SECOND EDITION

NUTRITION and DIABETES

Pathophysiology and Management

Edited by

EMMANUEL C. OPARA
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Nutrition and Diabetes

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SECTION II Pathophysiology and Treatment of Diabetes

Foreword

There has been no let-up in the acquisition of knowledge regarding the epidemiology, pathogenesis, and treatment of diabetes and obesity since the first edition of this book was published more than a decade ago. The resulting change in the way many people think about them makes a new edition timely.

The idea that overweight and (moderate) obesity is unhealthy, even in physically fit people, has been challenged. It is, they contend, not the overweight itself that is unhealthy but its associated morbid conditions—namely diabetes, hypertension, and cardiovascular disease. In support of this notion, there is some evidence that they are linked in a Mendelian fashion. Others—the majority—still believe the link is causal and that obesity is often the primary abnormality. The distinction is important, for, if the former view is correct, there is no need to treat overweight people providing they are happy and do not also suffer from diabetes, hypertension, or cardiovascular disease, whereas, if the latter view is correct, even modest overweight is clinically relevant.

Contrary to what Malthus would have had us believe, population growth has not yet outgrown humankind's ability to supply itself with food. Distribution and the wherewithal to purchase food do, however, remain problems that are beyond medicine's ability to resolve, and undernutrition in the midst of plenty is still with us.

While the general principles of good nutrition were established almost a century ago, the details are still poorly understood. The key role of energy supply and its relationship to body weight, and the importance of vitamins and trace elements and quality of dietary proteins to health, have long been recognized, but much remains to be learned. The relevance of differences in the nature of dietary carbohydrates and whether they are associated with dietary fiber has, however, a much shorter history—as has recognition of subtle differences in the nature and origin of dietary fats.

There is some evidence, for example, to suggest that the incidence of type 2 diabetes can be reduced by substituting camel's for cow's milk in the diet. But even after decades of anecdotal evidence and 20 or so years of scientific study—mostly outside the United States and Western Europe—the matter is undecided. This reflects just how difficult the truly scientific study of even comparatively minor changes in dietary practice on population health is to resolve—not least because of the logistics involved in conducting long-term research on human beings.

Since the last edition of the book, there has been more understanding of the part played by the gastrointestinal tract as an endocrine organ in the control of appetite and metabolic processes.

The desire and ability to eat is, and presumably always has been, controlled by a number of factors, one of which—namely, the availability and affordability of food, regardless of quality—is no longer a consideration in the developed, and increasingly the developing, world. This leaves physiological and societal factors as major determinants of the distribution and prevalence of obesity in and between nation states. That genetic considerations play an important role has long been recognized, but exactly how they manifest themselves is only slowly being elucidated.

Undoubtedly one of the most important advances in our understanding and treatment of type 2 diabetes has been the growth of bariatric surgery. Once reserved solely for treating diet-resistant, life-threatening obesity, it is now increasingly being used for the treatment of patients with type 2 diabetes who are only modestly overweight (body mass index [BMI] of at least 30). The resulting remarkable change in metabolism, leading in many cases to an immediate improvement or total remission of the diabetic diathesis, cannot be attributed to weight loss. It is thought largely to result from changes in the enteroinsular axis, most notably in the secretion of GIP and GLP-1(7-36) amide. How long the improvement, or cure in some cases, continues awaits lifelong follow-up studies.

Concurrently, developments in peptide synthesis have seen the introduction of GLP-1 analogues for the clinical treatment of type 2 diabetes and the experimental use of GIP analogues for the

treatment of obesity. A similar approach using analogues with either agonistic or antagonistic actions to the hormones controlling appetite can confidently be expected.

Glucagon, whose role in the adverse manifestations of diabetes has largely been ignored, has recently come to share center stage with insulin deficiency in the pathogenesis of the diabetic syndrome. It is currently being investigated as the target for a therapeutic approach that involves either impeding glucagon secretion or blocking its receptors.

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Preface

Diabetes and obesity are two common disorders that have come to be appropriately recognized as enormous burdens both to the afflicted individuals and modern society in general. What is even more striking is the relationship between obesity and impaired glucose regulation that predominantly results in overt diabetes. Consequently, as the incidence of obesity has risen in virtually every population, so has that of type 2 diabetes. Perhaps more alarming is the increasing incidence of obesity and type 2 diabetes among children. Given the chronic nature of both conditions and their known associations with cardiovascular and other serious complications, the coexistence of obesity and diabetes in children portends a dire future for society.

The purpose of *Nutrition and Diabetes: Pathophysiology and Management* is to provide a unique forum that highlights the link between the problems of obesity and diabetes. The pathophysiological underpinnings and pertinent aspects of each disorder are discussed exhaustively in relevant sections of the book. Importantly, the inter-relationships in the pathobiology and manifestations of obesity and diabetes come into focus, and many areas of overlap become obvious in the various chapters of the book. Enormous efforts have been made by the different contributors to first provide an overview of each topic, then discuss the mechanistic aspects of the given problem, and finally link these pathophysiological processes to the rationale of therapeutic strategies. The second edition of this book has been reorganized and is divided into two sections: Pathophysiology and Treatment of Obesity, and Pathophysiology and Treatment of Diabetes. New topics have been added in each section, including the emerging roles of probiotics in the pathogenesis and treatment of both obesity and diabetes.

Nutrition and Diabetes: Pathophysiology & Management is intended to be a reference handbook for physicians, nutritionists, dietitians, and other healthcare workers who deal daily with the various problems associated with obesity and diabetes. Researchers who desire a deeper understanding of the current state of knowledge, as well as gaps in our knowledge, would find this volume a worthwhile companion. Furthermore, the book should be of interest to experts and officials involved in formulating health policies in both advanced economies and developing countries. Finally, members of the general literate public (especially individuals afflicted with obesity and/or diabetes) would find this book a valuable source of actionable information to augment advice provided by their healthcare team.

The editors would like to thank all the chapter authors for the generous gift of their time to discuss important aspects of diabetes and obesity and make this a unique book. Each of you has helped to make this second edition a worthwhile project, and we are greatly indebted to you.

Emmanuel C. Opara and Sam Dagogo-Jack

Editors

Emmanuel C. Opara, PhD, received his MSc degree in Clinical Biochemistry at the University of Surrey in England and a PhD in Medical Biochemistry from the University of London. After a World Health Organization–sponsored research fellowship at the Mayo Clinic in Rochester, Minnesota, he was a visiting fellow at the National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health in Bethesda, Maryland before spending 15 years on faculty at the Duke University Medical Center in Durham, North Carolina. He subsequently was research professor in the Biomedical Engineering program at the Illinois Institute of Technology, Chicago while serving as a senior investigator in the Human Islet Transplant program at the University of Chicago. He accepted his present position as a professor at the Wake Forest Institute for Regenerative Medicine in 2009 while also serving as a professor in the Center on Obesity, Diabetes & Metabolism at the Wake Forest School of Medicine. He also serves as professor and graduate program director at the Wake Forest University campus of the joint Virginia Tech-Wake Forest School of Biomedical Engineering & Sciences.

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Section I

Pathophysiology and Treatment of Obesity

1 Central and Peripheral Modulators of Appetite and Satiety

Gabrielle Page-Wilson and Sam Dagogo-Jack

CONTENTS

1.1 INTRODUCTION

Regulation of energy homeostasis is critical to the survival of any species. Therefore, intricate behavioral, metabolic, and neuroendocrine mechanisms have evolved to integrate energy intake and dissipation. A delicate balance between intake and expenditure of energy is required to maintain

healthy weight. Perhaps for teleological reasons, the mechanisms that regulate energy homeostasis are biased in favor of net positive energy and are geared toward defense of weight loss rather than prevention of obesity. Hence, spontaneous weight loss in the absence of disease is rare, and the experience of progressive weight gain in free-living humans is common.

The adaptations that defend against weight loss eventually become maladaptive when obesity and its related metabolic and cardiovascular complications supervene, as in the present era (Bray 1996, Flegal et al. 2002). At its core, obesity signifies chronic disequilibrium between food consumption and energy expenditure. Total energy expenditure (TEE) comprises basal or resting energy expenditure (REE), thermic effect of food (TEF), mandatory physical activities of daily living (ADL), and volitional physical activity or exercise (Macdonald 1998). The contributions of TEF and ADL to TEE are rather modest, and the rate of voluntary physical activity is universally low among humans, leaving REE the energy-expenditure mode of choice for most people.

