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Obesity and the Regulation of Energy Balance

Review

Bruce M. Spiegelman*‡ and Jeffrey S. Flier†‡
*Dana-Farber Cancer Institute and the Department of Cell Biology
Harvard Medical School
Boston, Massachusetts 02115
†Beth Israel Deaconess Medical Center
Division of Endocrinology and the Department of Medicine
Harvard Medical School
Boston, Massachusetts 02115

Obesity is defined medically as a state of increased body weight, more specifically adipose tissue, of sufficient magnitude to produce adverse health consequences. There has been an alarming increase recently in the prevalence of this heterogeneous group of disorders in the Western world (Kuczmarski et al., 1994). Fully one-third of the American population is now considered obese, and the prevalence of obesity in children is escalating dramatically, presaging even greater medical harm in the decades to come (Troiano and Flegal, 1999). What accounts for this epidemic of energy storage? Body weight and composition, and the storage of energy as triglyceride in adipose tissue, are determined by the interaction between genetic, environmental, and psychosocial factors. These influences ultimately act by changing the energy balance equation, that is, the long-term balance between energy intake and expenditure. Physiologic studies had previously suggested that body weight and energy stores are homeostatically regulated, with either weight loss or gain producing concerted changes in energy intake and expenditure that resist the initial perturbation. Recent cloning of several obesity genes has revealed the initial molecular components of a coherent physiologic system for energy homeostasis (Barsh et al., 2000). Studies of obesity pathogenesis must now attempt to explain the disorder in the context of this physiologic system.

Although the role of genes in body fat regulation is now established, it is safe to assume that the rising prevalence of obesity has not been due to a recent change in the genetics of the Western world. The propensity for obesity must have been in our midst for a long time, only to emerge recently on a large scale as a result of changes in the environment, in particular the availability and composition of food and reduced requirement for physical exertion. It is very likely that the ability to store fat in times of nutritional abundance was a positive trait selected over many thousands of years of human evolution. The idea that humans evolved to efficiently store excess energy as fat to deal with periodic famine has been given a name — the "thrifty gene" hypothesis (Neel, 1999). An obese human of approximately 250 lbs. has the energy stores to survive a total fast of approximately 150 days! This impressive energy reserve is due both to the high energy content of triglycerides versus polysaccharides, and the fact that triglycerides are stored in essentially anhydrous form; polysaccharides such as glycogen are hydrated in storage form, decreasing their efficiency as fuel.

To understand obesity, one must understand the concept of energy balance (Figure 1). Assuming that an individual has no problem with the absorption of nutrients, stored energy will increase *only* if energy intake exceeds total body energy expenditure. Energy expenditure takes the form of physical activity, basal metabolism, and adaptive thermogenesis. Physical activity refers to all voluntary movement, while basal metabolism refers to the myriad biochemical processes necessary to sustain life. Adaptive thermogenesis refers to energy dissipated in the form of heat in response to environmental changes, such as exposure to cold and alterations in diet. It should be pointed out that the boundary between what is considered basal metabolism versus adaptive thermogenesis is not always clear-cut. Mammals often live in climates with temperatures below body temperature, sometimes far below body temperature. Thus, the determination of energy expenditure in response to cold versus that which is considered part of basal metabolism can be quite arbitrary. Traditionally, the basal metabolism rate is defined as the energy expenditure of a subject relaxed and at rest, at thermoneutrality, 8–12 hr after the last food ingestion. Metabolic rates of mammals will obviously vary as a continuum depending upon precise environmental conditions to which the organism is exposed.

Various cellular events can generally cause or prevent obesity *only* if they affect the overall energy equation of the individual. For example, absent the concept of energy balance, it might be supposed that obesity and its complications could be ameliorated by directly limiting adipose tissue development. Experimental models in which fat cell differentiation, development, and survival have been directly reduced have shown that this is not the case. Far from creating healthy, lean mice, the resulting animals appear to suffer from lack of an appropriate depot in which to deposit excess energy, and loss of key adipocyte-derived hormones (Ross et al., 1993; Moitra et al., 1998; Shimomura et al., 1998). This results in increased levels of blood lipids, accumulation of fat in the liver, diabetes, and death. Conversely, when a genetically altered animal is found to be leaner, this *does not* necessarily imply that the targeted gene has a role in fat development, per se. Rather, a defect is created in some component in the total energy balance scheme—usually food intake or energy expenditure.

A precise understanding of the contribution of diet to obesity has been confounded by the difficulty of obtaining accurate measurements of food intake in free-living individuals. Obese individuals tend to underreport food intake by as much as 30% (Lichtman et al., 1992), and most obese individuals are believed to ingest more calories than lean individuals (matched for exercise and other features), to maintain their increased weights.

‡E-mail: bruce_spiegelman@dfci.harvard.edu (B. M. S.); jflier@caregroup.harvard.edu (J. S. F.).

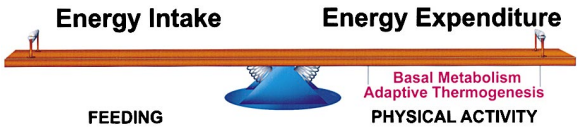

Figure 1. Key Component of the Energy Balance System

Obesity develops only if energy intake, in the form of feeding, chronically exceeds total body expenditure. Energy expenditure includes physical activity, basal metabolism, and adaptive thermogenesis.

Apart from the obvious effect of availability of palatable foods, the study of the influence of macronutrient composition, such as the balance of protein, carbohydrate, and fat on long-term body weight is in its infancy, and gene–diet interactions in the feeding response to diets of varying composition are just beginning to be studied. Because of the central notion of energy balance, food intake can be thought of as inadequate or excessive only in the context of that individual's energy expenditure.

It is clear that different individuals have a certain genetic propensity to store excessive caloric intake as fat. In a classic study, Bouchard and collaborators overfed pairs of monozygotic twins by precisely calibrated amounts (Bouchard et al., 1990). Different sets of twins showed remarkable differences in the degree to which these calories were stored as fat, but the tendency toward increased adiposity within each set of twins was remarkably similar. Since overfeeding above basal needs was controlled, the likely difference in fat accretion *between* sets of twins was likely due to differences in some component of energy expenditure, perhaps basal metabolic rates or adaptive thermogenesis. Further evidence that differences in metabolic rate are important variables in human obesity is provided by prospective studies done in Pima Indians (Ravussin, 1995), a group of Native Americans with a high predilection for obesity. When individuals were typed as to metabolic rates and then followed, those with lower metabolic rates had a greater incidence and magnitude of obesity.

CNS Control of Energy Intake and Body Weight

The central nervous system (CNS) influences energy balance and body weight through three mechanisms: (1) effects on behavior, including feeding and physical activity; (2) effects on autonomic nervous system activity, which regulates energy expenditure and other aspects of metabolism; and (3) effects on the neuroendocrine system, including secretion of hormones such as growth hormone, thyroid, cortisol, insulin, and sex steroids. The identity and coordination of these complex systems has been the subject of intense study, and much recent progress.

