the creation of a small pouch of stomach just below the esophagus that empties into a loop of jejunum [95]. Roux-en-Y gastric bypass produces the greatest degree of weight loss, and independently improves diabetes and dyslipidemia [96].

Vertical banded gastroplasty (VBG) is a restrictive procedure involving the placement of a staple line parallel to the lesser curvature, and a ring at the end of this narrowing which delays the entry of food into the stomach. Laparoscopic adjustable gastroplasty (LAG) involves the placement of an adjustable ring that narrows the opening between the upper and lower stomach. Pancreaticobiliary diversion (BPD) is rarely performed nowadays, and involves the formation of two parallel intestinal limbs, one of which empties the stomach and the other into the pancreatic and biliary secretions, both of which are emptied near the ileocecal valve [96]. BPD produces severe weight loss, likely through malabsorption [95, 96].

In a randomized controlled trial comparing patients with diabetes and a BMI between 30 and 40 kg/m<sup>2</sup> assigned to either gastric banding or a lifestyle program, patients in the gastric banding group lost 20% of their initial body weight compared to 1.4% in the lifestyle group, after 2 years [97]. Diabetes remission occurred in 73% of those in the surgical group, compared to 13% in the lifestyle modification group [97].

The Swedish Obese Subjects Study (SOS) found that cardiovascular risk factors were reduced in patients who had bariatric surgery than those in the control group



in the first 2 years after surgery [98]. The incidence of new cases of diabetes at 2 and 10 years was drastically reduced by bariatric surgery [98]. In a follow up SOS study, gastric bypass surgery resulted in 29% reduction in mortality after nearly 11 years compared with the control group [99]. Another study over an 8-year period showed that gastric bypass surgery decreased mortality in severely obese patients by 40% compared to the control group [100].

*Effects of bariatric surgery on diabetes.* Interest in the use of bariatric surgery as a primary treatment for diabetes is growing. A meta-analysis involving 136 studies and 22,094 patients found an overall 77% remission of diabetes after bariatric surgery, 84% for RYGB, 48% for LAG, 68% for VBG, and 98% for BPD [101]. Case series focusing on diabetes outcomes after RYGB have shown a decrease in fasting glucose and HbA1c levels to near normal levels [102, 103]. BPD also normalized glucose levels [104]. Laparoscopic sleeve gastrectomy decreased weight and led to resolution of diabetes at 12 months after surgery, comparable with the results of RYGB [105]. The multicenter SOS study compared bariatric surgery and medical treatment in well-matched obese patients [98, 99]. Bariatric surgery caused an average 16.1% weight loss at 10 years, compared to a small weight increase in controls. Weight loss was greater after RYGB (25.0 kg) than after LAG (13.2 kg) or VBG (16.5 kg). The fasting blood glucose increased in non-surgical controls (18.7%), but decreased substantially in RYGB patients at 2 years (13.6%) and 10 years (2.5%) [98, 99]. The underlying mechanisms for the hypoglycemic effects of RYGB are unclear, but current evidence suggests that changes in GLP-1 and PYY postsurgery may contribute to glucose regulation [106].

*Morbidity and mortality of bariatric surgery.* Despite the concerns about the safety of bariatric surgery, the operative mortality is very low. In a meta-analysis involving 361 studies and 85,048 patients, the total mortality was 0.28% by 30 days postsurgery, and 0.35% between 30 days and 2 years [101]. Similar outcomes were obtained in the longitudinal assessment of bariatric surgery (LABS) Consortium, a 10-center prospective trial involving 4,776 patients undergoing bariatric surgery [107]. Indeed, the mortality rates of bariatric surgery are lower than those of common abdominal operations, including laparoscopic cholecystectomy, which has an operative mortality of 0.3–0.6%. According to the Agency for Healthcare Research and Quality (AHRQ), the number of bariatric surgery procedures in the USA increased ninefold between 1998 and 2004, while death rates declined by 79%, from 0.89 to 0.19% [108]. Between 2002 and 2006, complication rates after bariatric surgery in the United States fell from 24 to 15%, infection rates fell by 58%, and abdominal hernias, staple leakage, respiratory failure, and pneumonia decreased by 29–50% [109, 110]. However, the rates of ulcers, dumping, hemorrhage, wound reopening, deep-venous thrombosis, pulmonary embolism, heart attacks, and strokes did not change [109, 110]. The decline in mortality and morbidity rates for bariatric surgery may be attributable to laparoscopic methods, improved quality-of-care mechanisms, and multidisciplinary team approaches. Common complications of bariatric surgery include anastomotic leaks, wound infections, cardiopulmonary events, and hemorrhage [110]. Dumping syndrome can be problematic [111]. Rarely, patients develop neuroglycopenia after Roux-en-Y gastric bypass surgery, which may require pancreatectomy [112, 113].

Nutritional complications can occur in the long-term following bariatric surgery [114, 115]. Protein malnutrition is seen more often following BPD, but rare after the standard RYGB procedure. Intestinal bypass surgery that circumvents the duodenum and proximal jejunum can cause micronutrient deficiencies, e.g., iron, calcium, and vitamin D. The incidence of iron deficiency after RYGB is 6–33%, and calcium and vitamin D deficiency occurs in 10–51% of patients. Vitamin B<sub>12</sub> and folate deficiency is very common and affects 30 and 60% of gastric bypass patients, respectively. Fat-soluble vitamin deficiency is more common after BPD than Roux-en-Y gastric bypass surgery. Nutritional deficiencies should be anticipated and treated with proper supplementation.

## References

1. Ogden, C. L., Carroll, M. D., Curtin, L. R., McDowell, M. A., Tabak, C. J., & Flegal, K. M. (2006). Prevalence of overweight and obesity in the United States, 1999–2004. *JAMA*, 295(13), 1549–1555.
2. Freedman, D. M., Ron, E., Ballard-Barbash, R., Doody, M. M., & Linet, M. S. (2006) Body mass index and all-cause mortality in a nationwide US cohort. *International Journal of Obesity*, 30(5), 822–829.
3. McTigue, K., Larson, J. C., Valoski, A., et al. (2006). Mortality and cardiac and vascular outcomes in extremely obese women. *JAMA*, 296(1), 79–86.
4. Knowler, W. C., Barrett-Connor, E., Fowler, S. E., et al. (2002). Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *The New England Journal of Medicine*, 346(6), 393–403.
5. Tuomilehto, J., Lindstrom, J., Eriksson, J. G., et al. (2001). Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. *The New England Journal of Medicine*, 344(18), 1343–1350.
6. Sjostrom, C. D., Lissner, L., & Sjostrom, L. (1997). Relationships between changes in body composition and changes in cardiovascular risk factors: The SOS Intervention Study. Swedish Obese Subjects. *Obesity Research*, 5(6), 519–530.
7. Moore, L. L., Vioni, A. J., Qureshi, M. M., Bradlee, M. L., Ellison, R. C., & D’Agostino, R. (2005). Weight loss in overweight adults and the long-term risk of hypertension: The Framingham study. *Archives of Internal Medicine*, 165(11), 1298–1303.
8. Stevens, V. J., Obarzanek, E., Cook, N. R., et al. (2001). Long-term weight loss and changes in blood pressure, results of the Trials of Hypertension Prevention, phase II. *Annals of Internal Medicine*, 134(1), 1–11.
9. Farooqi, I. S., & O’Rahilly, S. (2007). Genetic factors in human obesity. *Obesity Reviews*, 8(suppl 1), 37–40.
10. Greenfield, J. R., Miller, J. W., Keogh, J. M., et al. (2009). Modulation of blood pressure by central melanocortinergic pathways. *The New England Journal of Medicine*, 360(1), 44–52.
11. Lawlor, D. A., Smith, G. D., O’Callaghan, M., et al. (2007). Epidemiologic evidence for the fetal overnutrition hypothesis: Findings from the mater-university study of pregnancy and its outcomes. *American Journal of Epidemiology*, 165(4), 418–424.
12. Reilly, J. J., Armstrong, J., Dorosty, A. R., et al. (2005). Early life risk factors for obesity in childhood: Cohort study. *BMJ*, 330(7504), 1357.
13. Toschke, A. M., Montgomery, S. M., Pfeiffer, U., & von Kries, R. (2003). Early intrauterine exposure to tobacco-inhaled products and obesity. *American Journal of Epidemiology*, 158(11), 1068–1074.
14. Latner, J. D., & Clyne, C. (2008). The diagnostic validity of the criteria for binge eating disorder. *The International Journal of Eating Disorders*, 41(1), 1–14.