REE declines with age, which explains the tendency to positive energy balance and obesity in older persons. The TEE also is correlated positively with body surface area and is higher in obese than lean individuals. However, the REE compensation for obesity is ineffective in inducing weight loss or restoring normal weight. Therefore, REE, the major component of energy expenditure for most sedentary persons, is a practically nonmodifiable factor in energy homeostasis. This leaves restriction of food intake and volitional exercise as the main strategies for effective weight control.

1.2 APPETITE AND SATIETY

The "afferent" limb of the energy homeostasis loop is food consumption. Food intake is driven by appetite and terminated by satiety. Hunger is the physiological response to appetite, whereas meal termination occurs in response to satiety signals. The exact mechanisms controlling the primal instincts of appetite and hunger are incompletely understood. It is known, however, that a host of behavioral, environmental, cognitive, and situational influences can modify responses to hunger. Thus, appetite triggers hunger, but the latter can be overridden or suppressed to enable delay of food intake to a more appropriate time. Similarly, several organic and psychiatric disorders are associated with perturbations of appetite. The present review focuses on the neuro-hormonal regulation of food intake and attempts to integrate seminal experimental findings in rodents with current and future directions in human metabolic research and antiobesity drug development.

1.3 CENTRAL NERVOUS SYSTEM LOCALIZATION OF FEEDING CONTROL

The hypothalamus integrates diverse signals, including brain neurotransmitters, peripheral neurohumoral afferents, adipocyte-derived signals, gastrointestinal peptides, and other afferent inputs, to regulate energy homeostasis. The medial basal hypothalamus (MBH), which includes the ventromedial and arcuate nuclei, plays a critical role in the regulation of energy balance. The arcuate nucleus (ARC), situated at the base of the hypothalamus, houses two well-described neuronal populations, including proopiomelanocortin (POMC) and neuropeptide Y (NPY)/agouti-related peptide (AgRP) neurons that are important regulators of feeding. These neurons express receptors for hormones and neuropeptides and respond to peripheral changes in energy balance (Schwartz et al. 2000). The paraventricular nucleus (PVN) in the anterior hypothalamus, the major site of corticotropin-releasing hormone (CRH) and thyrotropin-releasing hormone (TRH) secretion, receives rich projections from the ARC. The PVN is involved in the integration of paracrine and endocrine metabolic signals, with classical neuroendocrine pathways mediated through the thyroid and hypothalamic-pituitary-adrenal axes. In addition to these nuclei, the brainstem also plays a role in appetite regulation, processing satiety signals that are conveyed following meal ingestion by vagal afferent projections that

converge in the nucleus tractus solitarius (NTS) of the hindbrain (Williams et al. 2001). Neuronal projections from the NTS to the PVN and lateral hypothalamus link the brainstem with the hypothalamus (Grill and Hayes 2012). However, studies in decerebrate rats, in which the hindbrain and forebrain are disconnected, demonstrate that the brainstem can independently control meal size in response to satiety signals like cholecystokinin (CCK), showing that the hindbrain and forebrain may both contribute to energy balance (Grill and Smith 1988).

1.4 HYPOTHALAMIC NEUROPEPTIDES THAT STIMULATE FOOD INTAKE

The hypothalamic orexigenic signals include NPY, AgRP, and the hypocretins/orexins.

1.4.1 Neuropeptide Y

NPY is a 36-amino acid polypeptide that rapidly stimulates food intake following intracerebroventricular (ICV) injection in rodents. The appetite-stimulating effects of NPY lead to sustained hyperphagia and weight gain in mice receiving chronic ICV administration. The specificity of NPY's effect has been established in studies that employed co-administration of NPY antagonists or its antibodies, both of which inhibited food intake in rats (Kalra et al. 1999). The role of NPY as a central physiological trigger of meal initiation is suggested by studies showing a rapid increase in hypothalamic NPY expression in the PVN before meal times, and persistence of NPY gene expression throughout the period of enforced hunger. The orexigenic action of NPY is mediated by interactions with Y1 and Y5 receptors (Henry et al. 2005). NPY also has diverse actions that enhance activation of the HPA axis, amplify the stress response, and alter seizure threshold (Beck 2006), which are obvious limitations to a direct deployment of NPY for treatment of cachexia and eating disorders. Interestingly, NPY expression in the arcuate nucleus is potently antagonized by the anorexigenic hormone leptin, as well as by insulin. Furthermore, activation of the Y2 receptor subtype on NPY neurons triggers inhibitory presynaptic signals. Consequently, central administration of PYY3-36 (a Y2 receptor agonist secreted by intestinal endocrine L cells) into the arcuate elicits a marked inhibition of food intake. Also, Y2 receptor knock-out mice lose their responsiveness to the anorectic effect of PYY3-36 (Batterham et al. 2002). The longstanding interest in the therapeutic potential of NPY antagonism was revived by the discovery of Y2R and Y4R receptor agonists and Y5R antagonists. To date, the success of these small molecules has been limited in clinical trials in humans by side effects or lack of efficacy (Brothers and Wahlestedt 2010).

1.4.2 Agouti-Related Protein

AgRP is expressed exclusively in the arcuate nucleus of the hypothalamus and co-localizes to the same neurons that secrete NPY (Goldstone et al. 2002). Several reports have confirmed that administration of AgRP potently enhances appetite in rodents, with central administration of AgRP increasing food intake for up to 7 days (Rossi et al. 1998). In contrast to the potent but relatively short-lived effect of NPY, chronic administration of AgRP results in sustained hyperphagia and obesity (Small et al. 2001). AgRP exerts its orexigenic effect by antagonizing the effect of POMC-derived peptide α-MSH at MC3 and MC4 receptors, increasing food intake and decreasing energy expenditure. The arcuate neurons that co-secrete NPY/AgRP are stimulated by fasting and potently inhibited by leptin and insulin (Breen et al. 2005). Several other important regulators of AgRP expression have been identified. Glucocorticoids impact AgRP expression, as illustrated by rodent studies demonstrating a decline in AgRP expression following adrenalectomy that is reversed by glucocorticoid replacement (Savontaus et al. 2002). The gut hormone ghrelin has also been shown to stimulate AgRP gene expression in the ARC, and ghrelin orexigenic effects have been shown to be partially mediated by NPY/AgRP (Chen et al. 2004).

1.4.3 Hypocretins/Orexins

The hypothalamic peptides hypocretins-1 and hypocretins-2 were discovered in 1998 by subtractive polymerase chain reaction (de Lecea et al. 1998). In the same year, homologous hypothalamic peptides named orexins 1 and 2 were discovered by Sakurai et al. (Sakurai et al. 1998) and shown to potently stimulate food intake in rats (Sakurai et al. 1998). The hypocretins/orexins stimulate food intake in rodents, an effect that is blocked by neutralizing antibodies to endogenous hypocretins (Willie et al. 2001).

In addition to directly stimulating food intake, the hypocretins/orexins may also influence energy homeostasis in other ways. For example, hypocretin levels increase in response to exercise, neuroglycopenia, and enforced wakefulness (Nishino 2003). Hypocretin-secreting neurons localize exclusively to the lateral hypothalamus, the region of the brain long known to integrate appetite signals. Although intriguing as modulators of food intake, interest in the hypocretins/orexins shifted to their role in sleep regulation when the genes for hypocretins/orexins were found to be the loci for narcolepsy (Chemelli et al. 1999, Lin et al. 1999). Documented mutations in the human hypocretin/orexin genes are rare among patients with sleep disorders, but nearly 90% of patients with narcolepsy-cataplexy have subnormal cerebrospinal fluid hypocretin levels (Nishino et al. 2001).

