Obesity Wars: Molecular Progress Confronts an Expanding Epidemic

Review

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The worldwide prevalence of obesity is increasing at an alarming rate, with major adverse consequences for human health. This "obesity epidemic" is paralleled by a rapid and substantive increase in our understanding of molecular pathways and physiologic systems underlying the regulation of energy balance. While efforts to address the environmental factors that are responsible for the recent "epidemic" must continue, new molecular and physiologic insights into this system offer exciting possibilities for future development of successful therapies.

The past ten years have been the golden age of obesity research. While long understood that the amount of body weight and fat are determined by an interplay of inheritance and environment, we are for the first time learning the identity of some of the responsible genes. Not surprisingly, the protein products of these genes define critical physiologic pathways for the regulation of energy balance. Among the most consequential discoveries were the ob gene product leptin (Zhang et al., 1994) and the leptin receptor, the latter first reported in *Cell* in 1995 (Tartaglia et al., 1995). These discoveries and others have transformed a former research backwater into a hotbed of modern science and drug discovery. It seems likely that insights into weight-regulatory pathways will accelerate identification of molecular targets that will eventually produce safe and effective pharmaceuticals for obesity and its complications. However, therapeutic optimism is occurring not a moment too soon, as an epidemic of obesity rages on, oblivious to these scientific achievements. Why is this happening? Obesity-promoting changes in our diet and reduced physical activity are accelerating on a global scale, outpacing scientific progress. Nevertheless, many individuals manage to resist obesity. Accordingly, a key goal of future research must be to identify mechanisms by which environmental factors interact with specific genes, either to promote, or facilitate resistance to obesity.

Overview of Regulated Energy Balance

Over the past decade, we have reached consensus that a physiologic system exists, the prime function of which is to maintain homeostasis of energy stores in response to variable access to nutrition and demands for energy expenditure. This system has both afferent sensing components, and efferent effector limbs. The afferent limb of this system includes several kinds of signals. One reflects short-term events such as those related to onset or termination of individual meals; another senses the long-term status of body energy stores. Although these long and short-term signals have often been viewed as operating independently, it now appears that they functionally overlap. Both converge on brain centers, most importantly within the hypothalamus, where the signals are integrated, and the direction and magnitude of efferent responses are determined. The efferent elements of the physiologic system include those regulating the intensity of hunger and subsequent food seeking behavior, the level of energy expenditure, including basal and that determined by physical activity, the levels of key circulating hormones such as insulin and glucocorticoids, and to a much smaller degree factors that influence the relative sizes of lean and fat mass in the body. Some of these same signals also regulate processes such as reproduction and growth that are linked to nutritional sufficiency (Figure 1).

Since survival is more acutely threatened by starvation than obesity, it should come as no surprise that this system is more robustly organized to galvanize in response to deficient energy intake and stores than to excess energy (Ahima et al., 1996). Indeed, the efficient storage of energy as fat promotes survival when food supplies are scarce, and evolution would be expected to favor such "thrifty genotypes" (Neel, 1999). Nonetheless, increased energy stores promote adaptive responses that resist obesity in experimental animals and humans. These "obesity avoidance" responses are characterized by suppression of appetite and increased energy expenditure (Weigle, 1994), and involve the same effector mechanisms that respond in an opposite direction to starvation, as though a switch can be thrown from starvation avoidance to obesity avoidance modes depending on the environment. Unfortunately, circuits that suppress appetite and increase energy expenditure in response to obesity-promoting aspects of the current environment are insufficiently robust to prevent obesity and its complications in a large and increasing fraction of the population. On the other hand, many individuals do resist obesity despite exposure to a common "toxic" environment. The variable susceptibility to obesity in response to environmental influences is undoubtedly modulated by specific genes. Existing knowledge at this interface is still fragmentary and is likely to be an area of great future progress.