15. Stunkard, A. J., Allison, K. C., Geliebter, A., Lundgren, J. D., Gluck, M. E., & O'Reardon, J. P. (2009). Development of criteria for a diagnosis: Lessons from the night eating syndrome. *Comprehensive Psychiatry*, 50(5), 391–399.
16. Gable, S., Chang, Y., & Krull, J. L. (2007). Television watching and frequency of family meals are predictive of overweight onset and persistence in a national sample of school-aged children. *Journal of the American Dietetic Association*, 107(1), 53–61.
17. Whitlock, E. P., Williams, S. B., Gold, R., Smith, P. R., & Shipman, S. A. (2005). Screening and interventions for childhood overweight: A summary of evidence for the US Preventive Services Task Force. *Pediatrics*, 116(1), e125–e144.
18. Williamson, D. F., Madans, J., Anda, R. F., Kleinman, J. C., Kahn, H. S., & Byers, T. (1993). Recreational physical activity and ten-year weight change in a US national cohort. *International Journal of Obesity and Related Metabolic Disorders*, 17(5), 279–286.
19. Gallagher, D., Heymsfield, S. B., Heo, M., Jebb, S. A., Murgatroyd, P. R., & Sakamoto, Y. (2000). Healthy percentage body fat ranges: An approach for developing guidelines based on body mass index. *The American Journal of Clinical Nutrition*, 72(3), 694–701.
20. Price, G. M., Uauy, R., Breeze, E., Bulpitt, C. J., & Fletcher, A. E. (2006). Weight, shape, and mortality risk in older persons: Elevated waist-hip ratio, not high body mass index, is associated with a greater risk of death. *The American Journal of Clinical Nutrition*, 84(2), 449–460.
21. Grundy, S. M., Cleeman, J. I., Daniels, S. R., et al. (2005). Diagnosis and management of the metabolic syndrome: An American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. *Circulation*, 112(17), 2735–2752.
22. Laaban, J. P., Daenen, S., Leger, D., et al. (2009). Prevalence and predictive factors of sleep apnoea syndrome in type 2 diabetic patients. *Diabetes & Metabolism*, 35(5), 372–377.
23. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection. (2001). Evaluation, and treatment of high blood cholesterol in adults (adult treatment panel III). *JAMA*, 285(19), 2486–2497.
24. Wadden, T. A., Womble, L. G., Sarwer, D. B., Berkowitz, R. I., Clark, V. L., & Foster, G. D. (2003). Great expectations: “I’m losing 25% of my weight no matter what you say”. *Journal of Consulting and Clinical Psychology*, 71(6), 1084–1089.
25. Rosenbaum, M., Murphy, E. M., Heymsfield, S. B., Matthews, D. E., & Leibel, R. L. (2002). Low dose leptin administration reverses effects of sustained weight-reduction on energy expenditure and circulating concentrations of thyroid hormones. *The Journal of Clinical Endocrinology and Metabolism*, 87(5), 2391–2394.
26. Klem, M. L., Wing, R. R., McGuire, M. T., Seagle, H. M., & Hill, J. O. (1997). A descriptive study of individuals successful at long-term maintenance of substantial weight loss. *The American Journal of Clinical Nutrition*, 66(2), 239–246.
27. Wadden, T. A., Butryn, M. L., & Byrne, K. J. (2004). Efficacy of lifestyle modification for long-term weight control. *Obesity Research*, 12(suppl), 151S–162S.
28. Orchard, T. J., Temprosa, M., Goldberg, R., et al. (2005). The effect of metformin and intensive lifestyle intervention on the metabolic syndrome: The Diabetes Prevention Program randomized trial. *Annals of Internal Medicine*, 142(8), 611–619.
29. Fuller, P. R., Perri, M. G., Leermakers, E. A., & Guyer, L. K. (1998). Effects of a personalized system of skill acquisition and an educational program in the treatment of obesity. *Addictive Behaviors*, 23(1), 97–100.
30. Harvey-Berino, J. (1998). Changing health behavior via telecommunications technology: Using interactive television to treat obesity. *Behavior Therapy*, 29(3), 505–519.
31. Wadden, T. A., Van Itallie, T. B., & Blackburn, G. L. (1990). Responsible and irresponsible use of very-low-calorie diets in the treatment of obesity. *JAMA*, 263(1), 83–85.
32. Tsai, A. G., & Wadden, T. A. (2006). The evolution of very-low-calorie diets: An update and meta-analysis. *Obesity (Silver Spring)*, 14(8), 1283–1293.
33. Tsai, A. G., & Wadden, T. A. (2005). Systematic review: An evaluation of major commercial weight loss programs in the United States. *Annals of Internal Medicine*, 142(1), 56–66.
34. Rolls, B. J., & Bell, E. A. (2000). Dietary approaches to the treatment of obesity. *The Medical Clinics of North America*, 84(2), 401–418, vi.

35. Lichtman, S. W., Pisarska, K., Berman, E. R., et al. (1992). Discrepancy between self-reported and actual caloric intake and exercise in obese subjects. *The New England Journal of Medicine*, 327(27), 1893–1898.
36. Heymsfield, S. B., van Mierlo, C. A., van der Knaap, H. C., Heo, M., & Frier, H. I. (2003). Weight management using a meal replacement strategy: Meta and pooling analysis from six studies. *International Journal of Obesity and Related Metabolic Disorders*, 27(5), 537–549.
37. Li, Z., Hong, K., Saltsman, P., et al. (2005). Long-term efficacy of soy-based meal replacements vs an individualized diet plan in obese type II DM patients: Relative effects on weight loss, metabolic parameters, and C-reactive protein. *European Journal of Clinical Nutrition*, 59(3), 411–418.
38. Hannum, S. M., Carson, L., Evans, E. M., et al. (2004). Use of portion-controlled entrees enhances weight loss in women. *Obesity Research*, 12(3), 538–546.
39. Hannum, S. M., Carson, L. A., Evans, E. M., et al. (2006). Use of packaged entrees as part of a weight-loss diet in overweight men: An 8-week randomized clinical trial. *Diabetes, Obesity & Metabolism*, 8(2), 146–155.
40. Metz, J. A., Stern, J. S., Kris-Etherton, P., et al. (2000). A randomized trial of improved weight loss with a prepared meal plan in overweight and obese patients: Impact on cardiovascular risk reduction. *Archives of Internal Medicine*, 160(14), 2150–2158.
41. Wing, R. R., Jeffery, R. W., Burton, L. R., Thorson, C., Nissinoff, K. S., & Baxter, J. E. (1996). Food provision vs structured meal plans in the behavioral treatment of obesity. *International Journal of Obesity and Related Metabolic Disorders*, 20(1), 56–62.
42. Makris, A. P., & Foster, G. D. (2005). Dietary approaches to the treatment of obesity. *The Psychiatric Clinics of North America*, 28(1), 117–139, viii–ix.
43. Foster, G. D., Wyatt, H. R., Hill, J. O., et al. (2003). A randomized trial of a low-carbohydrate diet for obesity. *The New England Journal of Medicine*, 348(21), 2082–2090.
44. Nordmann, A. J., Nordmann, A., Briel, M., et al. (2006). Effects of low-carbohydrate vs low-fat diets on weight loss and cardiovascular risk factors: A meta-analysis of randomized controlled trials. *Archives of Internal Medicine*, 166(3), 285–293.
45. Samaha, F. F., Iqbal, N., Seshadri, P., et al. (2003). A low-carbohydrate as compared with a low-fat diet in severe obesity. *The New England Journal of Medicine*, 348(21), 2074–2081.
46. Stern, L., Iqbal, N., Seshadri, P., et al. (2004). The effects of low-carbohydrate versus conventional weight loss diets in severely obese adults: One-year follow-up of a randomized trial. *Annals of Internal Medicine*, 140(10), 778–785.
47. Dumesnil, J. G., Turgeon, J., & Tremblay, A., et al. (2001). Effect of a low-glycaemic index–low-fat–high protein diet on the atherogenic metabolic risk profile of abdominally obese men. *The British Journal of Nutrition*, 86(5), 557–568.
48. Ludwig, D. S., Majzoub, J. A., Al-Zahrani, A., Dallal, G. E., Blanco, I., & Roberts, S. B. (1999). High glycemic index foods, overeating, and obesity. *Pediatrics*, 103(3), E26.
49. McMillan-Price, J., Petocz, P., Atkinson, F., et al. (2006). Comparison of 4 diets of varying glycemic load on weight loss and cardiovascular risk reduction in overweight and obese young adults: A randomized controlled trial. *Archives of Internal Medicine*, 166(14), 1466–1475.
50. Raatz, S. K., Torkelson, C. J., Redmon, J. B., et al. (2005). Reduced glycemic index and glycemic load diets do not increase the effects of energy restriction on weight loss and insulin sensitivity in obese men and women. *The Journal of Nutrition*, 135(10), 2387–2391.
51. Sloth, B., Krog-Mikkelsen, I., Flint, A., et al. (2004). No difference in body weight decrease between a low-glycemic-index and a high-glycemic-index diet but reduced LDL cholesterol after 10-wk ad libitum intake of the low-glycemic-index diet. *The American Journal of Clinical Nutrition*, 80(2), 337–347.
52. Brand-Miller, J., Hayne, S., Petocz, P., & Colagiuri, S. (2003). Low-glycemic index diets in the management of diabetes: A meta-analysis of randomized controlled trials. *Diabetes Care*, 26(8), 2261–2267.
53. Hu, F. B., Willett, W. C., Li, T., Stampfer, M. J., Colditz, G. A., Manson, J. E. (2004). Adiposity as compared with physical activity in predicting mortality among women. *The New England Journal of Medicine*, 351(26), 2694–2703.