Regulation of Energy Intake: Short- and Long-Term Control

Feeding behavior lies at the interface between free will and physiology, and is influenced by many factors. In addition to food availability, feeding is affected by metabolic, neural, and endocrine factors, and is modified by powerful visual, olfactory, emotional, and cognitive inputs. Ultimately, all of these factors must be integrated, so that decisions to begin and end periods of feeding will result (Schwartz et al., 2000). The regulation of feeding behavior may be divided into short- and long-term control systems. Short-term control involves the initiation and termination of meals. The major determinant of meal size is the onset of satiety, a response to neural and endocrine factors, such as gut distension and release of the gut peptide cholecystokinin (CCK) that are generated during the course of meal ingestion (Moran, 2000). These signals are transmitted to the caudal brainstem via the vagus nerve, where integration with other inputs occurs, leading to meal termination. Regulation of individual meal size by factors induced by meal ingestion is insufficient to account for energy balance over long periods of time since mice repeatedly injected with CCK maintain weight by ingesting more meals of smaller size. Long-term signals that reflect the status of energy stores, such as the fat-derived hormone leptin, provide information to the CNS that further regulates feeding behavior to promote energy homeostasis. Not surprisingly, these short- and long-term systems are interrelated (Emond et al., 1999), such that the feeding response to energy deficit is accomplished predominantly through increased meal size.

Role of the Hypothalamus

The hypothalamus is a region of the brain critical for regulation of homeostatic processes such as feeding, thermoregulation, and reproduction (Elmquist et al., 1999). To accomplish these ends, the hypothalamus senses neural, endocrine, and metabolic signals, integrates these inputs, and engages distinct effector pathways, resulting in behavioral, autonomic, and endocrine responses. In addition to the hypothalamus, central control of appetite and energy balance clearly involves widely distributed neural systems in the brainstem, cerebral cortex, olfactory areas, and elsewhere. The central role of the hypothalamus in appetite and satiety was determined early on by lesion studies. Lesions in the ventromedial hypothalamus cause obesity, while lesions in the lateral hypothalamus cause leanness (Elmquist et al., 1999). The inherent crudeness of physical lesions and the absence of defined molecular and anatomic pathways underlying them limited the interpretation of this paradigm, however, and other approaches were taken. For example, the effects of centrally administered neurotransmitters such as norepinephrine, dopamine, and serotonin, and hypothalamic neuropeptides such as NPY and CRH on food intake, autonomic output, metabolism, and energy balance were assessed. These studies established the existence of several CNS ligand-receptor pathways capable of modifying energy intake, energy balance, and metabolic status.

Early Studies of NPY

Examination of the neuropeptide Y (NPY) pathway best exemplifies such studies. NPY is widely and abundantly expressed within the nervous system. The arcuate nucleus of the hypothalamus is one site of particularly dense expression, and nutritional regulation (increased with starvation) is uniquely observed at that site. When administered into cerebral ventricles or specific hypothalamic nuclei, NPY robustly and rapidly increases feeding and suppresses energy expenditure, and thereby promotes obesity (Stanley et al., 1986; Billington et al., 1994). NPY was therefore viewed as an excellent candidate for an endogenous regulator of energy balance, promoting anabolism in response to energy deficits. The

biggest gap in knowledge regarding NPY, and the many other neurochemicals similarly shown to stimulate or suppress feeding, was our limited understanding of the physiologic system in which these powerful neural circuits participated. Specifically, how did nutritional status communicate information to these central pathways for integration and control? One obvious candidate signal was the hormone insulin (Schwartz et al., 2000). Insulin levels do reflect energy balance and stores, as they fall with starvation and rise with obesity. Insulin is transported into the brain through a saturable process, and when centrally administered, suppresses both food intake and arcuate NPY expression. However, the absence of insulin, as in type 1 diabetes, is associated with weight loss rather than gain. Furthermore, evidence that physiologic changes in peripheral insulin levels affected energy balance through the brain was limited, so most investigators believed that insulin was not the dominant peripheral signal to the CNS for regulation of energy balance. That signal was yet to be discovered.

Monogenic Obesities and the Discovery of Leptin

The field of energy balance advanced rapidly with the cloning of genetic loci responsible for several of the previously identified monogenic obesity syndromes in mice. The most dramatic and consequential advance resulted from the identification of the basis for syndromes of obesity in ob/ob and db/db mice (Zhang et al., 1994; Chen et al., 1996). Prior studies used the technique of parabiosis, in which mice are surgically joined to permit passage of molecules from one to the other. These led to the suggestion 30 years ago that ob/ob mice might be deficient in a circulating signal of satiety, while db/db mice might be deficient in its cognate receptor. This prescient prediction was fully born out with cloning of these genes in 1994 and 1995. The ob gene encodes a unique member of the cytokine family now named leptin, from the Greek root leptos, for thin. The dominant site from which leptin is secreted is the adipocyte, and the protein is truncated and biologically inactive in mutant mice (Zhang et al., 1994). The obesity syndrome in ob/ob mice is corrected by administration of the missing hormone (Halaas et al., 1995; Friedman and Halaas, 1998). Regulated expression of leptin in other sites, such as skeletal muscle (Wang et al., 1998), placenta, and stomach has been reported, and may ultimately be proven to be physiologically important, although this is not yet established. The signaling form of the leptin receptor, ObRb, is deleted in db/db mice (Chen et al., 1996; Friedman and Halaas, 1998), which are consequently unresponsive to endogenous or exogenous leptin. The identification of these two proteins establishes the first well-documented components of a powerful nutritional feedback loop from adipose tissue to the brain.

Leptin Physiology

Despite the remarkable ability of leptin to reverse obesity in leptin deficient ob/ob mice, and to cause leanness in wild-type mice, the function of the leptin pathway may not be simply understood as an antiobesity axis. Indeed, substantial data suggest that basal levels of leptin in the fed state serve as a signal of energy sufficiency (Reviewed in Ahima and Flier, 2000). Withdrawal of the leptin signal occurs quite rapidly with food restriction, exceeding the rate at which fat stores are reduced (Ahima et al., 1996). Reduced leptin entrains a complex neural response characteristic of starvation that includes hunger/food seeking behavior, efficient metabolism (clearly demonstrated in rodents), and an array of neuroendocrine responses that favor survival during periods of limited energy, such as suppression of reproduction, linear growth, and thyroid hormone levels (Ahima et al., 1996). Reintroduction of energy supplies rapidly raises leptin levels and suppresses this starvation program. Absence of the leptin signal in the presence of sufficient energy promotes obesity in both rodents and humans by producing an internal perception of starvation in the midst of plenty. In addition to its actions through CNS circuits, leptin appears to exert several effects directly on peripheral tissues through leptin receptor signaling. One important effect may involve suppression of triglyceride accumulation in nonadipose tissue, such as muscle and liver, which contributes to insulin resistance (Lee et al., 2000). Potent effects of leptin have also been seen on the immune system, the vascular system, and even on bone turnover (Ducy et al., 2000). Although it clearly serves as the switch from the starved to the fed states, leptin has limits in controlling obesity. As fat mass increases, further rises in leptin have a limited ability to suppress food intake and prevent obesity, as seen by the prevalence of obesity despite high levels of circulating leptin (Considine et al., 1996). Thus, the antiobesity role of leptin might have been limited through evolutionary pressure to promote fat storage in times of plenty.