Leptin and Energy Balance

Eight years after the positional cloning of the ob gene (Zhang et al., 1994) and the subsequent discovery that the encoded protein, leptin, is an adipocyte-derived cytokine whose administration reverses obesity caused by leptin deficiency in mice (Halaas et al., 1995) and humans (Farooqi et al., 1999), the biological role of leptin continues to be actively explored. Leptin was initially viewed as an adipocyte-derived signal that functioned primarily to prevent obesity, and that was the basis for its being named leptin, from the Greek root leptos for thin (Halaas et al., 1995). It is now understood that leptin also serves as an important signal from fat to brain

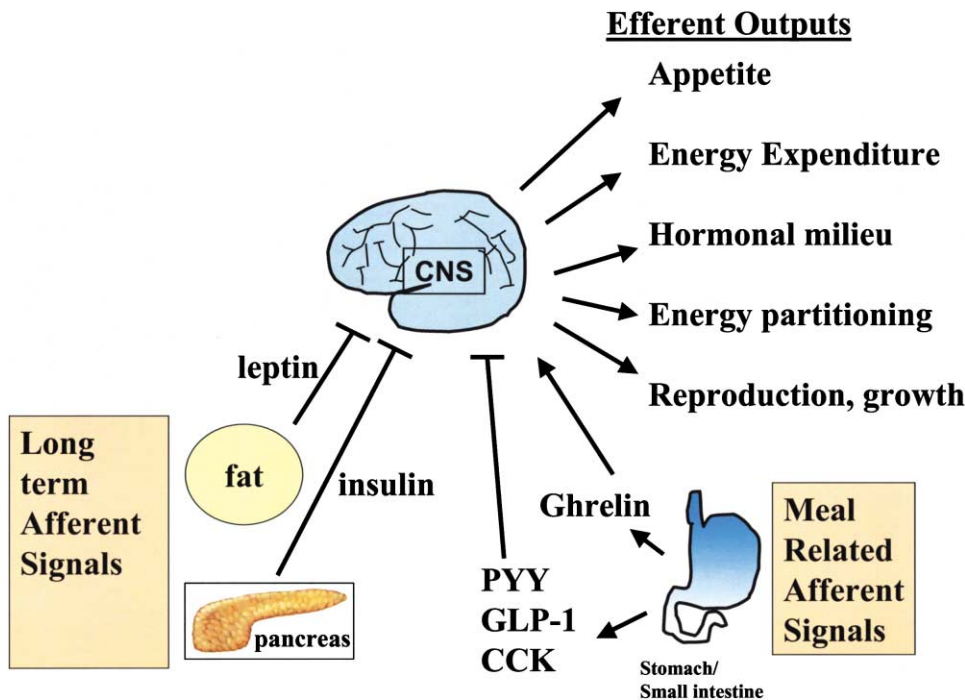*Correspondence: jflier@bidmc.harvard.edu

Figure 1. Components of the Energy Balance System

The energy balance system involves long-term afferent signals from fat (leptin) and pancreatic β cells (insulin) and short-term, meal-related afferent signals from the gut, including inhibitors of feeding (PYY, GLP-1, and CCK), and the stimulator of feeding (ghrelin). These inputs are integrated within the brain. Efferent outputs regulate appetite, energy expenditure, hormonal milieu, energy partitioning, and the status of reproduction and growth.

informing the brain, by its suppression, that the body is starving (Ahima et al., 1996), and this function is likely to be as important, or more important, than its antiobesity role. In the absence of leptin, the brain senses starvation despite massive obesity. Leptin deficiency drives hunger, suppresses energy expenditure, and inhibits reproductive competence, all advantageous adaptations in the context of starvation. The most sensitive area of the leptin dose response curve resides between the low levels brought about by food restriction (starvation) and the rising levels induced by refeeding. Additional effects of very high leptin levels that develop with obesity are at best of modest magnitude, and resistance to the action of leptin to suppress body weight is prone to develop in many individuals.

Some important differences between mice and humans deficient in leptin are now apparent. Leptin deficient mice have clearly reduced energy expenditure and efficient metabolism such that obesity develops even without overeating. In contrast, efficient metabolism has not yet been documented in several totally leptin deficient humans; hyperphagia appears to be the sole or dominant factor (Farooqi et al., 2002). Likewise, leptin deficient mice have a marked activation of the hypothalamic-pituitary-adrenal (stress) axis that contributes to the obese and diabetic phenotypes, but this is not observed in leptin deficient humans (Farooqi et al., 2002). Despite these species differences, mice and humans deficient in leptin are united by markedly increased appetite and consequent obesity, plus failure to initiate puberty; both of these abnormalities are dramatically suppressed by therapy with recombinant leptin. (Farooqi et al., 2002).