54. Lee, C. D., Blair, S. N., & Jackson, A. S. (1999). Cardiorespiratory fitness, body composition, and all-cause and cardiovascular disease mortality in men. *The American Journal of Clinical Nutrition*, 69(3), 373–380.
55. Stevens, J., Cai, J., Evenson, K. R., & Thomas, R. (2002). Fitness and fatness as predictors of mortality from all causes and from cardiovascular disease in men and women in the lipid research clinics study. *American Journal of Epidemiology*, 156(9), 832–841.
56. Jakicic, J. M., Winters, C., Lang, W., & Wing, R. R. (1999). Effects of intermittent exercise and use of home exercise equipment on adherence, weight loss, and fitness in overweight women: A randomized trial. *JAMA*, 282(16), 1554–1560.
57. Slentz, C. A., Duscha, B. D., Johnson, J. L., et al. (2004). Effects of the amount of exercise on body weight, body composition, and measures of central obesity: STRRIDE—a randomized controlled study. *Archives of Internal Medicine*, 164(1), 31–39.
58. Wing, R. R., & Hill, J. O. (2001). Successful weight loss maintenance. *Annual Review of Nutrition*, 21, 323–341.
59. Andersen, R. E., Wadden, T. A., Bartlett, S. J., Zemel, B., Verde, T. J., & Franckowiak, S. C. (1999). Effects of lifestyle activity vs structured aerobic exercise in obese women: A randomized trial. *JAMA*, 281(4), 335–340.
60. King, A. C., Haskell, W. L., Young, D. R., Oka, R. K., & Stefanick, M. L. (1995). Long-term effects of varying intensities and formats of physical activity on participation rates, fitness, and lipoproteins in men and women aged 50 to 65 years. *Circulation*, 91(10), 2596–2604.
61. Wadden, T. A., Foster, G. D., Letizia, K. A., & Mullen, J. L. (1990). Long-term effects of dieting on resting metabolic rate in obese outpatients. *JAMA*, 264(6), 707–711.
62. James, W. P., Astrup, A., Finer, N., et al. (2000). Effect of sibutramine on weight maintenance after weight loss: A randomised trial. STORM Study Group. Sibutramine Trial of Obesity Reduction and Maintenance. *Lancet*, 356(9248), 2119–2125.
63. McMahon, F. G., Fujioka, K., Singh, B. N., et al. (2000). Efficacy and safety of sibutramine in obese white and African American patients with hypertension: A 1-year, double-blind, placebo-controlled, multicenter trial. *Archives of Internal Medicine*, 160(14), 2185–2191.
64. Finer, N., Bloom, S. R., Frost, G. S., Banks, L. M., & Griffiths, J. (2000). Sibutramine is effective for weight loss and diabetic control in obesity with type 2 diabetes: A randomised, double-blind, placebo-controlled study. *Diabetes, Obesity & Metabolism*, 2(2), 105–112.
65. Fujioka, K., Seaton, T. B., Rowe, E., et al. (2000). Weight loss with sibutramine improves glycaemic control and other metabolic parameters in obese patients with type 2 diabetes mellitus. *Diabetes, Obesity & Metabolism*, 2(3), 175–187.
66. Berkowitz, R. I., Fujioka, K., Daniels, S. R., et al. (2006). Effects of sibutramine treatment in obese adolescents: A randomized trial. *Annals of Internal Medicine*, 145(2), 81–90.
67. Berkowitz, R. I., Wadden, T. A., Tershakovec, A. M., & Cronquist, J. L. (2003). Behavior therapy and sibutramine for the treatment of adolescent obesity: A randomized controlled trial. *JAMA*, 289(14), 1805–1812.
68. Godoy-Matos, A., Carraro, L., Vieira, A., et al. (2005). Treatment of obese adolescents with sibutramine: A randomized, double-blind, controlled study. *The Journal of Clinical Endocrinology and Metabolism*, 90(3), 1460–1465.
69. Munro, J. F., MacCuish, A. C., Wilson, E. M., & Duncan, L. J. (1968). Comparison of continuous and intermittent anorectic therapy in obesity. *British Medical Journal*, 1(5588), 352–354.
70. Sjostrom, L., Rissanen, A., Andersen, T., et al. (1998). Randomised placebo-controlled trial of orlistat for weight loss and prevention of weight regain in obese patients. European Multicentre Orlistat Study Group. *Lancet*, 352(9123), 167–172.
71. Davidson, M. H., Hauptman, J., DiGirolamo, M., et al. (1999). Weight control and risk factor reduction in obese subjects treated for 2 years with orlistat: A randomized controlled trial. *JAMA*, 281(3), 235–242.
72. Hauptman, J. (2000). Orlistat: Selective inhibition of caloric absorption can affect long-term body weight. *Endocrine*, 13(2), 201–206.
73. Torgerson, J. S., Hauptman, J., Boldrin, M. N., & Sjostrom, L. (2004). XENical in the prevention of diabetes in obese subjects (XENDOS) study: A randomized study of orlistat as an