The latter finding is inconsistent with a primary orexigenic role of the hypocretins/orexins as the mechanism for the increased prevalence of obesity, insulin resistance, and type 2 diabetes among patients with narcolepsy (Nishino et al. 2001). These metabolic disorders are more likely the result of the physical hypoactivity associated with narcolepsy. It must be noted, however, that the hypocretin/orexin system functions centrally as the major integrator of excitatory impulses from monoaminergic (dopamine, norepinephrine, serotonin, histamine) and cholinergic fibers that maintain wakefulness and vigilance (Taheri et al. 2002). Thus, besides a direct orexigenic effect, the hypocretins/orexins could exert metabolic effects through modulation of central autonomic outflow. However, the hypocretins/orexins are less attractive candidates for drug development, because their reported effects on food intake are less robust and consistent compared with the orexigenic effects of NPY.

1.5 ANOREXIGENIC NEUROPEPTIDES

As a central integrator of energy homeostasis, the hypothalamus also is a source of neuropeptides that inhibit food intake or induce satiety. The hypothalamic anorexigenic agents include the melanocortins, cocaine- and amphetamine-regulated transcript (CART), and serotonin.

1.5.1 Melanocortins

The melanocortins are derived from site-specific post-translational cleavage of the precursor parent molecule POMC. Cleavage of POMC within the anterior pituitary gives rise to adrenocorticotropic hormone (ACTH), which acts through the melanocortin 2 (MC2) receptor to stimulate adrenal steroidogenesis. Elsewhere in the brain, POMC is cleaved to another melanocortin α-MSH, which is an agonist for the MC3 and MC4 receptors. Administration of α-MSH (ICV) in rodents results in weight loss through inhibition of food intake and stimulation of energy expenditure (Neary et al. 2004). These actions are mediated through activation of two neuronal melanocortin receptor subtypes (MC3r and MC4r) and antagonized by an adjacent subset of hypothalamic neurons that express AgRP and NPY. Thus, the MC4 receptor plays a critical role on body-weight regulation. The NPY/AgRP neurons are inhibited by leptin and insulin.

The integrated physiology of the interactions of these opposing neuropeptides is evident from their weight-related alterations. Following weight loss, the decreasing levels of insulin and leptin lead to activation of NPY/AgRP neurons and inhibition of POMC neurons (Flier 1998, Schwartz et al. 2000). These counterregulatory changes induce accelerated food intake and accumulation of fat. Defects along the melanocortin signaling pathway, such as those seen in transgenic mice with targeted

disruption of the MC4 receptor (knock-outs), result in hyperphagia and obesity (Huszar et al. 1997). Recently, fairly widespread functional mutations of the human MC4 receptor has been demonstrated to patients with severe childhood obesity (Farooqi et al. 2000). It should be noted, however, that the majority of obese patients have no demonstrable mutations in MC4, yet such persons may possibly benefit from future therapies targeting activation of MC4 pathways. While intranasal administration of a melanocortin fragment (MSH/ACTH 4–10) initially looked promising in that regard, MSH/ACTH 4–10 failed to have an anorexigenic effect in obese males, despite its inducing modest weight loss in individuals of normal weight (Fehm et al. 2001, Hallschmid et al. 2006). Similarly, the use of a more selective MC4R agonist (MK-0493) did not result in weight loss in obese humans (Krishna et al. 2009). However, a newly developed MC4R agonist (RM-493) may prove more promising, as recent data suggests that it effectively increases REE in obese adults (Chen et al. 2015).

1.5.2 Cocaine- and Amphetamine-Regulated Transcript

CART is widely expressed in the brain, especially in the hypothalamic nuclei and in the anterior pituitary. Within the arcuate nucleus, POMC co-localizes to neurons that also express CART. Injection of CART (1–100 pmol, ICV) resulted in dose-dependent inhibition of food intake in rats. The effect was observed within 20 minutes and lasted approximately 4 hours. The decrease in food intake following treatment with CART was accompanied by inhibition of gastric emptying and reduction of oxygen consumption. The arcuate POMC/CART neurons act as downstream effectors of the anorexigenic action of leptin and are markedly stimulated by ICV injection of leptin (Cowley et al. 2001). Interactions between CART and the endogenous opioid, serotoninergic (Rothman et al. 2003), and cannabinoid (Cota et al. 2003) systems provide additional mechanisms for the anorexigenic effects of CART.

1.5.3 Serotonin

The amino acid derivative 5-hydroxytryptamine (serotonin, 5-HT) has ubiquitous neurotransmitter functions on numerous central nervous system (CNS) targets (Blundell 1984). Receptors for 5-HT are widely expressed in regions including the limbic system, raphe nucleus, and the hypothalamus. Activation of 5-HT receptors (especially the 5-HT2c subtype) is associated with inhibition of food intake. A similar anorexigenic effect is observed following augmentation of serotonin abundance through inhibition of its re-uptake. Conversely, studies in rodents have shown that deletion of the serotonin 5-HT2c gene results in marked hyperphagia (Tecott et al. 1995). Unfortunately, clinical experience with selective serotonin reuptake inhibitors shows modest and inconsistent effects on body weight, which indicates that the serotonergic pathway is overridden by more powerful orexigenic impulses under normal physiological conditions. Nonetheless, sibutramine, a selective agonist of the serotonin 2C receptor, is an effective weight-loss medication. (Sibutramine was withdrawn from use in 2010 because of increase risk of cardiovascular events.)

While serotoninergic effects on food intake were once thought to be mediated primarily by central 5-HT pathways, recent studies utilizing genetic mouse models have underscored the importance of the melanocortin system in mediating the anorexigenic effect of 5-HT. Serotonin has been shown to impact satiety by inhibiting AgRP neuronal activity and decreasing inhibition of POMC neurons via 5-HT1B receptor activation (Heisler et al. 2006). 5-HT2cRs on POMC neurons have also been shown to play a role in mediating serotonin's capacity to suppress food intake (Xu et al. 2008). Lorcaserin, a 5-HT2c agonist, was approved by the FDA in 2012 for treatment of obesity.

1.5.4 Brain-Derived Neurotrophic Factor

Brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin family of secreted peptides that acts at the tropomysin-related kinase B (TrkB) receptor to activate phospholipase C gamma, mitogen-activated protein kinase, and phosphatidylinositol-3 kinase (PI3-K) intracellular

TABLE 1.1
Selected Central Neuropeptides That Modulate Food Intake

Orexigenic	Anorexigenic
Neuropeptide Y	α-Melanocyte stimulating hormone
Agouti-related protein	Corticotropin-releasing hormone
Orexin a	Cocaine-amphetamine regulated transcript
Orexin b	Serotonin

signaling pathways (Reichardt 2006). While neurotrophins are most well-known for their impact on neuronal survival and differentiation (Chao 2003), there is evidence to support an important role for BDNF in energy homeostasis. Central administration of BDNF has been shown to reduce body weight in rodents (Lapchak and Hefti 1992), and reduced BDNF levels in heterozygous BDNF mice have been associated with increased food intake and obesity (Lyons et al. 1999). Similarly, decreased BDNF signalling, resulting from loss of a functional BDNF allele (Gray et al. 2006) or a de novo mutation in TrkB (Yeo et al. 2004), has been associated with hyperphagia and obesity in humans. Moreover, large-scale, genome-wide association studies of single nucleotide polymorphisms in BDNF genes have been associated with obesity (Hotta et al. 2009, Thorleifsson et al. 2009). BDNF's role as an anorexigenic factor is further supported by studies in rodents demonstrating reduced hypothalamic BDNF expression with fasting and increased BDNF gene expression following the administration of either glucose or melanocortin agonists (Xu et al. 2003, Unger et al. 2007). Continued studies designed to elucidate the role of BDNF in energy balance may lead to mechanistic insights that can be harnessed for developing novel treatment strategies for obesity in the future. Table 1.1 shows a list of orexigenic and anorexigenic central neuropeptides.

1.6 PERIPHERAL SIGNALS IN THE REGULATION OF FOOD INTAKE

The peripheral hormones that regulate food intake include several gastrointestinal, pancreatic, and adipocyte-derived peptides (Table 1.2). Based on extensive studies in rodents and limited human data, these peptides can be classified as having orexigenic (e.g., ghrelin) or anorexigenic (e.g., insulin, peptide YY, glucagon-like polypeptide, cholecystokinin, leptin) effects.