Is there a syndrome of partial leptin deficiency, more common than the extremely rare homozygous loss of function mutants of leptin or its receptor? Relatives of the leptin-deficient patients who are heterozygous for leptin deficiency do have a phenotype, comprising modest obesity and leptin levels below that expected for their increased fat mass (Farooqi et al., 2001). Such individuals will likely respond to leptin therapy with weight loss, although this has not yet been reported. In patients with ordinary obesity, leptin levels are increased in proportion to body fat (Considine et al., 1996), and this hyperleptinemia, together with the meager response of body weight in such individuals to therapeutic recombinant leptin (Heymsfield et al., 1999), defines a state of leptin resistance. Despite this correlation between fat mass and leptin level in populations, leptin levels in individuals with the same degree of obesity can vary widely, suggesting that a subpopulation might have relative leptin deficiency, which might predict leptin responsive obesity. Studies with recombinant leptin have to date provided no direct evidence for this hypothesis.

Leptin Resistance
The mechanism for resistance to the weight reducing effects of leptin in most obesity has received considerable attention, but is still unclear eight years after the first identification of the leptin receptor (Tartaglia et al., 1995). Two general molecular mechanisms have been identified. The first may involve a defect in leptin trans-

port across the blood brain barrier, to sites of action within the CNS critical for leptin regulation of energy balance. The existence of a transport defect is supported by the enhanced potency of leptin administered within the brain (as opposed to peripherally) in a mouse model of diet-induced obesity (Van Heek et al., 1997). Short isoforms of the leptin receptor lacking the sequences required for STAT activation, such as the original species cloned by Tartaglia et al. in 1995, are expressed at high levels in brain microvessels of mice, and contribute to leptin transport into the CNS (Hileman et al., 2002). Whether the Vmax of leptin transport is inherently inadequate (in some or all obese individuals) to permit high leptin levels to act at certain sites within the CNS, or an initially effective transport process becomes impeded by unknown nutritional or environmental factors is as yet unclear. It is also unresolved to what degree leptin action in specific regions of the hypothalamus or extra-hypothalamic brain requires transport across the blood brain barrier (BBB). Some important leptin target neurons in the arcuate nucleus (vide infra) are thought to reside outside the BBB, directly exposed to the peripheral circulation. To the extent that these sites are key loci of leptin action, a defective leptin transport mechanism should not be important. On the other hand, it is possible that the importance, in isolation, of these target sites in the arcuate nucleus, or their exposure to the peripheral circulation, have been overestimated.

The long isoform of the leptin receptor, known as ObRb, is a potent activator of JAK/STAT signaling (Vaisse et al., 1996). In normal mice exposed to high fat diets that promote obesity, the ability of leptin to activate STAT3 in hypothalamus is diminished (El-Haschimi et al., 2000). Potential mechanisms for suppression of proximal leptin signaling have therefore been sought. SOCS3 is an antagonist of leptin signaling that is rapidly induced within key hypothalamic neurons in a leptin dependent manner (Bjorbaek et al., 1999). Although there are suggestions that SOCS3 expression is induced in some tissues in models of nutritional or age-associated obesity (Wang et al., 2000), the role of this factor in the etiology of obesity has been difficult to resolve with certainty through genetic approaches, as Socs3$^{-/-}$ mice are embryonic lethal. Other approaches to creating SOCS3 deficiency in order to assess the effect on weight regulation and leptin action are needed. Recent studies indicate that haploinsufficiency of SOCS3 creates mice that are more sensitive to leptin, and resistant to obesity and its metabolic complications produced by high fat diet (B.J.C., J.K.H., L.O., I.T., C.B., and J.S.F., unpublished data). PTP1b is another candidate regulator of leptin sensitivity. Mice deficient in this tyrosine phosphatase have increased insulin sensitivity, and also resist obesity, and PTP1b deficient mice are more sensitive to leptin, possibly by enhanced JAK kinase activity (Zabolotny et al., 2002; Cheng et al., 2002). Whether SOCS3 and PTP1b have additive effects to suppress leptin signaling has not yet been evaluated.