- adjunct to lifestyle changes for the prevention of type 2 diabetes in obese patients. *Diabetes Care*, 27(1), 155–161.
74. Chanoine, J. P., Hampl, S., Jensen, C., Boldrin, M., & Hauptman, J. (2005). Effect of orlistat on weight and body composition in obese adolescents: A randomized controlled trial. *JAMA*, 293(23), 2873–2883.
75. Hill, J. O., Hauptman, J., Anderson, J. W., et al. (1999). Orlistat, a lipase inhibitor, for weight maintenance after conventional dieting: A 1-y study. *The American Journal of Clinical Nutrition*, 69(6), 1108–1116.
76. Despres, J. P., Golay, A., & Sjostrom, L. (2005). Effects of rimonabant on metabolic risk factors in overweight patients with dyslipidemia. *The New England Journal of Medicine*, 353(20), 2121–2134.
77. Le Foll, B., Gorelick, D. A., & Goldberg, S. R. (2009). The future of endocannabinoid-oriented clinical research after CB1 antagonists. *Psychopharmacology*, 205(1), 171–174.
78. Goldstein, D. J., Rampey, A. H., Jr., Roback, P. J., et al. (1995). Efficacy and safety of long-term fluoxetine treatment of obesity—maximizing success. *Obesity Research*, 3(suppl 4), 481S–490S.
79. Gadde, K. M., Parker, C. B., Maner, L. G., et al. (2001). Bupropion for weight loss: An investigation of efficacy and tolerability in overweight and obese women. *Obesity Research*, 9(9), 544–551.
80. Jain, A. K., Kaplan, R. A., Gadde, K. M., et al. (2002). Bupropion SR vs. placebo for weight loss in obese patients with depressive symptoms. *Obesity Research*, 10(10), 1049–1056.
81. Anderson, J. W., Greenway, F. L., Fujioka, K., Gadde, K. M., McKenney, J., & O'Neil, P. M. (2002). Bupropion SR enhances weight loss: A 48-week double-blind, placebo-controlled trial. *Obesity Research*, 10(7), 633–641.
82. Wilding, J., Van Gaal, L., Rissanen, A., Vercruyse, F., & Fitchet, M. (2004). A randomized double-blind placebo-controlled study of the long-term efficacy and safety of topiramate in the treatment of obese subjects. *International Journal of Obesity and Related Metabolic Disorders*, 28(11), 1399–1410.
83. Gadde, K. M., Francis, D. M., Wagner, H. R., II, & Krishnan, K. R. (2003). Zonisamide for weight loss in obese adults: A randomized controlled trial. *JAMA*, 289(14), 1820–1825.
84. Fontbonne, A., Charles, M. A., Juhan-Vague, I., et al. (1996). The effect of metformin on the metabolic abnormalities associated with upper-body fat distribution. BIGPRO Study Group. *Diabetes Care*, 19(9), 920–926.
85. Riddle, M. C., & Drucker, D. J. (2006). Emerging therapies mimicking the effects of amylin and glucagon-like peptide 1. *Diabetes Care*, 29(2), 435–449.
86. Ratner, R. E., Dickey, R., Fineman, M., et al. (2004). Amylin replacement with pramlintide as an adjunct to insulin therapy improves long-term glycaemic and weight control in Type 1 diabetes mellitus: A 1-year, randomized controlled trial. *Diabetic Medicine*, 21(11), 1204–1212.
87. Roth, J. D., Roland, B. L., Cole, R. L., et al. (2008). Leptin responsiveness restored by amylin agonism in diet-induced obesity: Evidence from nonclinical and clinical studies. *Proceedings of the National Academy of Sciences of the United States of America*, 105(20), 7257–7262.
88. DeFronzo, R. A., Ratner, R. E., Han, J., Kim, D. D., Fineman, M. S., & Baron, A. D. (2005). Effects of exenatide (exendin-4) on glycemic control and weight over 30 weeks in metformin-treated patients with type 2 diabetes. *Diabetes Care*, 28(5), 1092–1100.
89. Edwards, C. M., Stanley, S. A., Davis, R., et al. (2001). Exendin-4 reduces fasting and postprandial glucose and decreases energy intake in healthy volunteers. *American Journal of Physiology. Endocrinology and Metabolism*, 281(1), E155–E161.
90. Heine, R. J., Van Gaal, L. F., Johns, D., Mihm, M. J., Widell, M. H., & Brodows, R. G. (2005). Exenatide versus insulin glargine in patients with suboptimally controlled type 2 diabetes: A randomized trial. *Annals of Internal Medicine*, 143(8), 559–569.
91. Vilsboll, T., Zdravkovic, M., Le-Thi, T., et al. (2007). Liraglutide, a long-acting human glucagon-like peptide-1 analog, given as monotherapy significantly improves glycemic control and lowers body weight without risk of hypoglycemia in patients with type 2 diabetes. *Diabetes Care*, 30(6), 1608–1610.

92. Weintraub, M., Sundaesan, P. R., Schuster, B., et al. (1992). Long-term weight control study. II (weeks 34 to 104). An open-label study of continuous fenfluramine plus phentermine versus targeted intermittent medication as adjuncts to behavior modification, caloric restriction, and exercise. *Clinical Pharmacology and Therapeutics*, 51(5), 595–601.
93. Connolly, H. M., Crary, J. L., McGoon, M. D., et al. (1997). Valvular heart disease associated with fenfluramine-phentermine. *The New England Journal of Medicine*, 337(9), 581–588.
94. Palmieri, V., Arnett, D. K., Roman, M. J., et al. (2002). Appetite suppressants and valvular heart disease in a population-based sample: The HyperGEN study. *The American Journal of Medicine*, 112(9), 710–715.
95. Elder, K. A., & Wolfe, B. M. (2007). Bariatric surgery: A review of procedures and outcomes. *Gastroenterology*, 132(6), 2253–2271.
96. Buchwald, H., Avidor, Y., Braunwald, E., et al. (2004). Bariatric surgery: A systematic review and meta-analysis. *JAMA*, 292(14), 1724–1737.
97. Dixon, J. B., & O'Brien, P. E. (2002). Health outcomes of severely obese type 2 diabetic subjects 1 year after laparoscopic adjustable gastric banding. *Diabetes Care*, 25(2), 358–363.
98. Sjostrom, L., Lindroos, A. K., Peltonen, M., et al. (2004). Lifestyle, diabetes, and cardiovascular risk factors 10 years after bariatric surgery. *The New England Journal of Medicine*, 351(26), 2683–2693.
99. Sjostrom, L., Narbro, K., Sjostrom, C. D., et al. (2007). Effects of bariatric surgery on mortality in Swedish Obese Subjects. *The New England Journal of Medicine*, 357(8), 741–752.
100. Adams, T. D., Gress, R. E., Smith, S. C., et al. (2007). Long-term mortality after gastric bypass surgery. *The New England Journal of Medicine*, 357(8), 753–761.
101. Buchwald, H., Estok, R., Fahrbach, K., Banel, D., & Sledge, I. (2007). Trends in mortality in bariatric surgery: A systematic review and meta-analysis. *Surgery*, 142(4), 621–632; discussion 632–635.
102. Pories, W. J., Swanson, M. S., MacDonald, K. G., et al. (1995). Who would have thought it? An operation proves to be the most effective therapy for adult-onset diabetes mellitus. *Annals of Surgery*, 222(3), 339–350; discussion 350–352.
103. Schauer, P. R., Burguera, B., Ikramuddin, S., et al. (2003). Effect of laparoscopic Roux-en Y gastric bypass on type 2 diabetes mellitus. *Annals of Surgery*, 238(4), 467–484; discussion 484–485.
104. Scopinaro, N., Marinari, G. M., Camerini, G. B., Papadia, F. S., & Adami, G. F. (2005). Specific effects of biliopancreatic diversion on the major components of metabolic syndrome: A long-term follow-up study. *Diabetes Care*, 28(10), 2406–2411.
105. Vidal, J., Ibarzabal, A., Romero, F., et al. (2008). Type 2 diabetes mellitus and the metabolic syndrome following sleeve gastrectomy in severely obese subjects. *Obesity Surgery*, 18(9), 1077–1082.
106. Thaler, J. P., & Cummings, D. E. (2009). Minireview: Hormonal and metabolic mechanisms of diabetes remission after gastrointestinal surgery. *Endocrinology*, 150(6), 2518–2525.
107. Flum, D. R., Belle, S. H., King, W. C., et al. (2009). Perioperative safety in the longitudinal assessment of bariatric surgery. *The New England Journal of Medicine*, 361(5), 445–454.
108. Zhao, Y., & Encinosa, W. (2007). Bariatric surgery utilization and outcomes in 1998 and 2004, Statistical brief #23. <http://www.hcup-us.ahrq.gov/reports/statbriefs/sb23.pdf>.
109. Encinosa, W. E., Bernard, D. M., Du, D., & Steiner, C. A. (2009). Recent improvements in bariatric surgery outcomes. *Medical Care*, 47(5), 531–535.
110. Nguyen, N. T., Hinojosa, M., Fayad, C., Varela, E., & Wilson, S. E. (2007). Use and outcomes of laparoscopic versus open gastric bypass at academic medical centers. *Journal of the American College of Surgeons*, 205(2), 248–255.
111. Tack, J., Arts, J., Caenepeel, P., De Wulf, D., & Bisschops, R. (2009). Pathophysiology, diagnosis and management of postoperative dumping syndrome. *Nature Reviews. Gastroenterology & Hepatology*, 6(10), 583–590.
112. Goldfine, A. B., Mun, E. C., Devine, E., et al. (2007). Patients with neuroglycopenia after gastric bypass surgery have exaggerated incretin and insulin secretory responses to a mixed meal. *The Journal of Clinical Endocrinology and Metabolism*, 92(12), 4678–4685.



113. Service, G. J., Thompson, G. B., Service, F. J., Andrews, J. C., Collazo-Clavell, M. L., & Lloyd, R. V. (2005). Hyperinsulinemic hypoglycemia with nesidioblastosis after gastric-bypass surgery. *The New England Journal of Medicine*, 353(3), 249–254.
114. Brolin, R. E., Gorman, R. C., Milgrim, L. M., & Kenler, H. A. (1991). Multivitamin prophylaxis in prevention of post-gastric bypass vitamin and mineral deficiencies. *International Journal of Obesity*, 15(10), 661–667.
115. Halverson, J. D. (1986). Micronutrient deficiencies after gastric bypass for morbid obesity. *The American Surgeon*, 52(11), 594–598.