TABLE 1.2
Selected Peripheral Modulators of Food Intake

Signal	Main Targets
Anorexigenic	
Leptin	Hypothalamus
Peptide YY	Hypothalamus
Pancreatic polypeptide	Hypothalamus
Insulin	Hypothalamus
Cholecystokinin	Brainstem/vagus
GLP-1	Local GI/diverse
Oxyntomodulin	Local GI/diverse
Orexigenic	
Ghrelin	Hypothalamus

GI, gastrointestinal; GLP-1, glucagon-like peptide 1.

1.6.1 Adipocyte-Derived Signals

There is a mature and growing body of literature on the roles of several adipocyte products (including nonesterified fatty acids, adipocytokines, and leptin) in the regulation of metabolic fuel economy, energy balance, glucoregulation, food intake, and body weight. Products such as nonesterified fatty acids have long been proposed as mediators of obesity-associated insulin resistance and glucose dysregulation (Randle et al. 1988, Paolisso et al. 1998, Unger 1995, Laaksonen et al. 2002), as discussed elsewhere in this book.

1.6.2 Adipocytokines

The adipocytokine TNF-α (also known as *cachectin*, for its association with cachexia or wasting) is a mediator of insulin resistance and is secreted in higher amounts by adipocytes from obese subjects (Hotamisligil et al. 1993, 1994, 1996, Kern et al. 1995, Norman et al. 1995, Moller 2000). Other circulating and adipose-derived proinflammatory cytokines also have been implicated in the pathogenesis of obesity-associated insulin resistance and diabetes (Grimble 2002, Freeman et al. 2002). On the other hand, adiponectin is secreted in abundant amounts by fat cells from insulin-sensitive persons and is deficient in persons with obesity or insulin resistance (Stefan et al. 2002). Thus, numerous adipose tissue products serve as markers, signals, or modulators of energy balance, fuel economy, intermediary metabolism, glucoregulation, and other metabolic events that intersect with food intake and body-weight homeostasis. Of these numerous adipose tissue products, leptin is perhaps the best characterized in terms of its role in the regulation of food intake and related mechanisms.

1.6.3 Leptin

The positional cloning of the mouse (*ob*) gene and its human homologue (Zhang et al. 1994) represents a major milestone in obesity research. Two separate mutations of the *ob* gene result in either a premature stop codon or complete absence of *ob* mRNA in the *ob/ob* mouse (Zhang et al. 1994). The resultant absence of a normal *ob* gene product leads to overfeeding, massive obesity, delayed sexual maturation (Chehab et al. 1996), and immune defects (Matarese 2000) in *ob/ob* mice. The human *ob* or *lep* gene is transcribed and translated into a secreted protein mainly in white adipose tissue, but activity can also be reported in brown adipose tissue, gastric epithelium, and placental tissue (Masuzaki et al. 1997, Bado et al. 1998). Circulating leptin levels are increased by feeding, decreased during fasting or following weight loss, and altered by a variety of hormonal and physiological factors (Krempler et al. 2000, Page-Wilson et al. 2017).

A pedigree with severe childhood obesity associated with deletion of a guanine nucleotide in codon 133 of the human *lep* gene was the first human example of congenital leptin deficiency to be identified (Montague et al. 1997). A missense *lep* mutation in codon 105 has also been identified in a Turkish pedigree (Strobel et al. 1998). Three individuals (two female, one male) homozygous for this mutation have the phenotype of hypoleptinemia, marked hyperphagia, massive obesity, and hypothalamic hypogonadism. Excluding these rare reports, common forms of human obesity do not appear to be caused by discernible *lep* mutations (Considine et al. 1995, Maffei et al. 1996). Treatment with recombinant leptin results in a marked reduction in food intake and profound weight loss in *ob/ob* mice (Halaas et al. 1995, Pelleymounter et al. 1995, Campfield et al. 1995, Weigle et al. 1995, Chehab et al. 1996, Matarese 2000). Leptin therapy is also remarkably effective in correcting obesity in humans with congenital leptin deficiency (Pelleymounter et al. 1995, Farooqi et al. 1999, 2002, Licinio et al. 2004).

1.6.3.1 Mechanism of Action

Leptin exerts its effects through interaction with cognate cell membrane receptors (lep-r) (Tartaglia et al. 1995). One full length (isoform-b) and several alternatively spliced forms (a, c, d, e, f) of lep-r

have been identified in brain and peripheral tissues (Takaya et al. 1996, Lee et al. 1996). Lep-r is a member of the class 1 cytokine receptor family (Vaisse et al. 1996, Baumann et al. 1996, Wang et al. 1997). This receptor family mediates gene transcription via activation of the *jak-stat* pathway (Ghilardi and Skoda 1997). The long isoform, lep-r (b), expressed in the hypothalamus, mediates the central effects of leptin. Intracellular signaling pathways arising from activated lep-r (b) include the phosphorylation and activation of the signal transducer and activator of transcription (STAT) pathways mediated by STAT3 and STAT5, the SOCS3-mediated feedback inhibition pathway, and the extracellular regulated kinase (ERK) pathway (Bjorbaek et al. 1999, Robertson et al. 2008). The shorter isoforms of the leptin receptor are truncated in the cytoplasmic domain but can bind leptin and probably mediate some peripheral actions (Takaya et al. 1996, Lee et al. 1996, Kieffer et al. 1996).

Leptin-receptor activation results in decreased expression of NPY, thereby inhibiting the powerful orexigenic effects of NPY (Ghilardi and Skoda 1997). Leptin's action to suppress food intake is mediated through an elaborate neuronal circuitry that involves suppression of orexigenic signals (NPY, AgRP, MCH, hypocretins 1 and 2/orexins a and b) and activation of anorexigenic (α-MSH, MC4, CRH, CART) neuronal pathways (Schwartz et al. 2000). Mutations in the lep-r gene result in obesity and leptin resistance in *db/db* mice (Chen et al. 1996) and *fa/fa* rats (Phillips et al. 1996), respectively. Human lep-r mutations associated with morbid obesity have been described in a French kindred (Clement et al. 1998). Adipose tissue *lep* mRNA (Lonnqvist et al. 1995, Hamilton et al. 1995) and circulating leptin (Considine et al. 1996, Dagogo-Jack et al. 1996) levels are elevated in obese subjects, suggesting that obese persons are not responding optimally to the weight-regulating effects of leptin. The basis of this "leptin resistance" is unclear but may be related to alterations in circulating leptin-binding proteins (Lou et al. 2010), impaired blood-to-brain leptin delivery (Schwartz et al. 1996), or defects in leptin receptor signaling (Tartaglia et al. 1995, Bjorbaek et al. 1999, Ozcan et al. 2009, Myers et al. 2012).

1.6.3.2 Leptin and Insulin Action

Replacement doses of recombinant leptin, administered systemically, normalized plasma glucose and insulin levels in hyperglycemic, hyperinsulinemic *ob/ob* mice (Halaas et al. 1995, Weigle et al. 1995) and in leptin-deficient subjects with diabetes and insulin resistance (Licinio et al. 2004). Low doses of leptin administered intravenously or into the cerebral ventricles increased glucose utilization and decreased hepatic glycogen storage in wild-type mice (Kamohara et al. 1997). Furthermore, leptin therapy selectively depletes visceral fat stores and stimulates insulin sensitivity in rats (Barzilai and Gupta 1999). These findings indicate that leptin may be a naturally occurring insulin sensitizer. Indeed, addition of leptin to cultured human hepatocytes stimulates signaling along the phosphatidyl inositol 3′ kinase pathway, one of the mediators of insulin action (Cohen et al. 1996). Reversal of lipotoxicity may be another mechanism for the insulin-sensitizing effects of leptin (Bai et al. 1996, Shimabukuro et al. 1997). There is a marked variability in plasma leptin levels (even among persons of comparable adiposity), which may be partially related to differences in insulin sensitivity (Dagogo-Jack et al. 1997, Askari et al. 2010).