Although resistance to leptin action on body weight and STAT3 signaling are seen in typical obesity, it is clear that this is both less severe than that seen in rare cases where the receptor is totally lacking, and less global, since the leptin signal required to maintain repro-

duction is maintained in usual obesity, but absent when receptors are absent. There is therefore increased interest in the precise signaling pathways downstream of the leptin receptor that mediate its actions on energy balance and endocrine function. Initial attention focused on the JAK STAT pathway, and consequent regulation of gene expression. However, additional pathways downstream of JAK, including MAP kinase and PI3 kinase, are capable of being activated by leptin in vitro and in vivo (Kim et al., 2000). Recently, a knockin mouse has been created (Lepr Y1138S) in which the leptin receptor is incapable of activating STAT 3, but retains ability to activate other pathways downstream of JAK (Bates et al., 2003). The resulting mouse has severe obesity and many other features of complete leptin resistance seen in lepr$^{db/db}$ mice with mutation of the leptin receptor gene, but interestingly this mouse has preserved reproductive capacity, suggesting that some actions downstream of the leptin receptor employ signaling pathways independent of STAT 3. Studies with enzyme inhibitors suggest that PI3 kinase may be necessary for some of the actions of leptin on food intake (Niswender et al., 2003). Actions of leptin to suppress appetite and regulate endocrine function may therefore involve both PI3 kinase and STAT3 signaling, as well as other pathways as yet undefined.

A Role for Insulin in Energy Balance?

Is leptin the only peripheral hormone whose levels fall to signal starvation, and rise in proportion to increasing obesity to limit weight gain? The extreme obesity of leptin deficiency indicates that lack of leptin cannot be substantially compensated by other hormonal signals. On the other hand, other less potent signals might coexist with leptin and play a role in energy balance. Insulin is a product of the pancreatic β cells and is the master metabolic switch between the fed and fasted states as relates to metabolic fuel disposition and use. Its levels are well known to fall with fasting, and rise with obesity, similar to leptin. This led to the proposal many years before the cloning of the ob gene, that insulin might be the dominant signal of fuel status to the brain (Woods et al., 1985). A transport system of insulin across the BBB was described, and administration of pharmacologic doses of insulin within the CNS suppresses food intake in rodents and subhuman primates, and regulates expression of hypothalamic neuropeptides that influence appetite (Schwartz et al., 2000). The discovery of leptin put this subject on the back burner, but insulin is receiving renewed attention. Targeted deletion of the insulin receptor in neurons produces obesity in mice (Bruning et al., 2000), but this is very mild compared to the severe obesity that develops when leptin receptors are deleted from neurons (Cohen et al., 2001). Interestingly, suppression of insulin signaling within the brain using antisense reduces the ability of peripherally administered insulin to suppress hepatic glucose production (Obici et al., 2002b). Insulin had been thought to exert its actions on the liver directly on peripheral organs. Thus, CNS insulin receptors may influence both energy balance and peripheral metabolic pathways, perhaps through signaling in key integrative neurons that also respond to leptin and other signals. Since rats with
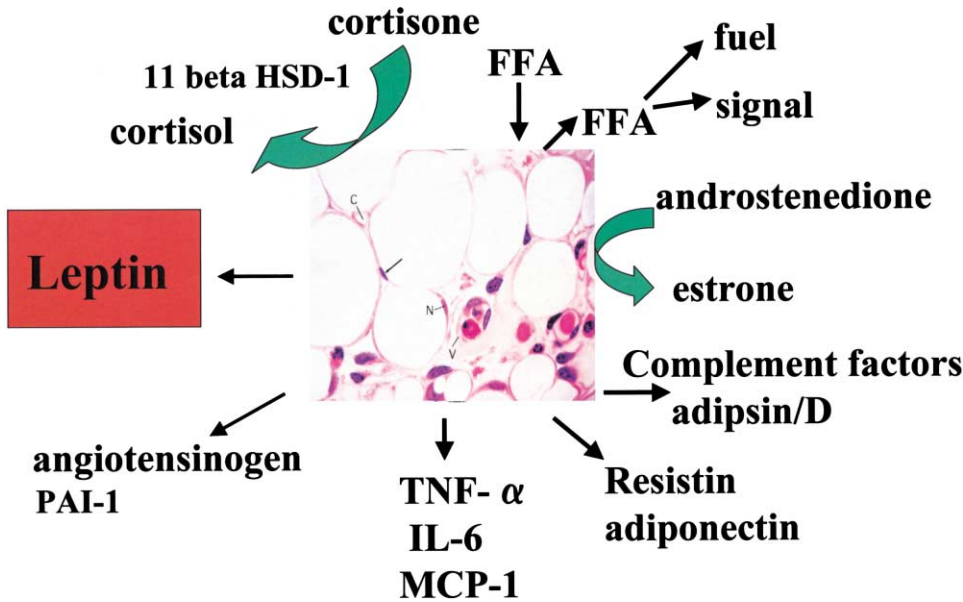
Figure 2. The Adipocyte as an Endocrine Cell