# Index

## A

- Acipimox, 351
- Adenosine triphosphate (ATP), 1
- Adipokines
  - adipocytes, 70
  - adiponectin
    - CNS action, 78–79
    - and metabolic syndrome, 77–78
    - regulation, 76
    - signaling, 76–77
  - autocrine and paracrine mechanism, 70
  - leptin
    - glucose metabolism, 75
    - immunity effects, 75
    - in neurons, 74–75
    - regulation, 71
    - resistance, 73–74
    - signaling, 71–73
  - proinflammatory cytokines
    - interleukin-6, 82
    - TNF- $\alpha$ , 81–82
  - resistin
    - CNS effects, 80
    - human resistin, 80–81
    - regulation, 79–80
  - retinol binding protein-4, 82
  - white adipose tissue, 69
- Adiponectin
  - cardiac dysfunction, 272–273
  - CNS action, 78–79
  - enhanced lipolysis, 298
  - long-term regulation,
    - energy stores, 100
  - and metabolic syndrome, 77–78
  - pancreatic  $\beta$ -cell function, 207–208
  - regulation, 76
  - signaling, 76–77
- Adipose leukocyte infiltration
  - macrophages
    - human obesity, 159
    - infiltration and activation, 158–159
    - in mice, 159
  - T-cell modulation, 160–161
- Adipose stem cells (ASCs), 57
- Adipose tissue
  - anatomical distribution and structure, 55–57
  - biochemical properties, 53–54
  - brown adipose tissue
    - differentiation programme, 61
    - function, 61
    - in human adult, 63–64
    - in vivo regulation, 62–63
  - dysfunction and inflammation (see Inflammation)
  - functions, 53
  - pathogenesis, PCOS, 334
  - physiological system, 54–55
  - white adipose tissue
    - differentiation programme, 58–59
    - progenitor identification, 60–61
    - in vivo regulation, 57
- Adipose tissue macrophages (ATMs), 56
- Adipose triglyceride lipase (ATGL), 41, 297
- Agouti-related peptide (AGRP), 72, 90–91
- Ahlstrom syndrome, 360
- Amino acid metabolism, 45–47
- AMP-activated protein kinase (AMPK)
  - activation, SIRT1 system, 48
  - cellular metabolism changes, 47
  - leptin signaling, neurons, 74
  - lipolysis inhibition, 298
  - metabolic regulation, 47–48

**Amylin**

- diabetic drug, 370
- islet–hypothalamic axis regulation, 204–205
- pancreatic hormone, 116–117
- short-term regulation, 99
- weight loss, 370

**Apolipoprotein C-III (apoC-III), 301****Arginine vasopressin (AVP), 203****Atherogenic dyslipidemia**

- apolipoprotein-B synthesis, 301
- hepatic lipogenesis, 299–300
- hypercatabolism, HDL, 302–303
- impaired catabolism, lipoprotein remnants, 301–302
- visceral adipose tissue, 293
- VLDL overproduction
  - adipose tissue fatty acid storage, 296–297
  - adipose tissue, liver, 295
  - enhanced lipolysis, 296, 297–298
  - FFA availability, 294
  - lipoprotein lipase activity, 295–296
  - mechanism, 294–295

**B*****Bacteroides thetaiotaomicron*, 312*****Bacteroidetes*, 317****Bardet-Biedl syndrome, 360****Bariatric surgery**

- effects, 20–21
- morbidity and mortality, 21–22

**Basal metabolic rate (BMR), 3****Blood-brain barrier (BBB), 92****Body mass index (BMI), 127, 258, 359****Brown adipose tissue (BAT)**

- differentiation, 61
- function, 55, 61
- haematoxylin–eosin stained section, 55
- in human adult, 63–64
- in vivo regulation, 62–63

**Brown fat uncoupling protein, 39–40****Bupropion, 370****C****Carbohydrate and energy metabolism**

- brown fat and uncoupling proteins, 39–40
- citric acid cycle, 32
- fructose, 31
- gluconeogenesis, 32–34
- glucose transport and phosphorylation, 27–28

**glycogen, 35–37****glycolytic pathway, 29–31****oxidative phosphorylation, 37–39****pyruvate dehydrogenase, 31–32****Carcino-embryonic antigen-related****cell adhesion molecule 1****(CEACAM1), 219****Cardiac adiposity, 262–263****Cardiac dysfunction**

- clinical consideration, 278–279
- co-morbidities
  - diabetes (hyperglycemia), 264–265
  - hypertension, 265–266
  - sleep apnea, 266

**dyslipidemia, 257****functional changes**

- LV diastolic function, 260–261
- LV systolic function, 259–260
- RV function, 261
- vascular function, 261

**heart failure, obesity, 258–259****mechanical changes, animal model**

- altered cardiac substrate metabolism, 267
- lipotoxicity, 269–271
- mitochondrial dysfunction, 268–269
- mitochondrial uncoupling, 267–268
- in obesity and diabetes, 266–267

**multiple mechanisms, 279****obesity paradox, 263–264****postulated mechanism**

- adiponectin effect, 272–273
- cardiomyocyte apoptosis, 276–277
- ECM and fibrosis, 275–276
- inflammation, 277
- leptin effect, 273–275
- neurohumoral activation, 271

**sleep disorder, 257****structural changes, obesity**

- cardiac adiposity, 262–263
- cardiac tissue composition, 262
- left atrial size, 262
- left ventricular hypertrophy, 261
- right ventricular cardiac hypertrophy, 262
- valvular heart disease, 262

**Cardiometabolic disease**

- circadian misalignment, 235
- feeding time, 235–236
- gene, metabolic regulation, 237–238
- gene, sleep/wake phenotypes, 236–237
- sleep quantity and quality impact, 234–235

**Cardiomyocyte apoptosis, 276–277**

## Chemokines

- adipose tissue, 160
- secretome, 164

## Cholecystokinin (CCK)

- feeding and energy homeostasis, 97
- short-term regulation, 98
- upper gastrointestinal tract, 111–112

## Chyme, 2

## Circadian rhythms

- cardiometabolic diseases
  - circadian misalignment, 235
  - feeding time, 235–236
  - gene and sleep/wake phenotypes, 236–237
  - metabolic regulation and gene, 237–238
  - sleep quantity and quality impact, 234–235
- and metabolism
  - amino acids, 244
  - carbohydrates, 244
  - cellular energy status, 244–246
  - core clock molecular network, 230–233
  - endotoxins, 247–248
  - FAA, 241
  - fatty acids/lipids, 243–244
  - ghrelin, 248
  - hormonal mediators, 246–247
  - lipid messenger, 247
  - peripheral clocks, regulation, 233–234
- neurophysiological structure
  - energy center and sleep/wakefulness center, 240–241
  - sleep centers, 238–240
  - sleep quantity and quality impact, 234–235

## Citric acid cycle, 32

## c-Jun N-terminal kinase (JNK), 156

Classical hormones. *See* Specific hormones

## Cocaine and amphetamine-regulated transcript (CART), 72

## Cohen syndrome, 360

## Copy number variation (CNV), 129

## Cori cycle, 34

## Cushing's syndrome

- causes, 143
- diagnosis, 143–144
- glucocorticoids (GC)
  - (*see* Glucocorticoids (GC))
- linking cortisol and metabolic syndrome, 13–14
- sign and symptoms, 143
- types, 143
- vs. pseudo-Cushing's syndrome, 144–145

## Cytokines, 163–164

**D**

- Data Collection on Adverse Events of Anti-HIV Drugs (DAD), 348
- Dehydroepiandrosterone sulfate (DHEAS), 333
- Dexamethasone suppression test (DST), 144
- Diabetes (hyperglycemia), 264–265
- Diabetes Control and Complications Trial (DCCT), 141
- Diabetes Prevention Program (DPP), 360
- Dietary-induced thermogenesis (DIT), 5
- Dinitrophenol, 39. *See also* Intermediary metabolism
- DNA microarray, 314
- DNA sequencing technology, 325
- Dorsomedial nucleus (DMN), 96
- Dyslipidemia. *See also* Atherogenic dyslipidemia
  - coronary atherosclerosis, 257
  - free fatty acid flux, 294, 295
  - ischemic heart disease, 257
  - metabolic atherogenic triad, 293
  - treatment, HIV-infected patients, 350, 351