Central Neural Circuits Regulating Energy Balance

Knowledge of the neural circuits that coordinate the response to leptin and other inputs has increased at a rapid rate. Through positional cloning of rodent genes, targeted gene deletion, identification of mutant genes in human obesity, and follow-up studies using the techniques of functional neuroanatomy, a complex and extensive central circuit for regulation of energy balance has been defined. Although several sites in the brain express leptin receptors and respond to this hormone and various neuropeptides with changes in energy intake and expenditure, the best characterized and most clinically relevant circuit is simply described as a leptin-regulated central melanocortin circuit. The major components of this circuit are presented in Figure 2, where 7 unique proteins in a single pathway have been shown to contribute to weight regulation and obesity.

The Central Melanocortin Pathway

Leptin acts through ObRb receptors on two distinct populations of neurons in the arcuate nucleus. One population coexpresses the orexigenic (feeding-inducing) neuropeptides NPY and AgRP, and leptin action reduces their expression (Elias et al., 1999; Elmquist et al., 1999; Schwartz et al., 2000). The other population coexpresses mRNAs encoding anorexigenic peptides, cocaine and amphetamine related transcript (CART) and α-MSH (derived from proopiomelanocortin [POMC]), and leptin induces their expression (Elias et al., 1999; Elmquist et al., 1999; Schwartz et al., 2000). Thus, leptin suppresses two orexigenic peptides and induces two anorexigenic peptides through direct action on arcuate neurons. The pathway just described is incestuous, as AgRP and α-MSH are antagonistic ligands for a common receptor,

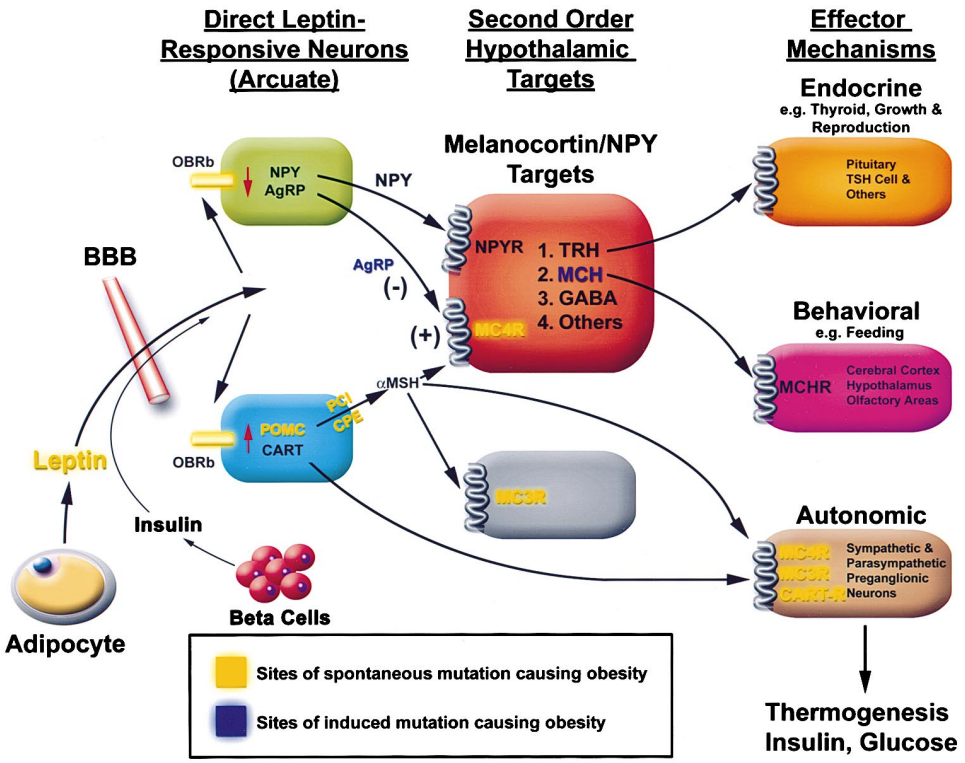

Figure 2. A Leptin-Regulated Melanocortin Circuit Influences Energy Homeostasis and Body Weight

The adipocyte hormone leptin crosses the blood brain barrier (BBB) and acts directly on two populations of neurons within the arcuate nucleus that express NPY and AgRP or POMC and CART. Leptin stimulates production of α-MSH, an agonist for the MC4 receptor (as well as CART), and inhibits production of AgRP, an antagonist for this receptor (as well as NPY). MC4 receptor–expressing neurons receive these leptin-regulated signals, as well as others, such as NPY. Such MC4R neurons are just now being chemically and functionally identified, and include TRH neurons in the paraventricular nucleus (PVH) that regulate the thyroid, MCH neurons in the lateral hypothalamus that regulate feeding, GABAergic neurons in the PVH that modify other as yet unidentified neurons tied into energy balance, and others. Several outputs of the MC4R-expressing neurons include: endocrine outputs such as thyroid, growth and reproduction, through control of pituitary function; behavioral outputs, including feeding; autonomic output, regulating energy expenditure; insulin secretion; and glucose homeostasis. Sites in the pathway at which spontaneous loss of function mutations have caused obesity in rodents and humans are indicated in yellow, as are sites at which induced mutations have caused obesity in rodents (in blue). Not shown here are potential direct actions of leptin on peripheral tissues.

the melanocortin 4 receptor (MC4R), which is expressed primarily in the brain (Cone, 1999). Activation of MC4R by MSH reduces food intake, while suppression of MC4R signaling through this receptor by the endogenous antagonist AgRP or pharmacologic antagonists increases feeding and diminishes the hypophagic response to leptin (Fan et al., 1997). This pathway was discovered through the convergence of several prior lines of investigation. The dominant obesity syndrome of the A^y mouse was shown to be due to a gene rearrangement causing ectopic expression of the coat color regulating protein agouti (Bultman et al., 1992). Through transgenic and pharmacologic experiments, it became apparent that agouti produced obesity by antagonizing the action of α-MSH on MCR4s within the brain (Lu et al., 1994; Fan et al., 1997; Ollmann et al., 1997; Graham et al., 1997), thereby mimicking the hypothalmic agouti homolog AgRP.