Basal (fasting) plasma leptin levels are similar in patients with diabetes compared with body mass index (BMI)- and gender-matched nondiabetic subjects (Sinha et al. 1996, Liu et al. 1999), but dynamic leptin response to secretagogues is attenuated in patients with diabetes (Liu et al. 1999, Dagogo-Jack et al. 2000). We have postulated that the increased leptin secretory response to food (as well as insulin and glucocorticoids) represents a counterregulatory attempt to limit hyperphagia and weight gain (Dallman et al. 1993, Dagogo-Jack et al. 1997) (Figure 1.1). This adaptation may be of physiological relevance, because feeding stimulates the secretion of insulin and cortisol (Rosmond et al. 2000), which in turn stimulate secretion of the satiety hormone leptin. Theoretically, a defect in leptin secretion could permit hyperphagia, promote weight gain, and aggravate insulin resistance. If impaired leptin secretion is confirmed as a general feature of diabetes, such diabetic dysleptinemia may provide a rationale for evaluation of leptin therapy. Indeed, patients with lipodystrophic

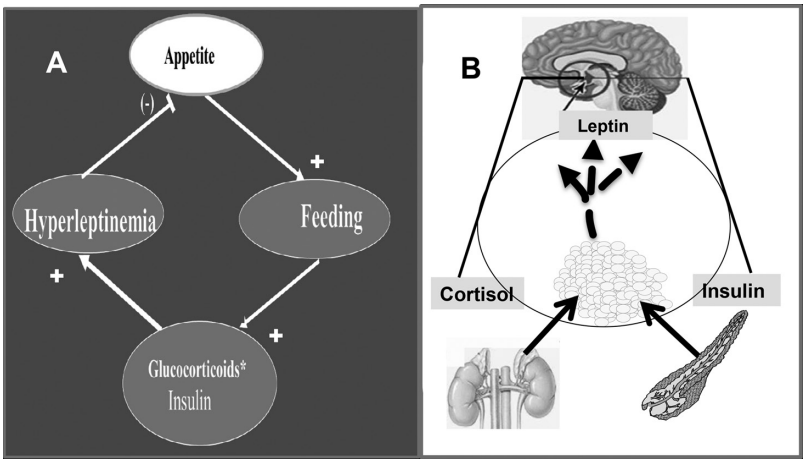

FIGURE 1.1 Increased appetite and consequent feeding induce postprandial plasma insulin and cortisol levels; glucocorticoids and insulin stimulate leptin secretion by adipocytes, which can induce satiety and prevent hunger between meals and during sleep (*A*). Leptin, glucocorticoids, and insulin have central effects on hypothalamic hunger and satiety centers (*B, small circle*), in part, by altering neuropeptide Y expression. Peripherally, a feedback loop (*B, wider circle*) involving direct suppression of glucocorticoid and insulin secretion by leptin ensures homeostasis. Impaired insulin- and glucocorticoid-stimulated leptin secretion in patients with diabetes would be permissive of increased caloric intake, weight gain, insulin resistance and hyperglycemia. (Adapted from Dagogo-Jack S. 2015. Dynamic leptin secretion in obesity and diabetes. In *Leptin: Regulation and Clinical Applications.* Edited by S. Dagogo-Jack, 189-202. New York: Springer.)

diabetes and leptin deficiency respond remarkably well to leptin replacement and often achieve independence from insulin and oral hypoglycemic agents (Oral et al. 2002).

1.6.3.3 Exogenous Leptin Therapy for Human Obesity

Administration of low physiological doses of recombinant methionyl-human-leptin (0.01–0.04 mg/kg) produced dramatic results in morbidly obese, leptin-deficient patients (Montague et al. 1997, Farooqi et al. 1999, 2002, Licinio et al. 2004). Following daily subcutaneous injection of recombinant leptin, significant weight loss was reported within 2 weeks. The weight loss was maintained at a rate of approximately 1–2 kg per month, without evidence of tachyphylaxis, throughout 12–18 months of treatment (Farooqi et al. 1999, Licinio et al. 2004). Analysis of body composition indicated that selective fat depletion accounted for approximately 95% of the total weight loss. Daily food consumption (and food-seeking behavior) decreased within 1 week of initiation of leptin replacement, and 95% of the total weight loss was accounted for by selective body fat depletion (Farooqi et al. 1999). Basal energy expenditure decreased (due to weight loss), but dynamic energy expenditure increased by approximately the same amount during leptin treatment, the latter being due to increased physical activity (Farooqi et al. 1999). The stimulatory effect of recombinant leptin on physical activity was first noted in ob/ob mice and is probably mediated by activation of the sympathetic nervous system (Haynes et al. 1997). Similar but less dramatic benefits on weight reduction were observed following leptin augmentation in a cohort of 54 lean and 73 obese men and women with normal leptin genotype (as indicated by baseline serum leptin levels > 10 ng/mL) (Heymsfield et al. 1999). The subjects were randomized to daily self-injection with placebo or different doses (0.01, 0.03, 0.10, or 0.30 mg/kg) of recombinant methionyl human leptin. The mean weight changes at 24 weeks ranged from -0.7 ± 5.4 kg for the 0.01 mg/kg dose, to -7.1 ± 8.5 kg for the 0.3 mg/kg dose.

As in patients with congenital leptin deficiency, loss of fat mass accounted for most of the weight loss following leptin treatment (Heymsfield et al. 1999). Notably, there was marked individual variability in the response to recombinant leptin among subjects with normal leptin genotypes. Subsequent studies of leptin treatment in obese individuals and post-Roux-en-Y gastric bypass (RYGB) patients failed to demonstrate weight loss (Zelissen et al. 2005, Korner et al. 2013). Similarly, leptin treatment did not influence body weight in obese individuals with type 2 diabetes (Moon et al. 2011). Leptin-deficient patients are clearly more sensitive to leptin treatment than patients with common forms of obesity.

The enthusiasm surrounding leptin's therapeutic potential for reversing obesity has waned due to its lack of consistent efficacy in obese populations. Nonetheless, the possibility of restoring leptin responsiveness by using it in conjunction with agents like exendin-4 has shown promise in rodents and is a strategy worthy of exploration in human obesity (Muller et al. 2012). Notably, recombinant methionyl human leptin (metreleptin) was recently approved by the U.S. Food and Drug Administration for the treatment of congenital or acquired general lipodystrophy. The currently known metabolic and behavioral effects of leptin are summarized in Table 1.3.

1.6.4 Pancreatic Signals

1.6.4.1 Insulin

Insulin was the first peripheral signal shown to regulate food intake through interaction with central hypothalamic neurons (Kennedy 1953). Protagonists of popular diets have claimed in the lay press that limitation of insulin secretion is the mechanism for hunger control in subjects fed low-carbohydrate, ketotic diets, yet the scientific evidence strongly disputes that claim. Insulin fulfills the role (shared by leptin) of serving as a marker of adipose tissue mass and is secreted in direct proportion to fat mass. Insulin secretion also serves as an acute response to caloric influx: Increased secretion begins within minutes of initiation of feeding, is maintained for the duration of food intake, and returns to basal secretory rate in the post-absorptive period. If insulin were an appetite stimulant (like ghrelin), its secretion would have preceded, not followed, ingestion of food.

The timing and pattern of postprandial insulin secretion suggest a role in the regulation of satiety and meal termination. Indeed, direct administration of insulin to the CNS suppresses food intake in rodents (Baura et al. 1993). Because circulating insulin reaches the CNS via receptor-mediated transport across the blood-brain barrier, it is possible that peak insulin levels attained

TABLE 1.3
Behavioral and Metabolic Effects of Leptin

Behavioral
- Inhibition of food intake
- Stimulation of physical activity

Body Composition
- Induction of fat-mass loss
- Preservation of lean-muscle mass
- Preservation of bone mass

Glucose and Fat Metabolism
- Improvement in insulin sensitivity
- Improvement in glucose tolerance
- Partitioning of triglycerides to adipocytes
- Increased fat oxidation
- Reduction of hepatic steatosis

during feeding trigger central mechanisms that mediate satiety. Postprandial insulin secretion also is a potent signal for leptin secretion (Bado et al. 1998). Thus, in addition to a direct effect, insulin could exert anorectic effects via a leptin-mediated mechanism. Paradoxically, patients with diabetes who are treated with exogenous insulin or medications that increase insulin secretion or sensitivity tend to gain weight. Although several mechanisms explain the weight gain during intensive diabetes therapy, additional putative mechanisms include "central" insulin resistance and diabetic dysleptinemia (Liu et al. 1999, Dagogo-Jack et al. 2000, 2008).