**E**

- Electron transport chain. *See* Oxidative phosphorylation
- Endogenous cannabinoids, 118
- Endothelial lipase (EL), 303
- Energy balance and obesity
  - carbohydrate, 13
  - chronic imbalance, 12
  - fat, 13, 14
  - homeostasis, 10, 11
  - macronutrient oxidation, 12
  - protein, 12–13
- Energy expenditure
  - basal metabolic rate (BMR)
    - body weight estimation, 3
    - databases, 3–4
    - fat-free mass, 4
    - regulation, SNS activity, 5
  - measurement
    - direct calorimetry, 8
    - doubly labeled water method, 10
    - factorial method, 10
    - indirect calorimetry, 8–10
  - physical activity
    - daily activities, 5–6
    - PAL, 6–7
  - resting metabolic rate (RMR), 3
  - thermic effect of food, 5
- Epigenetic factors, 57

Epigenetic modification  
 changes, gene expression, 133, 134  
 environmental effects, obesity, 134  
 genomic imprinting, 133

Exenatide, 371

Extracellular matrix (ECM), 275

Extracellular signal-regulated kinase  
 (ERK), 164

## F

Fat-free mass (FFM), 4

Fatty acid

oxidation

activation, 42

$\beta$ -oxidation, 43–44

translocation, 42–43

synthesis, 45

Fatty acid synthase (FAS), 223

Female reproductive health

childhood obesity, girls, 331

clinical data, 331

energy homeostasis

leptin, 332–333

white adipose tissue, 332

maternal and fetal complications, 331

pathogenesis, PCOS

adipose tissue, 334

cross talk between leptin and insulin

signaling pathway, 336–337

LH and FSH synthesis, 334

obesity, 334–336

*Firmicutes*, 317

Fluorescence-activated cell sorting (FACS), 60

Fluoxetine, 370

Follicle stimulating hormone (FSH), 332

Food anticipatory activity (FAA), 241

Food quotient (FQ), 12

Free fatty acid (FFA)

adipose inflammation, 158

adipose lipolysis, 53

availability, VLDL production, 294

TLR-4 activation, 321–322

Fructose metabolism, 31

## G

Galanin, 202

Gastrin-releasing polypeptide (GRP), 117

Gastrointestinal hormones

bombesin-related peptides

endogenous cannabinoids, 118

glucagon-like peptide-2 (GLP-2), 118

G-protein, 117

neuromedin U (NMU), 118

distal gut

oxyntomodulin (OXM), 114

peptide YY, 115

homeostasis, 110

hormonal interactions, 118–119

pancreatic hormones

amylin, 116–117

pancreatic polypeptide (PP), 116

physiological role, 110

upper gastrointestinal tract

cholecystokinin (CCK), 111–112

ghrelin, 110–111

incretins and enteroinsular axis,  
 112–114

Gene

candidate genes, 128–129

environmental interaction, 128

epigenetic modification, 133–134

heritability, 127–128

increase, obesity rates, 127

linkage, 130

obesity, prevention and therapy, 135

unanticipated gene, 133

variants, 129–130, 132–133

WGA studies

advantages, 130

genome-wide significance,  
 131–132

marker identification, 130

Genome wide association studies (GWAS),  
 57, 132

Genomic fingerprinting technique, 314

Ghrelin

circadian rhythms, 248

feature, 98

GHS-Rs, 205

Prader-Willi syndrome, 98

significance, islet  $\beta$ -cell, 205

treatment, 98

upper gastrointestinal tract,  
 110–111

Glitazones, 352

Glucagon-like peptide-1 (GLP-1),  
 112–113, 200

Glucagon-like peptide-2 (GLP-2), 118

Glucocorticoids (GC), 100

Cushing's syndrome

clinical manifestation, 143

diagnosis, 143–144

linking cortisol and metabolic  
 syndrome, 145–146

vs. pseudo-Cushing's syndrome,  
 144–145

receptor, 142

secretion of, 142

- Gluconeogenesis
    - citric acid cycle, 33, 34
    - cori cycle, 34
    - glycerol, 34
    - glycolytic regulation, 34
    - in liver, 32
    - pyruvate carboxylase synthesis, 33
    - uses, 32
  - Glucose-dependent insulinotropic polypeptide (GIP), 113–114
  - Glucose metabolism, 27–28
  - Glucose toxicity, 191
  - Glutamine, 47
  - Glycogen metabolism
    - breakdown by
      - phosphorylase, 35
      - regulatory hormones, 36–37
    - insulin stimulation, 37
    - liver and muscle, 19
    - synthesis, 35
  - Glycogen synthase kinase 3 (GSK3), 183
  - Glycolysis, 29–31
  - Gonadotropin releasing hormone (GnRH), 332
  - Growth hormone (GH)
    - deficiency, HIV-infected patients, 353
    - metabolic complication, GHD, 149–150
    - secretion, 149
  - Growth hormone deficiency (GHD), 149
  - Growth hormone releasing hormone (GHRH), 149, 353
  - Gut microbiome
    - characterization, 313–315
    - diet
      - alterations, composition of, 319
      - regulation, 319–320
      - Western diet, 319
    - ecology
      - B. thetaiotaomicron*, 312–313
      - inflammatory bowel disease, 312
      - role, host metabolism, 312
      - short chain fatty acids, 313
      - vitamin synthesis, 313
    - innate immunity and obesity
      - adipokines, 320
      - insulin resistance, 320
      - TLR-5 activation, 321
      - TLR-4 signaling, 321–322
      - toll-like receptors, 320
    - metabolomics analysis
      - metabolic reactions, 316
      - paradigm models, 317
      - proton NMR/mass spectroscopy, 316
  - metagenomic analysis
    - DNA sequences, 315
    - fecal samples, 315–316
    - metabolic function, 315
  - nitrogen balance
    - intake, dietary proteins, 324
    - loss of, 323
    - urea cycle disorders, 324–325
    - urea hydrolysis, 323
  - obesity
    - composition, twins, 318
    - DIO model, ob/ob mouse, 317–318
    - energy input, 317
    - future studies, 318–319
    - mechanism, 318
    - in murine systems, 317
    - physical activity, 317
    - short-chain fatty acids, 318
    - thermodynamic first law, 317
    - type 1 diabetes (T1D), 322–323
- ## H
- Hepatic insulin resistance, 299–300
  - Hepatic lipase (HL), 302
  - High density lipoprotein (HDL), 293
  - Highly active antiretroviral therapy (HAART), 343
  - HIV lipodystrophy. *See* Lipodystrophy, HIV patient
  - Homeostasis, 155
  - Hormone sensitive lipase (HSL), 297
  - Human energy metabolism
    - availability, 1
    - chyme, 2
    - definition, 1
    - energy balance and obesity
      - carbohydrate, 13
      - fat, 14
      - protein, 12–13
    - energy expenditure
      - basal and resting metabolic rate, 3–5
      - measurement, 8–10
      - physical activity, 5–7
      - thermic effect of food, 5
    - from food, 1–2
    - oxidative pathways, 1
    - risk factors, weight gain
      - adaptive thermogenesis, 17–18
      - low fat oxidation, 16
      - low metabolic rate, 14–15
      - low physical activity, 15–16
      - sympathetic nervous activity, 18
    - utility, 1, 2



11 $\beta$ -Hydroxysteroid dehydrogenase type 1 (11 $\beta$ -HSD1)  
 expression and activity, 145–146  
 metabolic syndrome, 145

Hyperinsulinemia  
 insulin clearance, 222  
 metabolic syndrome, 179–180  
 PCOS, 335

Hypertension, 265–264

Hypocretins. *See* Orexins

Hypothalamus  
 arcuate nucleus  
   circumventricular organ (CVO), 94  
   leptin and insulin signaling, 92  
   neuronal circuitry, 91  
   neuron populations, 90–91  
   pharmacological effects, 91, 93–95  
 dorsomedial nucleus (DMN), 96  
 lateral hypothalamic area, 97  
 paraventricular nucleus (PVN), 96  
 role, 89–90  
 ventromedial nucleus (VMN), 96

Hypothyroidism  
 causes, 148  
 cholesterol synthesis and insulin sensitivity, 139  
 obesity, 148–149  
 sign and symptoms, 147–148  
 treatment, 148