Gene deletion of the MC4R causes obesity in mice, and mice heterozygous for the knockout allele have moderate obesity as well (Huszar et al., 1997). Remarkably, 4%–5% of severe human obesity appears to be due to mutation at this locus, and most affected humans have a single mutant allele, which causes obesity through haploinsufficiency, rather than a dominant-negative mechanism (Farooqi et al., 2000). This suggests that this pathway is required for normal energy homeostasis and is extremely tightly regulated. The obesity in several other rare human and murine syndromes also converges on this pathway. For example, mutation in the POMC gene, which prevents production of POMC products including α-MSH, produces obesity in mice and humans (Krude et al., 1998). Likewise, mutation in neuropeptide processing enzymes PC-1 and carboxypeptidase E cause complex obesity syndromes in humans and mice respectively, very likely at least in part through effects on POMC processing (Reviewed in Barsh et al., 2000). Very recently, targeted deletion of the MC3R, a closely related receptor also restricted largely to the brain, was shown to produce obesity in mice (Butler et al., 2000). Interestingly, the obesity from this lesion occurs without the hyperphagia seen in MC4R mutants, and may be associated with a loss of lean body mass as well as increased adiposity. Thus, these two melanocortin receptors can cause obesity through distinct physiologic mechanisms.

Pathways Downstream of Melanocortin Receptors

The identification of a leptin-regulated melanocortin pathway provides a molecular and neuroanatomic link

between peripheral signals and CNS circuits, but leaves open the question of how these melanocortin signals produce downstream effects on appetite, energy expenditure, and neuroendocrine function. Several possible mechanisms are emerging. In one model, leptin-regulated arcuate melanocortin nerve terminals project onto neurons within the paraventricular hypothalamic nucleus (PVN) that have previously been described to respond in vivo to changes in nutritional status and leptin levels. The PVN can be viewed as a motor arm of the hypothalamus, as it regulates pituitary hormone secretion via specific neuropeptides released by projections to the median eminence, and regulates autonomic activity via projections to autonomic preganglionic neurons. One example is the TRH neuron in the PVN that regulates the pituitary–thyroid axis. TRH expression within the PVN is regulated through both melanocortin inputs from the arcuate nucleus acting through MC4Rs on TRH neurons, and by direct action via leptin receptors on these cells (reviewed in Flier et al., 2000). Similar mechanisms may account for leptin effects on other PVN neurons that influence endocrine status, autonomic function, or appetite. A second and parallel model involves direct projection of these arcuate melanocortinergic neurons (AgRP and α-MSH) onto neurons within the lateral hypothalamus that express the orexigenic neuropeptides MCH and orexin/hypocretin (Elias et al., 1999). MCH, the expression of which was discovered to be upregulated in ob/ob hypothalami, stimulates food intake (Qu et al., 1996). Deletion of the MCH gene causes a lean phenotype (Shimada et al., 1998), and transgenic overexpression promotes obesity. The first two models propose direct actions of melanocortins on TRH or MCH neurons. Additional mechanisms are likely to exist, since electrophysiologic evidence supports melanocortinergic neurons projecting to GABAergic interneurons in the PVN that are proposed to serve as integrators of numerous inputs (Cowley et al., 1999). It is likely that all of these mechanisms exist in concert.

Other Neuropeptides and Neurotransmitters

The melanocortin system, although extremely important, is not the only neuropeptide system involved in weight regulation. NPY acts through several species of GPCRs to regulate energy balance, and PVN neurons (either interneurons or neurons such as TRH neurons) may be sites where melanocortin and NPY signals are integrated. Although $NPY^{-/-}$ mice feed normally and have normal body weight, NPY deficiency ameliorates obesity and other features of ob/ob mice, indicating that NPY is necessary for the full response to leptin deficiency (Erickson et al., 1996a, 1996b). CART is expressed widely, and is coexpressed in many leptin-regulated arcuate POMC neurons. Among several projections, leptin regulated CART neurons in the arcuate nucleus project to autonomic sites in the spinal cord, providing a possible link to autonomic pathways (Elias et al., 1998). Many other neuropeptides, including CRH, GHRH, and galanin have been described to participate in these regulatory pathways, and most are beyond the scope of this review. Ghrelin is a peptide expressed in stomach and brain that was originally identified through its actions on the growth hormone axis. It is now clear that ghrelin promotes hyperphagia and obesity through actions in the brain, possibly on NPY neurons (Tschop et al., 2000). It is likely that additional peptides will be identified. In addition to neuropeptides and transmitters, the function of these circuits is also influenced by metabolic fuels. Neurons in the hypothalamus that respond to changes in glucose levels (low brain glucose promotes feeding) may be the same as, or functionally linked to those neurons that respond to leptin and express the peptides discussed above. A role for lipid mediators in metabolic sensing may be suggested by the recent observation that inhibitors of fatty acid synthase potently inhibit food intake through actions in the brain (Loftus et al., 2000).

The neurotransmitters norepinephrine, dopamine and serotonin are well known to be involved in central energy balance circuits. Serotonergic neurons within the caudal brainstem project widely within the brain, and drugs that increase serotonergic signaling suppress food intake and have been used to treat obesity. Mice with deletion of the $5HT_{2c}$ serotonin receptor subtype have modest obesity (Nonogaki et al., 1998). Leptin increases serotonin turnover, suggesting that these pathways can converge, but $5HT_{2C}$-deficient mice retain an anorectic response to leptin.

The Return of Insulin

Although the discovery of leptin overshadowed earlier interest in the role of insulin as a central regulator of energy balance, the venerable hormone has made a comeback. Not only is insulin a prominent positive regulator of leptin expression in the fat cell, but the mild obesity in mice with neuron-specific deletion of the insulin receptor (Bruning et al., 2000) or the insulin receptor substrate IRS-2 (Burks et al., 2000), support the idea that insulin and leptin may cooperate in central pathway regulation. It will be important to determine whether leptin and insulin signaling pathways converge on some of the same target cells, and by what mechanisms these signaling pathways might interact.

Leptin Resistance: Hard Wired or Acquired?

As discussed above, most obese humans and rodents develop obesity despite high leptin levels, and administration of additional leptin fails to reverse the obese state. It is possible that "leptin resistance" arose through evolution to permit energy storage in times of plenty. This still leaves open the question of mechanism. One potential mechanism involves a limitation of leptin transport across the blood brain barrier, which may operate through alternative splice variants of the leptin receptor highly expressed in brain microvessels. This mechanism is supported by the ability of leptin injected directly into the brain to both suppress food intake and induce hypothalamic signaling more effectively than leptin injected by the peripheral route in mice with diet-induced obesity (Van Heek et al., 1997; El-Haschimi et al., 2000). The molecular basis for this limitation is not yet defined. Leptin signaling within the hypothalamus may also be impaired in the obese state. In susceptible C57Bl mice with obesity induced by high fat diet, this signaling defect is acquired as obesity develops (El-Haschimi et al., 2000). Leptin resistance in these common states is not complete, however, since obesity is much less severe than seen in states of absent ligand or receptor, and these mice lack several features, such as neuroendocrine defects, that characterize mice completely lacking leptin or leptin receptors. This would make good evolutionary sense, since modifications of leptin action designed to permit energy storage would be best designed

to avoid the drastic consequences of starvation that result from total leptin lack. Currently, we do not understand the details of leptin signaling and targets that result in such discordant effects.