1.6.4.2 Pancreatic Polypeptide

Pancreatic polypeptide (PP) is secreted by specialized endocrine cells within the pancreatic islets of Langerhans. PP is also sparsely expressed in the endocrine cells of the small and large intestine (Cox 2007). PP is secreted post-prandially in proportion to meal size, and during insulin-induced hypoglycemia. The release of PP and its biologic effects on food intake are mediated primarily by Y4 receptors and require parasympathetic activation (Havel et al. 1992, Field et al. 2010). Postprandial PP levels probably add to the physiological satiety signaling cascade that limits hyperphagia and helps maintain interprandial intervals. Indeed, systemic administration of PP has been shown to reduce food intake in rodents (McLaughlin 1982), and reductions in energy intake ranging from 11% to 25% have been observed following intravenous infusions of PP in nonobese humans (Batterham et al. 2003b, Jesudason et al. 2007). Similarly, reductions in food intake have been observed following PP infusions in obese patients with Prader-Willi syndrome (Berntson et al. 1993). Furthermore, the administration of PP in patients with chronic pancreatitis has been shown to improve glucose tolerance (Brunicardi et al. 1996). Pharmacologic attempts to harvest PP's anorectic potential have been explored. However, to date, Y4 receptor agonists have not progressed beyond phase II studies (Valentino et al. 2010).

1.6.5 Gastrointestinal Peptides

1.6.5.1 Ghrelin

The polypeptide ghrelin was initially described and characterized as the endogenous ligand for the growth hormone secretagogue receptor in 1999 (Kojima et al. 1999). The ghrelin molecule contains 28 amino acids and an acyl radical, the latter being essential for biologic effect. The post-translational acylation of ghrelin is mediated by the enzyme gastric O-acyl transferase (GOAT) (Yang et al. 2008). Ghrelin is synthesized and secreted by the stomach, reaches the anterior pituitary via the circulation, and stimulates growth hormone secretion by the somatotrophs. The nutritional effects of ghrelin became evident when it was shown that central (ICV) administration potently increased food intake in rodents (Wren et al. 2000). A similar effect on food intake was observed following peripheral (IV) injection of ghrelin in rats (Wren et al. 2000). In humans, intravenous administration of ghrelin stimulates food intake by approximately 30% (Wren et al. 2001). Interestingly, ghrelin is the only metabotropic peptide thus far identified that stimulates food intake directly when administered peripherally.

Physiologic studies have indicated that ghrelin serves as a peripheral signal for hunger and meal initiation; blood levels peak sharply just before feeding and fall rapidly following food intake (Cummings et al. 2001). Prolonged administration of ghrelin in rodents leads to chronic hyperphagia and weight gain, yet obese individuals typically exhibit high plasma leptin and low ghrelin levels (Tschop et al. 2001), with peak levels in the morning and the nadir at night. The mechanism of action of ghrelin involves stimulation of hypothalamic neurons (Nakazato et al. 2001) and inhibition of gastric vagal afferent signals (Date et al. 2002). Based on the foregoing, it is plausible that ghrelin or its analogues could be candidates for future therapy for primary anorexia, as well as the anorexia and cachexia often seen in patients with HIV-AIDS, systemic disorders, and malignant diseases. Conversely, ghrelin antagonism is an attractive idea for drug development for obesity and hyperphagic disorders. Ghrelin O-acyltransferase (GOAT) is another potential therapeutic target, given that inhibition of its enzymatic activity could decrease circulating concentrations of active acyl ghrelin and decrease hunger (Gardiner and Bloom 2008).

1.6.5.2 Peptide YY

The gut-derived peptideYY (PYY) is a member of the neuropeptide Y family that includes PP and NPY (Larhammar 1996, Kalra et al. 1999, Wynne et al. 2004). PYY is synthesized by the mucosal endocrine L cells located in the small intestine and large bowel. PYY3-36 is the major isoform secreted into the circulation (Grandt et al. 1994). Feeding is a major stimulus for the release of PYY, which then serves as an anorectic/satiety signal from the intestinal cells. The potent anorectic effect of PYY3-36 has been demonstrated in rodent studies involving direct administration of PYY3-36 into the arcuate nucleus (Batterham et al., 2002). Similarly, the peripheral administration of PYY3-36 reduces appetite and food intake in both lean and obese humans inhibited food intake by approximately 30% compared with placebo (Batterham et al. 2003a).

PYY3-36 appears to exert its anorectic effect through coordinate inhibition of orexigenic NPY neurons and stimulation of POMC neurons in the arcuate nucleus. These molecular changes are observed following peripheral administration of PYY3-36 (Batterham et al. 2002). PYY3-36 is thought to exert its effect through interaction with high-affinity Y2 receptors in the hypothalamus, gaining direct access via an incomplete blood-brain barrier in the median eminence. Activation of the Y2 receptor subtype on NPY neurons triggers inhibitory presynaptic signals. Consonant with this mechanism, Y2 receptor knock-out mice lose their responsiveness to the anorectic effect of PYY3-36 (Batterham et al. 2002). Thus, circulating PYY3-36 appears to exert its anorectic effect by directly inhibiting orexigenic NPY neurons in the arcuate nucleus. Notably, the NPY neurons in the arcuate nucleus are the central integrating sites for numerous peripheral signals (including leptin, insulin, PYY3-36, and ghrelin) that regulate food intake. Studies indicate that PYY3-36 effectively suppresses appetite over the short term in lean and obese humans (Batterham et al. 2003a, Sloth et al. 2007), and modulates brain activity in hypothalamic appetite centers when administered intravenously (Batterham et al. 2007). However, nausea has been a dose-limiting effect of intravenous, intranasal, and oral administration (Sloth et al. 2007, Gantz et al. 2007, le Roux et al. 2008, Beglinger et al. 2008). Nonetheless, the anorectic effect of PYY3-36 is noteworthy, and this peptide and its analogues remain candidates of interest for obesity drug development.

1.6.5.3 Glucagon-Like Peptide 1

Glucagon-like peptide 1 (GLP-1) is derived from the precursor molecule prepro-glucagon. Site-specific cleavage of prepro-glucagon in the pancreas results in glucagon, whereas in the intestinal endocrine L cells, the result is GLP-1. Both GLP-1 and PYY are co-secreted by the intestinal L cells in response to the arrival of nutrients in the gut. Like PYY, GLP-1 also appears to serve as a gut-derived satiety signal. While GLP-1 has a short half-life and is rapidly degraded by the enzyme dipeptidyl peptidase-4 (DPP-4), central and peripheral administration of GLP-1 results in marked inhibition of feeding in rodents (Turton et al. 1996, Abbott et al. 2005). GLP-1 is often described as an incretin because of its effect in boosting postprandial insulin secretion. Additional glucoregulatory actions of GLP-1 include suppression of glucagon secretion and prolongation of gastric emptying (Kreymann et al. 1987). GLP-1 can induce modest weight loss by delaying gastric emptying, inducing satiety, and inhibiting food intake; the latter effect is likely mediated by direct GLP-1 stimulation of POMC/CART cells in the arcuate nucleus (Verdich et al. 2001, Secher et al. 2014). Accordingly, GLP-1's glycemic and anorectic properties have been harnessed therapeutically. Synthetic GLP-1 receptor agonists, which are resistant to degradation by the enzyme DPP-4, have been shown to effectively improve glycemic control in type 2 diabetes (Shyangdan et al. 2011). Exenatide, a long-acting GLP-1 receptor agonist that is approved for treatment of type 2 diabetes in patients inadequately controlled on oral agents, has been associated with dose-dependent weight loss (Kolterman et al. 2003, DeFronzo et al. 2005). A 20-week, randomized, double-blind, placebo-controlled trial of liraglutide, another long-acting GLP-1 analogue, as compared to an open-label orlistat arm, demonstrated weight loss of more than 5% of baseline weight in 61% of patients treated with liraglutide, as compared to 29.6% of placebo- and 44.2% of orlistat-treated patients (Astrup et al. 2009). While gastrointestinal side effects are common with liraglutide across

the dose spectrum, the medication was approved by the U.S. Food and Drug Administration for the treatment of obesity in 2014. In the pivotal 56-week randomized, controlled trial, treatment with liraglutide 3.0 mg resulted in a mean weight loss of 8.4 kg, compared to 2.8 kg in the placebo group, in patients with a BMI of 30 or higher, or 27 kg/m^2 or more, with dyslipidemia and/or hypertension (Pi-Sunyer et al. 2015).