Hypoxia, 157

## I

IGF binding protein 2 (IGFBP2), 75

Inflammation  
 adipose leukocyte infiltration  
   adipose macrophages, 158–159  
   T-cell modulation, 160–161  
 adipose-specific activation  
   adipocyte hypertrophy, 156  
   cellular stress, 158  
   free fatty acids, 158  
   hypoxia, 157  
 gene expression profiling, 166  
 insulin resistance, 188–190  
 insulin signaling pathway  
   effects, 161, 162  
   endotoxemia and adipose TLR signaling, 161–163  
   intracellular kinase, role, 164–165  
   secretome, 163–164  
   SOCS protein, 165  
   transcription factor, 165–166  
 proteomics, 167

systemic activation  
   homeostasis, 155  
   metabolic endotoxemia, 156  
   TLR, 155  
 therapeutic implications, 167  
   existing therapy, 167  
   leukocyte infiltration, 168–169  
   novel strategy, 167–168

Inflammatory bowel disease (IBD), 312

Innate immunity, gut microbiome  
 adipokines, 320  
 insulin resistance, 320  
 TLR-5 activation, 321  
 TLR-4 signaling, 321–322  
 toll-like receptors, 320

Insulin  
 action of, 140  
 carbohydrate metabolism regulator, 26  
 de novo lipogenesis, 141  
 glucose utilization, 140  
 islet-hypothalamic axis regulation, 203–204  
 lipid metabolism, 140–141  
 long-term regulation, energy stores, 100  
 obesity, 141–142  
 pathways, 140

Insulinoma, 142

Insulin resistance. *See also* Metabolic syndrome  
 clinical vs. molecular, 176–177  
 HIV lipodystrophy, 344  
 in humans, 192–193  
 insulin signaling pathway  
   Akt/protein kinase B, 182  
   AS160, 183  
   atypical PKCs, 182–183  
   CAP/Cbl Pathway, 182  
   FoxO1, 184  
   glycogen synthase kinase 3, 183  
   insulin receptor substrate proteins, 180–181  
   MAP-kinase, 181–182  
   mTOR, 183  
   network, 178  
   PI 3-kinase, 181  
   receptor, 178–180  
   SREBP-1c, 184–185  
 metabolic effects  
   IRS, 186–187  
   LIRKO mice, 186  
   PI 3-kinase, 187  
   PKC- $\lambda$  knockout, 187–188

- molecular mechanism
    - endoplasmic reticulum (ER) stress, 190–191
    - glucose toxicity, 191
    - inflammation, 188–190
    - IRS-1 Ser 307 phosphorylation, 191–192
    - lipotoxicity, 190
  - Insulin signaling pathway
    - effects, 161, 162
    - endotoxemia and adipose TLR signaling, 161–163
    - insulin resistance
      - Akt/protein kinase B, 182
      - AS160, 183
      - atypical PKCs, 182–183
      - CAP/Cbl Pathway, 182
      - FoxO1, 184
      - glycogen synthase kinase 3, 183
      - insulin receptor substrate proteins, 180–181
      - MAP-kinase, 181–182
      - mTOR, 183
      - network, 178
      - PI 3-kinase, 181
      - receptor, 178–180
      - SREBP-1c, 184–185
    - intracellular kinase, role
      - JAK/STAT system, 165
      - MAPK family, 164–165
      - NFκB, 164
    - secretome
      - chemokines, 163–164
      - cytokines, 164
    - SOCS protein, 165
    - transcription factors, 165–166
  - Insulin tolerance test (ITT), 150
  - Interferon regulatory factors (IRFs), 166
  - Interleukin-6 (IL-6), 82, 208–209
  - Intermediary metabolism
    - an integrated modulator of cellular metabolism (AMPK), 47–48
    - carbohydrate and energy metabolism
      - brown fat and uncoupling proteins, 39–40
    - citric acid cycle, 31–32
    - fructose, 31
    - gluconeogenesis, 32–34
    - glucose transport and phosphorylation, 27–28
    - glycogen, 35–37
    - glycolysis, 29–31
    - oxidative phosphorylation, 37–39
    - pyruvate dehydrogenase, 31–32
    - protein and amino acid, 45–47
    - regulation, 26–27
    - transgenic model
      - adipose insulin-stimulated glucose, 41
      - MIRKO mice, 40
    - triglyceride and fatty acid
      - fatty acid oxidation, 42–44
      - fatty acid synthesis, 45
      - ketone bodies, 44
      - lipolysis, 40–42
      - triglyceride synthesis, 44–45
  - Intramyocellular lipid (IMCL), 345
  - Islet amyloid polypeptide (IAPP). *See* Amylin
- J**
- JAK-2/STAT-3 pathway, 336
- L**
- Laparoscopic adjustable gastroplasty (LAG), 371
- Left ventricular hypertrophy (LVH), 261
- Leptin
  - adipokines
    - glucose metabolism, 75
    - immunity effects, 75
    - in neurons, 74–75
    - regulation, 71
    - resistance, 73–74
    - signaling, 71–73
  - cardiac dysfunction, 273–275
  - hypothalamic-pituitary axis
    - bimodal effects, 332
    - hypoleptinemic lipodystrophic, 333
    - nuclear and cytoplasmic maturation enhancement, 333
  - and insulin-signaling pathways, PCOS, 336–337
  - islet-hypothalamic axis regulation, 206–207
  - lipodystrophy treatment
    - (*see* Lipodystrophy, HIV patient)
  - long-term regulation, energy stores, 99–100
  - reproduction, 332
  - signaling, 333
- Leukocyte infiltration, 168–169
- Lipodystrophy, HIV patient
  - antiretroviral therapy, body composition and metabolic changes
  - metabolic functions, 346
  - NRTI, 347
  - PI effects, 346

- Lipodystrophy, HIV patient (*cont.*)
- cardiovascular risk
    - DAD study group, 348
    - factors, 349
    - Framingham risk equation, 348
    - IMT marker, 350
    - myocardial infarction rates, 349
    - SMART study, 349–350
    - vs. non-HIV-infected patients, 348–349
  - dyslipidemia treatment
    - acipimox, 351
    - changes, visceral adipose tissue, 353–354
    - GH deficiency, 353
    - GHRH usage, 353
    - glitazones, 352
    - HMGCoA reductase inhibitors, 351
    - leptin, 352–353
    - metformin, 351–352
    - niacin, 351
    - rosiglitazone, 352
    - thiazolidinediones, 352
  - etiology, 343
  - genetic polymorphism, 347
  - HAART, 343
  - metabolic and anthropometric changes
    - fat redistribution, 346
    - IMCL and insulin-stimulated glucose disposal, 345
    - insulin resistance, 344
    - lipolytic rates, 346
- Lipolysis
- free fatty acid flux, adipose tissue, 295
  - insulin resistance, 297–298
  - triglyceride metabolism, 40–42
- Lipopolysaccharide (LPS), 320
- Lipoprotein lipase (LPL), 295
- Lipotoxicity
- cardiac dysfunction, 269–270
  - molecular mechanism, 190
  - transgenic models, 270–271
- Liver-specific insulin receptor knockout
- mouse (LIRKO), 223
- Low density lipoprotein (LDL), 293
- Luteinizing hormone (LH), 332
- M**
- Matrix metalloproteinases (MMPs), 275
- Melanin-concentration hormone (MCH), 201
- Melanocortin-4 receptor (MC4R), 360
- Metabolic syndrome, 222.
- See also* Non-alcoholic fatty liver disease (NAFLD)
  - adiponectin, 77–78
  - diagnostic criteria, 175, 176
  - 11 $\beta$ -hydroxysteroid dehydrogenase type 1, 145
  - hyperinsulinemia, 179–180
  - insulin resistance (*see* Insulin resistance)
- Metabolomics analysis, gut microbiome
- metabolic reactions, 316
  - paradigm models, 317
  - proton NMR/mass spectroscopy, 316
- Metagenomic analysis, gut microbiome
- DNA sequences, 315
  - fecal samples, 315–316
  - metabolic function, 315
- Metformin, 335, 351–352
- Microsomal triglyceride transfer protein (MTP), 301
- Mitochondrial dysfunction, 268–269
- Mitochondrial uncoupling, 267–268
- Mitogen-activated protein kinase (MAPK)
- signaling pathway, 164, 336
- Multiple-hit hypothesis, 224
- Myocardial infarction (MI), 349
- N**
- N-acylphosphatidylethanolamine (NAPE), 247
- Neural control, feeding and energy homeostasis
- adiposity hormones, 99–100
  - biogenic amine pathways, 100–101
  - brain-gut-adipose interactions, 90
  - brainstem regulation, 97
  - hypothalamic regulation
    - arcuate nucleus, 90–95
    - dorsomedial nucleus, 96
    - lateral hypothalamic area, 97
    - paraventricular nucleus, 96
    - ventromedial nucleus, 96
  - hypothalamus role, 89–90
  - nutrients and gut hormones
    - amylin, 99
    - cholecystokinin, 98
    - ghrelin, 98
    - GLP-1 and GLP-2, 99
    - oxyntomodulin, 99
    - PYY, 99
- Neuromedin U (NMU), 118
- Neuropeptide regulations,
- pancreatic islet  $\beta$ -cells
    - AVP, 203
    - galanin, 202
    - MCH, 201–202
    - NPY and orexins, 202
    - somatostatin, 203