Leptin Signaling and Resistance

Leptin receptors are members of the class I cytokine receptor family that utilize associated Jak kinases for signal transduction (Tartaglia, 1997). The best studied aspect of leptin signaling is the Jak-dependent activation of the STAT 3 pathway, and subsequent regulation of target gene expression. It is also clear that leptin also induces, via Jak, activation of the MAPkinase pathway, one mechanism for which involves the participation of the phosphatase SHP-2. It has not yet been determined whether distinct effects of leptin, including rapid effects on ion channel activity, require specific downstream effector mechanisms. The control of cytokine signaling involves negative feedback signals. SOCS-3 is a member of one such family of negative regulatory proteins that is induced in leptin responsive cells by leptin signaling pathways, and can serve as a marker of cells responding directly to leptin (Bjorbaek et al., 1998, 1999). SOCS-3 inhibits leptin signaling by actions at the level of Jak as well as through binding to the receptor itself (Bjorbaek et al., 2000). It is not yet clear whether endogenous SOCS-3, or other regulators such as the STAT inhibitors PIAS-1/3 are responsible for leptin resistance in obesity. Since leptin and insulin may have some common sites of action, it is interesting to note that insulin signaling may also be antagonized by SOCS family members (Emanuelli et al., 2000).

Genes and the Environment

The cloning of genes responsible for the previously known rodent monogenic obesities (e.g., ob, db, and A^y) has led to the identification of regulatory pathways, and has indicated that these pathways are preserved in humans. The realization that severe obesity in humans can result from mutations in the ob, db, and MC4R loci, with the latter accounting for 4%–5% of severe cases, indicates the importance of these systems. However, the rarity of these mutations highlights the fact that most human obesity is polygenic rather than Mendelian, controlled by many genetic loci. Since the prevalence of obesity is increasing in industrialized societies, it is apparent that many of these genes must confer susceptibility to environmental factors, such as availability of food and composition of diets, and response to exercise, or lack of it. Efforts are underway to map genes that confer susceptibility to diet induced obesity in inbred strains of mice. Responsible genes may be part of already identified pathways, or may be previously unknown components of known pathways. In human populations, genome scans for linkage with obesity-related phenotypes are ongoing in many populations. One locus showing linkage to leptin levels in two populations is on the short arm of chromosome 2 at band 21 (2p21), a region that includes the POMC gene (Comuzzie et al., 1997). In addition to studies based on linkage, or detection of mutations in rare Mendelian disorders, many studies have sought evidence through a candidate gene approach. Although this approach can be successful when the candidate has a fundamental role (e.g., the MC4R locus), the list of plausible candidates is very long, and factors such as heterogeneity of populations and small biological impact of a given variation have led to inconsistent findings (e.g., the W64R variation in the β3 adrenergic receptor) (Reviewed in Barsh et al., 2000).

Control of Energy Expenditure—Adaptive Thermogenesis

The components of energy expenditure that can be readily altered, i.e., physical activity and adaptive thermogenesis, are of particular interest in the control of obesity. Since physical activity is more properly the realm of fitness gurus and psychologists, we will concentrate here on describing the current state of scientific thinking about adaptive thermogenesis. Work in this area has centered on two main aspects: the neural circuitry that activates thermogenesis and the peripheral tissues that actually oxidize fuels.

One might have assumed that neural pathways that control food intake and energy expenditure are completely distinct. However, both naturally occurring and targeted genetic lesions in mice have indicated that these pathways are tightly interrelated. Mutations in leptin and the leptin receptor, MCR4 and MCH all strongly influence *both* food intake and energy expenditure in a coherent way, so that fat storage is increased (or decreased) via both of these major components of the energy balance equation. Conversely, exogenous administration of leptin to leptin-deficient mice decreases food intake, but also keeps energy expenditure at a higher level than would be expected for that degree of food intake (Friedman and Halaas, 1998). Clearly, a major component of this brain-driven thermogenesis is dependent on output from the sympathetic nervous system to the peripheral tissues, especially brown fat and skeletal muscle. While the role of the sympathetic nervous system in activating brown adipose tissue (BAT)-mediated thermogenesis via β-adrenergic receptors and cyclic AMP (cAMP) is well established, the role in skeletal muscle is still a matter of some conjecture. That catecholamines infused into skeletal muscle increase energy expenditure without the performance of work is quite clear (Simonsen et al., 1992); whether the key step(s) involves control of vascular tone, or regulation of specific components involved in fuel oxidation is not established. However, the fact that mice genetically ablated for the production of catecholamines show greater sensitivity to cold than do mice mutated in UCP-1 (Enerback et al., 1997; Thomas and Palmiter, 1997) (see below), suggests strongly that other, non-BAT pathways of thermogenesis are important.

The mitochondrion is the cellular furnace where fuels (dervied from fatty acids and glucose) are oxidized and energy is either stored in the high-energy phosphate bonds of ATP or is released as heat. Figure 3 illustrates that as electrons are passed down, the energy gradient of the electron transport chain, protons are pumped out of the inner matrix of the mitochondria, generating an electrochemical gradient across the inner mitochondrial membrane. These protons have two probable fates: as described by Mitchell, they can reenter the mitochondrial matrix through ATP synthase, driving the synthesis of ATP. ATP production is thus linked to the consumption of oxygen and this is referred to as coupled respiration. Alternatively, protons may "leak" back across the

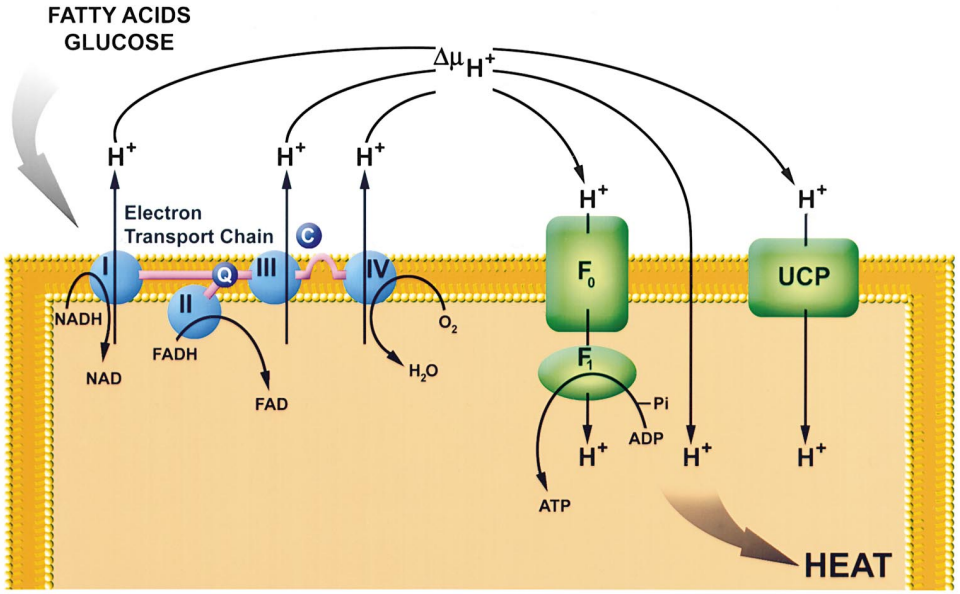

Figure 3. Mitochondrial ATP Metabolism and Thermogenesis through Proton Transport