Endogenous GLP-1 abundance can be augmented by inhibition of DPP4. Although oral DPP-4 inhibitors have been shown to improve glycemic control and are approved for the treatment of type 2 diabetes, their effects on body weight are neutral (Amori et al. 2007, Dicker 2011). Recently, efforts have been made to establish GLP-1 and glucagon receptor co-agonists for the treatment of obesity. These compounds harness the lipolytic and thermogenic effects of glucagon, while counteracting glucagon-induced hyperglycemia by the co-administration of GLP-1 (Sanchez-Garrido et al. 2017). In response to promising animal data, showing PEGylated GLP-1 and glucagon receptor co-agonism results in weight loss and improved glucose tolerance in diet-induced obese mice (Day et al. 2009, Clemmensen et al. 2014), early clinical trials in humans are now underway (Sanchez-Garrido et al. 2017).

1.6.5.4 Oxyntomodulin

Oxyntomodulin (OXM) is produced from prepro-glucagon, mainly in the endocrine L-cells of the gut, and is secreted with GLP-1 following nutrient ingestion (Drucker 2005). This peptide hormone is composed of 37 amino acids, with sequence homology to GLP-1 and glucagon (Holst 1983). OXM has an affinity for GLP-1 and the glucagon receptor (Gros et al. 1993, Holst 1997) and, in addition to acutely inhibiting gastric emptying and gastric and pancreatic exocrine secretion, OXM has been shown to inhibit food intake and increase energy expenditure in both rodents and humans (Schjoldager et al. 1988, Dakin et al. 2001, Wynne et al. 2005, 2006, Bagger et al. 2015). In a randomized, controlled trial, an average weight loss of 2.3 kg was observed in overweight and obese subjects treated with subcutaneous OXM for 4 weeks, as compared to a 0.5-kg weight loss with placebo. Additionally, OXM infusions have been shown to improve glucose metabolism in type 2 diabetes (Shankar 2013). Given the therapeutic potential for native OXM to promote weight loss and glucose control, the therapeutic possibilities of stimulating endogenous OXM secretion, or synthesizing analogues, are currently receiving attention (Pocai 2014).

1.6.5.5 Cholecystokinin

CCK is best known for its role in food digestion, namely stimulation of pancreatic enzyme secretion and gallbladder contraction. However, CCK has been recognized as a potent satiety factor for more than 3 decades (Gibbs et al. 1973). Peripheral and central mechanisms appear to mediate the anorectic/satiety effects of CCK. Peripherally, activation of CCK_A receptors on vagal nerve endings and on the pyloric sphincter reduces food intake (Moran 2000).

Centrally, interactions between CCK and leptin pathways elicit synergistic anorectic effects (Matson et al. 1997). An additional mechanism of action of CCK might also involve activation of brainstem neurons that regulate portion size. The effect of peripheral administration of CCK is transient and more consistent with a modulatory effect on satiety/meal termination than primary inhibition of meal initiation (West et al. 1987, Moran 2000). To induce durable inhibition of food intake, high doses and prolonged administration of CCK have been tried, but success has been limited by rapid development of tolerance (Crawley and Beinfeld 1983).

1.7 CONCLUSIONS

An elaborate network of central and peripheral neuro-hormonal signals (Figure 1.2) has evolved to regulate feeding, one of the primal activities necessary for survival and self-preservation. Despite decades of animal and human research, the full extent of the processes and humors involved in the

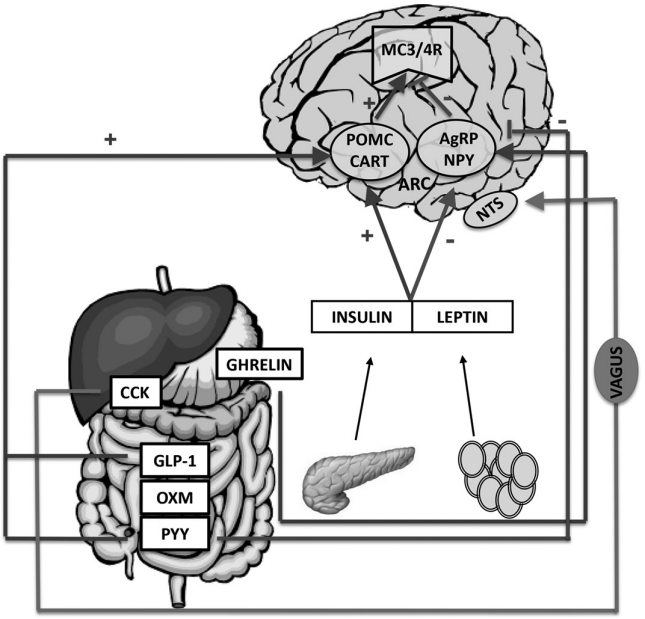

FIGURE 1.2 The hypothalamus and brainstem are critical regulators of appetite, integrating diverse hormonal signals from adipocytes (leptin), the pancreas (insulin), and the gastrointestinal tract (GLP-1, PYY, OXM, CCK, ghrelin) to regulate energy balance. Both insulin and the adipocyte-derived hormone leptin decrease food intake by acting in the arcuate nucleus (ARC) of the hypothalamus to stimulate POMC neurons and inhibit AgRP neurons, resulting in increased melanocortin receptor signaling (MC3/4R). GLP-1 and OXM are released from the L cells of the gut following nutrient ingestion and reduce food intake. GLP-1 directly stimulates POMC/CART neurons to suppress appetite. PYY decreases appetite by both inhibiting AgRP and stimulating POMC neurons. The gut hormone CCK, released primarily from the enteroendocrine cells of the duodenum, in response to nutrients and visceral stretch, stimulate satiety by way of the vagal afferent input to the nucleus tractus solitarius (NTS) of the hindbrain.

regulation of food intake remains to be elucidated. Current understanding indicates that energy homeostasis in health is predicated upon a balance between orexigenic and anorexigenic factors, both centrally and peripherally. Virtually all of the peripheral signals (e.g., insulin, PYY, leptin, CCK) are triggered by food ingestion and attenuated by fasting or starvation, indicating a response system that is tailored at satiety and meal termination. Ghrelin, the only peripheral signal that is activated pre-prandially, is unique in its role as a rare peripheral signal for hunger and meal initiation.

The rarity of peripheral hormonal signals that trigger meal initiation may be a reflection of the incompleteness of current understanding. However, a more plausible explanation is that appetite and hunger are under predominantly central control and are orchestrated by neuronal projections from various brain centers to POMC and AgRP/NPY expressing arcuate neurons. The central control of feeding is organized into an integrated neuroendocrine system that either stimulates or inhibits food intake. The orexigenic (e.g., NPY, AgRP) and anorexigenic (e.g., melanocortins) components of this system receive afferent neuroendocrine and metabolic signals from the periphery but may also be subject to local and paracrine influences, as well as inputs from higher brain centers. The coordinate regulation of these various opposing mechanisms leads to energy homeostasis that is physiologically skewed toward positive balance. An increased understanding of these mechanisms is a pre-requisite for the discovery of drug interventions that can dependably modulate food intake and prevent or treat obesity.