- Niacin, 351
- Nitrogen balance, gut microbiome  
 intake, dietary proteins, 324  
 loss of, 323  
 urea cycle disorders, 324–325  
 urea hydrolysis, 323
- Non-alcoholic fatty liver disease (NAFLD)  
 hepatic insulin resistance, 219, 221  
 history, 219, 220  
 insulin endocytosis, CEACAM1, 219, 221  
 intertwined paths, insulin and metabolism  
 CEACAM1, 222–223  
 fatty acid synthase, 223  
 hyperinsulinemia, 222  
 metabolic syndrome, 222  
 pathogenesis, NASH  
 Ceacam1 mutant mice, 224–225  
 hepatic steatosis, 219, 220
- Nonalcoholic steatohepatitis (NASH). *See*  
 Non-alcoholic fatty liver disease  
 (NAFLD)
- Nuclear factor kappa-light-chain-enhancer  
 of activated B cells (NFκB), 156
- Nucleoside reverse transcriptase inhibitor  
 (NRTI), 343
- Nucleus of the solitary tract (NTS), 74
- O**
- Obesity paradox, 263–264
- Operational Taxonomic Units (OTUs), 314
- Orexins, 202
- Orlistat  
 clinical trials, 15–16  
 diabetic patient, 16  
 dosage, 16–17  
 effective, weight loss, 16  
 obesity treatment, 278
- Oxidative phosphorylation, 37–39
- Oxyntomodulin (OXM), 99, 114
- P**
- Pancreatic islet β-cells  
 adipokines and cytokines  
 adiponectin, 207–208  
 interleukin-6, 208–209  
 leptin, 206–207  
 resistin, 208  
 gut peptide regulations  
 amylin, 204–205  
 ghrelin, 205  
 GLP-1, 205–206  
 insulin, 203–204  
 inhibition of, 200, 201  
 neuropeptide regulations  
 arginine vasopressin (AVP), 203  
 galanin, 202  
 MCH, 201–202  
 NPY, 202  
 orexins, 202  
 somatostatin, 203  
 potential therapeutic approaches, 209–210  
 stimulation of, 200
- Pancreaticobiliary diversion (BPD), 371
- Pancreatic polypeptide (PP), 116
- Paraventricular nucleus (PVN), 72, 96
- Partial insulin resistance, 299–300
- PCoA, geometric technique, 314
- Peptide YY (PYY), 99, 115
- Phosphofructo-2-kinase/fructose-2,6-bisphos-  
 phatase (PFKFB), 30
- Physical activity level (PAL), 6
- Pioglitazone, 352
- Polycystic ovary syndrome (PCOS)  
 androgen production, 334  
 childhood obesity, girls, 331  
 DHEAS, 333–334  
 etiology, 139  
 leptin and insulin signaling pathway,  
 336–337  
 LH and FSH synthesis, 334  
 obesity, 334–336
- Polymerase chain reaction (PCR)-based  
 analysis, 315
- Prader-Willi syndrome, 98, 360
- Principal coordinate analysis (PCoA), 314
- Proopiomelanocortin (POMC), 72, 91
- Protease inhibitor (PI), 343
- Protein kinase A (PKA), 30
- Protein metabolism, 45–47
- Pseudo-Cushing's syndrome, 144–145
- Pyruvate dehydrogenase (PDH), 31–32
- Q**
- Quantitative trait loci (QTL), 133
- R**
- Resistin  
 CNS effects, 80  
 islet-hypothalamic axis regulation, 208  
 in mice and human, 80–81  
 regulation, 79–80
- Resistin-like molecules (RELMs), 79
- Resting energy expenditure (REE), 148
- Resting metabolic rate (RMR), 3
- Right ventricular cardiac hypertrophy, 262
- Rimonabant, 370

Rosiglitazone, 352  
16S rRNA gene sequencing, 314

## S

Sarcopenic obesity, 258  
Sibutramine  
    clinical trial, 12–13  
    diabetic patient, 13  
    dosage, 14  
    feeding regulation and energy  
        homeostasis, 101  
    obese children, 14  
    obesity treatment, 278  
    side effect, 13  
Sleep. *See also* Circadian rhythms  
    clinical evidence  
        animal studies, 234–235  
        human studies, 234  
    genetic evidence, human and animal model  
        circadian genes, metabolism regulation,  
            237–238  
        circadian genes, wake phenotypes,  
            236–237  
    neurophysiological structure  
        centers, 238–240  
        energy center and wakefulness centers,  
            240–241  
Sleep apnea, 266  
Somatostatin, 203  
Specific dynamic action (SDA), 5  
Stavudine, 347  
Steroid hormone binding-globulin (SHBG),  
    335  
Sterol regulatory element-binding protein  
    1c (SREBP-1c), 222  
Strategies for Management of Antiretroviral  
    Therapy (SMART) study, 349  
Stromovascular fraction (SVF), 56  
Suppressor of cytokine signaling (SOCS),  
    165, 189  
Swedish Obese Subjects Study (SOS), 371  
Sympathetic nervous system (SNS), 4  
Sympathomimetic drugs, 368–369

## T

Therapy  
    amylin, 370  
    bariatric surgical treatment  
        effects, diabetes, 372  
        morbidity and mortality, 372  
        nutritional deficiency, 373  
        SOS study, 371–372

body-mass index, 359  
bupropion, 370  
clinical evaluation  
    history, 360–362  
    laboratory analysis, 363–364  
    physical examination, 362–363  
definition, obesity, 359  
dietary plan  
    conventional foods, 366  
    low calorie diet, 365  
    low glycemic index diets, 366  
    meal replacement, 366  
    total energy expenditure, 365  
    VLCD, 365  
exenatide, 371  
exercise, 366–367  
fluoxetine, 370  
lifestyle changes, 364–365  
orlistat, 369–370  
rimonabant, 370  
risk assessment and treatment, 364–365  
sibutramine, 367–368  
sympathomimetic drugs, 368–369  
topiramate, 370  
weight loss, 360  
Thermic effect of food (TEF), 5  
Thiazolidinedione (TZD), 210, 298, 352  
Thyroid hormone  
    effects, fatty acid metabolism, 147  
    functions, 146  
    hypothyroidism  
        clinical manifestation, 147–148  
        and obesity, 148–149  
    nuclear receptors, 146–147  
    synthesis and secretion, 146  
Thyrotropin releasing hormone (TRH), 146  
Toll-like receptor (TLR), 155, 320  
Topiramate, 370  
Triacylglycerols (TGs), 53  
Triglyceride  
    carbohydrate metabolism, 25  
    fatty acid oxidation  
        activation, 42  
         $\beta$ -oxidation, 43–44  
        translocation, 42–43  
    fatty acid synthesis, 45  
    ketone bodies, 44  
    lipolysis, 40–42  
    storage, 25  
    synthesis, 44–45  
    translocation, 28–30  
Triglyceride-rich lipoprotein (TRL),  
    295, 296  
Type 1 diabetes (T1D), 322–323

**U**

Urea cycle disorders (UCD), 324–325

**V**

Valvular heart disease, 262

Ventral tegmental area (VTA), 100

Ventromedial nucleus (VMN), 96

Vertical banded gastroplasty (VBG), 371

Very low calorie diet (VLCD), 365

Very low density lipoprotein  
(VLDL), 293

**W**

Weight management plan, 364

Wellcome Trust Case Control Consortium  
(WTCCC) study, 131

White adipose tissue (WAT)

differentiation programme, 58–59

distribution, 55–56

histology, 56

immune cells, 56

progenitors identification, 60–61

SVF, 56

in vivo regulation, 57

Whole genome association (WGA)  
studies, 130

**Z**

Zonisamide, 370