Fatty acids and glucose are oxidized to generate NADH and $FADH_2$, which donate electrons to the electron transport chain. Ubiquinone (Q) shuttles electrons from both complexes I and II to complex III, whereas cytochrome c (C) shuttles electrons from complex III to complex IV. Molecular oxygen (O_2) is the terminal electron acceptor. Protons are pumped out by complexes I, III, and IV of the electron transport chain, which creates a proton electrochemical potential gradient ($\Delta\mu_{H+}$). Protons may reenter the mitochondrial matrix through the F_0/F_1-ATPase, with energy being used to generate ATP from ADP and Pi. Protons may also reenter through an uncoupling protein (UCP), or the membrane itself, with energy being released in the form of heat. Abbreviations: complex I, NADH-ubiquinone oxidoreductase; complex II, succinate-ubiquinone oxidoreductase; complex III, ubiquinone-cytochrome-c oxidoreductase; and complex IV, cytochrome-c oxidase.

inner mitochondrial membrane in a manner not linked to ATP production. This uncouples energy storage from oxygen consumption and is referred to as uncoupled respiration. A certain degree of "leak" is an inherent property of several biological membranes, but this proton translocation can be greatly accelerated by the action of uncoupling proteins (UCPs), which function as specialized proton channels not linked to ATP production. In uncoupled respiration, energy is released as heat because these leaks, whether catalyzed by UCPs or not, disrupt the cycle and result in fuel oxidation in the absence of work.

The thermogenic function of BAT and UCP-1 have been extensively studied. Interestingly, mice genetically reduced in BAT are prone to obesity while mice deficient in UCP-1 have not shown a propensity to gain weight (Lowell and Flier, 1997). On the other hand, mice lacking UCP-1 are extremely sensitive to cold. These data suggested that there may well be additional mechanisms that can control energy expenditure and metabolic rates in addition to UCP-1, even in BAT.

The last several years have seen the identification of two other members of the UCP family: UCP2, widely expressed in many tissues, and UCP3, expressed primarily in BAT and skeletal muscle. Hopes have been high that these proteins may play major roles in whole body energy expenditure outside of BAT, particularly in skeletal muscle (Kozak and Harper, 2000; Ricquier and Bouillaud, 2000). While it seems clear that these proteins can uncouple oxidative phosphorylation at a cellular level and UCP-3 can protect against obesity when greatly overexpressed in the skeletal muscle of mice (Clapham et al., 2000), several pieces of data have suggested that the role of these proteins in energy balance and physiology may be complex. Most strikingly, fasting, which is associated with a major *reduction* in total body energy expenditure, is associated with an *increase* in the expression of UCP2 and UCP3 mRNA (Boss et al., 2000). More recently, mice deficient in UCP2 or UCP3 have been made and neither strain shows a significant propensity for hypothermia, reduced energy expenditure, or obesity (Arsenijevic et al., 2000; Gong et al., 2000; Vidal-Puig et al., 2000). Together, these data suggest that none of the known UCPs *alone* has clear-cut anti-obesity effects. Of course, it is still entirely possible that they can functionally compensate for each other in targeted mutations, so combined mutations must be created before absolute conclusions can be drawn.

Where does this leave us with regard to mechanisms of energy expenditure and adaptive thermogenesis in large animals like humans, where the mass of skeletal muscle vastly exceeds that of brown fat? There are several possibilities. First, since the inner membrane of mitochondria itself leaks protons, it is possible that adaptive thermogenesis is controlled in an important way by regulation of mitochondrial biogenesis and electron transport rates themselves. An increased electron transport system will drive a greater membrane potential as more protons are pumped out. It is well recognized that this greater membrane potential alone will result in increased proton leak and increase uncoupled respiration even in the absence of a UCP. Hence, UCP1 may be a very specialized molecule for defense against cold in small mammals, but large animals with smaller surface

to volume ratios may simply be able to use the inherent properties of biological membranes to regulate heat production and adaptive thermogenesis.

Alternatively, one can imagine that other "futile-cycles" can serve as specific thermogenic mechanisms regulating metabolic rates (reviewed in Lowell and Spiegelman, 2000). From a bioenergetic perspective, there is nothing magical about a futile cycle of proton pumping that could not, in principle, be accomplished through other means. Key examples exist—deep diving fish have a specially modified periocular muscle that is relatively devoid of cotractile elements. Depolarization of these muscle cells causes release of calcium from the sarcoplasmic reticulum. ATP is then consumed by the Ca^{2+}-ATPase, which pumps calcium back into the sarcoplasmic reticulum in a futile ion cycle. Ca^{2+} cycling results in fuel oxidation without work being performed, thus generating heat. Mammals apparently have a potential to cycle calcium in a similar way. Humans or pigs carrying mutations in the ryanidine receptor, the Ca^{2+} release channel of the sarcoplasmic reticulum, release Ca^{2+} in response to stress or an anesthesia. This results in futile calcium cycling and an intense thermogenesis that can be fatal. Such a mechanism is also observed in cold-adapted birds, suggesting that this could play a role in adaptive thermogenesis of other animals including humans.

Transcriptional Control of Thermogenesis

Several key steps of mammalian thermogenesis, particularly mitochondrial biogenesis and the expression of UCP1, have been the subject of considerable study at the transcriptional level. Much of this effort has been focused on the role of cAMP in inducing these processes.

The UCP1 gene has an enhancer element that is both brown fat selective and responsive to cyclic AMP stimulation (Cassard-Doulcier et al., 1993; Kozak et al., 1994). Perhaps surprisingly, there have been no reports to date of brown fat–specific transcription factors regulating this enhancer. Rather, there are binding sites for PPARγ, an important regulator of both white and brown fat cell differentiation, a potential thyroid hormone response element (TRE), a retinoic acid response element (RARE), and several potential cyclic-AMP response elements (Sears et al., 1996). As defined in knockout mice, the only transcriptional component shown to be required for BAT development is PPARγ (Barak et al., 1999).