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2 Genetic Determinants of Nutrient Processing

Nicholette D. Palmer

CONTENTS

2.1 INTRODUCTION

Success in the era of human genetic studies was first realized with the identification of rare variants underlying Mendelian forms of disease. Also termed *single-gene disorders*, these diseases are often the result of changes in DNA sequence (mutations) in one gene and have been observed to segregate within families. The first Mendelian disease for which the molecular basis was identified was sickle cell anemia [1]. Resulting from a nucleotide transversion (A→T), the codon at amino acid position 6 in the β-hemoglobin gene (*HBB*) is mutated from a glutamic acid to valine. The mutation produces a variant form of the β-chain of hemoglobin, termed *HbS*, which polymerizes in red blood cells after deoxygenation [2,3]. This aggregation of hemoglobin chains causes red blood cells to distort into a crescent or sickled shape. These defective red blood cells are unable to transport oxygen and cause obstructions in the vasculature, resulting in increased mortality. This discovery demonstrated a molecular basis for disease that has been successfully applied to many other Mendelian diseases (e.g., cystic fibrosis [4], Tay Sachs disease [5], and Huntington's disease [6].) The majority of Mendelian phenotypes identified to date result from protein coding mutations, with relatively few phenotypes attributed to variation outside the coding region [7]. As of November 2017, there were 5,132 Mendelian phenotypes with a known molecular basis, while 1,593 remain unknown, and many more Mendelian conditions have yet to be recognized [8].

In contrast to Mendelian disease, the genetic basis of common diseases including diabetes, obesity, and coronary artery disease are heterogeneous, with modest contributions from variants, inclusive of those that predispose to monogenic forms of disease [9,10]. In fact, many Mendelian diseases present as more phenotypically severe forms of common diseases [11]. For example, both type 2 diabetes (T2D) and maturity-onset diabetes of the young (MODY) share a common etiology (i.e., presence of hyperglycemia resulting from defective pancreatic beta-cell function). MODY is a Mendelian disease estimated to occur in 1/10,000 adults, with an observed autosomal-dominant inheritance pattern, and attributed to six known genes [12–17]. In contrast, T2D is a highly prevalent common disease in which the genetic variants identified explain a relatively small proportion of the heritability, while variants contributing to MODY have only a nominal impact on T2D [18].

Despite these shortcomings, studying the genes that cause related monogenic disorders has identified potential pathways involved in the molecular basis of common disease.

Great progress toward understanding the genetic basis of Mendelian and common disease has been enabled with the completion of the Human Genome Project. Launched in 1990, the Human Genome Project was an international effort to determine the nucleotide sequence of the human genome with identification and mapping of genes, the hereditary unit of the genome coding for proteins, and other molecules. Declared complete in 2003, the Human Genome Project identified approximately 30,000–40,000 protein-coding genes, a preponderance of segmental duplications in the euchromatic regions of the genome that make up 92% of the human genome—that is, heterochromatic regions, including centromeres and telomeres, were not sequenced as part of the Human Genome Project, and identified more than 1.4 million single-nucleotide polymorphisms (SNPs) [19].

Prior to the completion of the Human Genome Project, few genetic variants had been identified that were reproducibly associated with common diseases or quantitative phenotypes (e.g., lipid levels and blood pressure), limiting our insight into disease pathophysiology. Once completed, the catalog of genetic variation provided a basis for disease gene mapping. These advances paved the way for the first genome-wide association studies (GWAS), which allowed for a comprehensive and unbiased assessment of the contribution of common variation across the genome to diseases and related phenotypes. The first GWAS was reported in 2005, for age-related macular degeneration (AMD), and was conducted in 96 patients previously diagnosed with AMD and 130 age-matched control individuals who were AMD-free. Among the 103,611 SNPs examined, a single SNP in the promoter region of *HTRA1*, a serine protease gene, was identified as a major genetic risk factor for AMD [20].

Discovery has accelerated, with improvements in genotyping technologies and development of analytical methods. Today, genotyping arrays can simultaneously assess up to 4,284,426 SNPs (e.g., Illumina Omni5 array). A summation of work published to date is publicly available through the GWAS Catalog (www.ebi.ac.uk/gwas) and, as of the latest publication, includes 24,218 unique SNP-trait associations from 2,518 publications in 337 different journals [21]. Collectively, GWAS have identified thousands of genetic variants associated with disease and underlying complex traits, yet the majority of these variants make only nominal contributions to disease risk and explain only a small proportion of familial clustering. While this observation could suggest that cumulative variation could be more impactful, a sophisticated evaluation of environmental exposure has yet to be addressed [9].

Traditional epidemiologic studies documenting an increase in prevalence rates of common diseases also supports environmental contributions. As an example, in type 1 diabetes, a disease highly prevalent among European children, the number of prevalent cases is expected to increase by 70% by 2020 [22]. An increasing prevalence of obesity has also been observed in the United States, with rates rising from 15% in the late 1970s to 35% today [23,24]. Consistent with these increases are the increases in prevalence of T2D [24] and cardiovascular disease (CVD) [25]. Taken together, the nominal effect of genetic variation on disease risk and rapid increases in disease prevalence suggests the contribution of nongenetic factors.

2.2 NATURE VERSUS NURTURE DEBATE

The dichotomy of nature versus nurture was formally presented by Sir Francis Galton in 1874 with his publication of *English Men of Science: Their Nature and Nurture*. By historical definition, "nature is all that a man brings with himself into the world; nurture is every influence from without that affects him after his birth" [26]. Even early on, this subject area argued that a man's natural abilities (e.g., intelligence and character traits) were derived from hereditary factors. This assertion conflicted with the empiricist views of earlier scholars, such as Francis Bacon and John Locke, who

argued that man's resemblance to "white paper, void of all characters," with "all the materials of reason and knowledge" derived from experience [27].

This debate continued into the twentieth century, with a noticeable shift toward nature resulting from rediscovery of Mendel's laws of heredity and initial insights into population genetics, and culminating with the Human Genome Project. Contemporary research now appreciates that nature and nurture domains are intertwined—that is, genes influence our response to environment, and our environment and experiences modulate the expression of genes. Although conceptually more complicated, the codependent nature of these domains argues for infinitely more finite time points at which the trajectory and progression toward disease are amenable to intervention.

2.3 NUTRITION, GENETICS, AND METABOLIC DISEASE

Metabolic syndrome is the designation of a group of interrelated risk factors that increase the incidence of CVD and T2D. Inclusive of central obesity, insulin resistance, dyslipidemia (i.e., increased triglycerides and reduced high-density lipoprotein), and hypertension, these perturbations promote the progression and pathogenesis of diet-related diseases. With the prevalence of obesity, diabetes, and hypertension increasing, the prevalence of metabolic syndrome has increased, with more than one-third of U.S. adults meeting disease criteria, thereby increasing morbidity and mortality [28].

The selective advantage of metabolic disease and its associated risk factors can be explained, in part, by the "thrifty gene" hypothesis [29]. Specific to the example of T2D and obesity, the "thrifty gene" hypothesis posits that there was evolutionary selection of genes related to energy storage and fat deposition that conferred benefit in times of food scarcity but which are associated with deleterious effects in a Westernized environment that is dominated by physical inactivity and excess caloric consumption. In support of this hypothesis is the finding that obesity and T2D have risen to epidemic proportions in certain ethnic groups living in a Westernized environment, compared to their native environment (e.g., Pima Indians) [30,31]. These gene-diet interactions describe the effect of dietary changes on genotype to produce a resultant phenotype or disease. This is supported by the interindividual variability observed in response to dietary modification (i.e., gene-nutrition interaction). A summary of relevant gene-nutrient interaction studies across a range of genes and dietary factors and their impact on metabolic disease is presented in Table 2.1.

TABLE 2.1
Gene-Nutrient Interactions and Their Effects on Metabolic Disease

Locus	Variant (Location)	Dietary Factor	Observation	References
Peroxisome proliferator-activated receptor delta (*PPARD*)	rs2016520 (5′ UTR)	Total fat	C allele carriers were protected from metabolic syndrome (OR = 0.62) with further reduced risk among low dietary fat consumers (OR = 0.42)	[44]
Acetyl-CoA carboxylase beta (*ACACB*)	rs4766587 (intronic)	n-6 polyunsaturated fatty acid (PUFA)	A allele carriers had increased risk of metabolic syndrome (OR = 1.29), which was exacerbated on a high-fat diet (OR = 1.62), particularly when rich in n-6 PUFA (OR = 1.82)	[83]
Nitric oxide synthase 3 (*NOS3*)	rs1799983 (E298D)	n-3 polyunsaturated fatty acid (PUFA)	A allele carriers had increased triacylglycerol (TAG) with low plasma n-3 PUFA	[84]

(*Continued*)