The genes of mitochondrial biogenesis and the respiratory chain have been intensively studied, leading to the discovery of nuclear respiratory factor (NRF)-1 and -2 as key transacting elements. The vast majority of mitochondrial genes that are encoded in the nuclear genome have functional binding sites for NRF-1, NRF-2, or both (Virbasius and Scarpulla, 1994; Gugneja et al., 1996). The NRFs, in turn, regulate mitochondrial transcription factor A (mtTFA), which directs the transcription and replication of the mitochondrial genome. How external stimuli, such as cold or diet, affect the amount or activity of NRFs has not been well studied.

Recently, a transcriptional component, PPARγ coactivator (PGC)-1, that can coactivate and coordinate many transcription factors that participate in multiple aspects of adaptive thermogenesis has been described (Puigsever et al., 1998). PGC-1 is expressed in multiple tissues of rodents and man, but is cold inducible only in BAT and skeletal muscle. This cold induction is the result of the sympathetic nervous system acting via β-3 adrenergic receptors and cyclic cAMP (Boss et al., 1999). When PGC-1 is expressed in white fat or skeletal muscle cells, a broad program of thermogenesis begins, including induction of mitochondrial biogenesis, expression of an uncoupling protein (UCP-1 or UCP-2 in fat cells or muscle, respectively), and an increase in total cellular respiration. From the perspective of the adipose lineage, PGC-1 expression makes white fat cells more like brown cells (Puigserver et al., 1998; Wu et al., 1999).

PGC-1 can interact with and coactivate a large number of transcription factors in addition to PPARγ. Indeed, PGC-1 can interact with most nuclear hormone receptors (Puigserver et al., 1998; Knutti et al., 2000; Tsherepanova et al., 2000). PGC-1 docks on some receptors, such as PPARγ or PPARα, in a non-ligand-dependent way and interacts with other receptors, such as the estrogen receptor or glucocorticoid receptor, in a ligand-dependent manner. The ability of PGC-1 to promote mitochondrial biogenesis appears to be due to its ability to turn on the expression of both NRF-1 and NRF-2, and to directly coactivate NRF-1 through protein–protein interactions (Wu et al., 1999). PGC-1 loses most or all of its ability to activate mitochondrial biogenesis in the presence of a dominant-negative allele of NRF-1. The ability of PGC-1 to activate the UCP-1 enhancer appears linked to the coactivation of PPARγ, since PGC-1's activity on this enhancer is ablated by a mutation of the PPARγ binding site.

It is interesting to note that while most biological programs studied to date show dominant regulation at the level of the DNA binding transcription factor, adaptive thermogenesis shows remarkable regulation at the level of the coactivator. Presumably, this is due to the fact that this complex program, which involves multiple tissues, hormone sensitivities, and transcription factors, requires more genetic coordination than can be achieved at the level of a single DNA binding factor. A crucial question that must be answered is whether expression of PGC-1 itself determines or helps to determine whether cells are white fat cells or brown fat cells. Similarly, skeletal muscle fibers come in two types: slow twitch (type 1) that are very oxidative and are very rich in mitochondria, and fast twitch (type 2), which have a more glycolytic metabolism and have fewer mitochondria. Since slow twitch fibers are more oxidative, a greater number of these versus fast twitch fibers could well alter total body energy expenditure. The role of PGC-1 in both of these cell biological decisions must be determined via gain- and loss-of-function experiments in mice.

Linking Lipogenesis and Adipogenesis—A Role for ADD1/SREBP1?

A chronic imbalance between energy intake and energy expenditure can lead to an increase in both fat cell size and fat cell number. Recent studies have suggested a mechanism that can potentially link lipogenesis and adipogenesis.

Several recent reviews have been written on the transcriptional control of adipogenesis (Mandrup and Lane 1997; Rosen et al., 2000), so the details of this process will not be discussed here. What now seems very clear

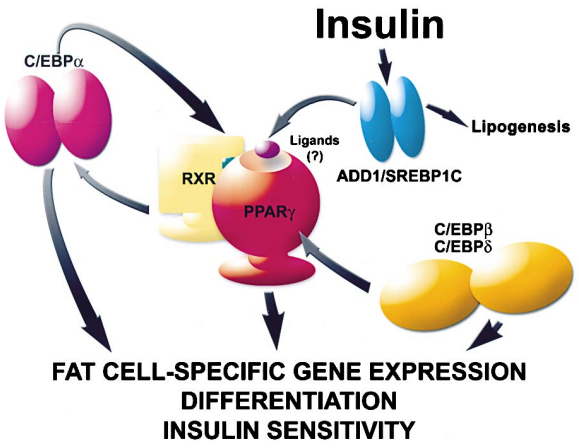

Figure 4. The Transcriptional Control of Adipogenesis Involves the Activation of Several Families of Transcription Factors

These proteins are expressed in a network in which C/EBPβ and C/EBPδ are detected first, followed by PPARγ, which in turn activates C/EBPα and a broad program of adipogenesis. C/EBPα exerts positive feedback on PPARγ to maintain the differentiated state. ADD1/SREBP1c is regulated by insulin in fat and can activate PPARγ by inducing its expression as well as by promoting the production of an endogenous PPARγ ligand. ADD1/SREBP1c also activates many genes of lipogenesis. All of these factors contribute to the expression of genes that characterize the terminally differentiated phenotype.

is that fat cell differentiation is regulated by the function and interplay between several members of the C/EBP transcription factor family, and the nuclear receptor PPARγ. This is summarized in Figure 4. A key question has been, how can a chronic imbalance between energy intake and energy expenditure trigger both fat cell hypertrophy and fat cell hyperplasia? There is no doubt concerning the dominant role played by insulin in the fed and chronically overfed states. There have been a number of transcription factors that can potentially regulate different genes of lipogenesis, but ADD1/SREBP1, a member of the basic helix-loop-helix family of factors, can activate a broad program of genes involved in fatty acid and triglyceride metabolism in both fat and liver (Kim and Spiegelman, 1996; Shimano et al., 1996). Importantly, the expression of ADD1/SREBP1 is regulated by fasting and feeding and this was shown to be regulated by insulin in fat (Kim et al., 1998a). Subsequent studies showed that insulin also regulated this factor in liver (Shimomura et al., 1999). ADD1/SREBP1 has been shown to be synthesized as a membrane-bound precursor that must be released by proteolysis (Brown and Goldstein, 1998; Brown et al., 2000). Cleavage of the ADD1/SREBP1 does not seem to be regulated by cholesterol levels, unlike SREBP2, and the pathway responsible for this activation for ADD1/SREBP1 is still unclear.

ADD1/SREBP1 can also accelerate adipogenesis. This factor alone cannot promote differentiation of non-adipogenic fibroblasts, but when coexpressed on fibroblasts expressing PPARγ, cell differentiation is enhanced (Kim and Spiegelman, 1996). Subsequent studies have shown that ADD1/SREBP1 can enhance the transcriptional activity of PPARγ, and indeed, can activate an isolated ligand binding domain of PPARγ fused to the DNA binding domain of the yeast Gal4 (Kim et al., 1998b). It is most likely that this occurs via transcriptional control of the enzymes required to make an endogenous, agonist ligand of PPARγ. This possibility is further strengthened by the fact that ADD1/SREBP1 controls several known enzymes of fatty acid metabolism, and all known biological ligands for PPARγ are fatty acids or fatty acid derivatives. The identity of the precise PPARγ ligands regulated by ADD1/SREBP1 are unknown but of great interest.

Therapeutic Issues and Opportunities

It is estimated that 300,000 people die annually in the United States as a result of obesity, and most of these deaths are due to the effect of obesity in promoting diabetes, hypertension, cardiovascular disease, and cancer (Kopelman, 2000). Therapy based on nutritional and behavioral counseling is capable of producing useful weight loss with attendant reduction of morbid consequences, but weight loss is usually partial, and almost always temporary. Existing pharmaceuticals target central serotonergic and adrenergic pathways, or inhibit intestinal fat absorption, and are of limited efficacy (Bray and Tartaglia, 2000). The approval of new therapies for obesity will require high standards for safety for several reasons. These include the likely need for chronic therapy, the history of toxicity of serotonergic drugs, concern about abuse by those seeking weight loss for purely cosmetic reasons, and the common, if erroneous view that obese individuals should be able to lose weight through personal effort rather than drugs. Despite these concerns, new insights into the molecular and physiologic pathways that underlie regulated energy balance have created many opportunities for drug discovery in the obesity field.

Obesity Therapy and Energy Balance

A successful obesity therapy must impact energy intake, energy expenditure, or both. Since several key targets (e.g., leptin receptors and MC4Rs) coordinately increase energy expenditure and suppress energy intake, it is possible to envisage therapies that accomplish both effects. Therapies that target energy intake or expenditure alone may initially produce weight loss, but the existence of a homeostatically defended feedback loop would be expected to resist further weight loss and limit efficacy. For example, if weight loss resulted in a fall in leptin levels, this might resist further weight loss through effects on central pathways to increase hunger and decrease energy expenditure. It is possible that combination therapies aimed at two or more distinct steps in the pathway of energy balance might be necessary, as is often the case in disorders such as hypertension and type II diabetes. Finally, we may eventually target specific therapies to individual lesions underlying obesity in specific cases. It is already evident that rare patients lacking leptin respond dramatically to this hormone, while patients lacking the receptor will be totally unresponsive, and patients with common obesity have a limited response.

Therapies Aimed at Suppressing Food Intake

Despite disappointing results of initial clinical trials, it may be possible to find strategies for administering leptin to patients with common obesity that will favor weight

loss or maintain weight loss brought about by other means (Reviewed in Mantzoros and Flier, 2000). The phenomenon of leptin resistance motivates the search for small molecule leptin receptor agonists that might bypass the blood brain barrier, or receptor sensitizers. Several cytokines suppress appetite and promote weight loss. Ciliary neurotrophic factor (CNTF) is a neurocytokine that has been found to reverse obesity in leptin resistant db/db mice, possibly by activating JAK/STAT signaling pathways in leptin responsive neurons (Gloaguen et al., 1997). Therapeutic trials in obesity are underway. Several gut-derived peptides that influence satiety, such as cholecystokinin (CCK) and glucagon-like peptide-1 (GLP-1) (Flint et al., 1998), or small molecule agonists for their receptors, are therapeutic candidates. Perhaps the greatest pharmaceutical effort is addressing the central neuropeptide pathways. Knockout experiments for NPY and its several receptors suggest a robust capacity for redundancy of these pathways, but small molecule antagonists for NPY Y-1 and Y-5 receptors have shown preclinical promise, and are under development (Gehlert, 1999). It should be stressed that the phenotypes of gene knockout mice may not always predict the response to inhibitors of the same pathway. Although less advanced than NPY antagonists, great effort is being expended to develop MC4R agonists and antagonists for the MCH receptor.

Therapies Aimed at Increasing Energy Expenditure

The fall in energy expenditure during weight loss limits the efficacy of diets, and therapies that could prevent this, or simply increase energy expenditure, would promote weight loss. Thyroid hormone promotes thermogenesis by as yet uncertain mechanisms, and it is well known to clinicians that increased thyroid hormone produces weight loss, but other adverse effects of thyroid excess including loss of lean body mass prevent its use as an obesity treatment. β-3 adrenergic agonists are highly effective at promoting thermogenesis and weight loss in animals, and if selectivity for β-3 receptors can be established, such drugs may be very effective (Himms-Hagen et al., 1994). Likewise, drugs capable of activating or increasing expression of the newly identified mitochondrial uncoupling proteins UCP-2 and UCP-3, or the transcriptional regulator PGC-1, would be interesting therapeutic candidates.

Therapies that May Limit Obesity by Uncertain Mechanisms

The results of several gene knockout experiments have produced mice with resistance to obesity that was not anticipated. Protein tyrosine phosphatase-1B (PTP 1-B) knockout mice have enhanced insulin sensitivity, and are also resistant to obesity caused by high-fat diet (Klaman et al., 2000). Although the mechanism for the effect on energy balance is uncertain, PTP-1B could be a target for a drug to treat both diabetes and obesity. The enzyme acyl CoA:diacylglycerol transferase (DGAT) mediates the final step in the glycerol phosphate pathway of triglyceride synthesis. Mice lacking DGAT are lean and resistant to obesity induced by high-fat diet, and surprisingly manifest increased energy expenditure that remains unexplained (Smith et al., 2000). DGAT may also be a target for anti-obesity therapy. Perilipin is an adipocyte protein that regulates lipolysis through effects on hormone sensitive lipase. A perilipin knockout mouse, in which adipocyte lipase is constitutively increased, is lean despite increased food intake, and has increased metabolic rate; absence of perilipin dramatically reduces obesity of db/db (Martinez-Botas et al., 2000). Although some aspects of the phenotype, such as increased energy expenditure, are yet to be explained, perilipin may be an interesting drug target.

Conclusions

Obesity and its antithesis, starvation, have always been part of the human condition, and for most of human history have been seen as resulting simply from availability of food, or acts of will related to attainment of desired body shape. Although this view persists in some quarters to this day, the last 5 years of the millennium have witnessed a dramatic increase in our understanding of the biology of regulated energy balance and body weight. Physiologic pathways whose existence was debated 10 years ago are now being characterized in molecular detail, with immediate implications for understanding of pathogenesis of human obesity and other disorders of energy balance. The roadmap provided by these advances establishes a clear direction for future research, but critical details remain to be discovered, and therapeutic applications remain to be realized. In particular, the mechanisms by which environmental factors, including diet and exercise, interact with molecular pathways in the common polygenic forms of obesity is largely unknown at present. Insights from the sequencing of the human genome and the coming advances in proteomics are likely to fuel the next wave of progress. It is likely that both new genes and new regulatory pathways will be identified. It may seem unlikely that the recent wave of progress can be matched in the early years of the current millennium, but we would not choose to make that bet.

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