In addition to releasing free fatty acids (FFA) which can function as fuels or signals, adipocytes can modify steroid hormones leading to production of estrogen and glucocorticoids, and release an increasing number of hormones, hormone precursors, hemostatic regulators, or cytokines including leptin, angiotensinogen, plasminogen activator inhibitor-1 (PAI-1), TNF-α, IL-6, resistin, adiponectin, and complement factor D, also known as adipsin.

mutations in the leptin receptor gene have a reduced response to insulin administered centrally, leptin action may influence insulin action pathways in the brain.

Direct Actions of Leptin Outside of the Brain

Many actions of leptin are exerted initially through binding to receptor sites on specific hypothalamic neurons, after which peripheral actions are exerted through consequences of reduced food intake, or changes in secretion of pituitary hormones and autonomic nerve activity. However, the long, signaling form of the leptin receptor (ObRb) is also expressed in many peripheral tissues, including liver, fat, heart, β cells of the pancreas, and immune cells. Several approaches have been employed to demonstrate that leptin can signal directly in peripheral cells, and the actions of leptin through such direct pathways may reverse some phenotypes of leptin deficiency, such as by promoting dissipation of intracellular fat (Unger, 2002). Since many aspects of leptin deficiency can be reversed by administration of leptin within the brain (Campfield et al., 1995), or by brain specific expression of leptin receptors in receptor deficient mice (Kowalski et al., 2001), the relative importance of central versus direct peripheral actions of leptin remains uncertain. Although not related directly to the actions of leptin on energy balance, leptin has potent actions on cells of the immune system (Lord et al., 1998) and the skeleton (Ducy et al., 2000). It is likely that actions on the immune system are predominantly direct, whereas, surprisingly, the actions of leptin on bone may be mediated largely by changes in sympathetic nerve activity within bone (Takeda et al., 2002). An action of leptin to activate the enzyme AMP kinase in peripheral tissues, which is important in regulating the oxidation of lipid fuels, is medi-

ated by both neural pathways and direct actions on peripheral tissues (Minokoshi et al., 2002).

The disorders of lipoatrophy, in which fat tissue fails to develop or is lost, have produced valuable insights into the mechanism for metabolic dysfunction that accompany leptin deficiency. Mice develop lipoatrophy when a truncated version of the nuclear protein SREBP-1c is transgenically expressed in adipose tissue (Shimomura et al., 1999). The insulin resistance and diabetes seen in these mice was reversed by recombinant leptin (Shimomura et al., 1999), supporting the view that deficient leptin caused by loss of its tissue of origin, is a major cause of this metabolic defect. Recombinant leptin therapy in patients with congenital lipodystrophy has also been shown to ameliorate insulin resistance, as well as reduce the accumulation of lipid within many tissues other than fat (Oral et al., 2002). To what extent these actions are mediated via the CNS or involve direct actions on peripheral organs is unresolved.

Leptin Is Not the Only Adipose Cell Product that Affects Systemic Metabolism

The adipose cell was previously viewed primarily as a site for energy to be stored as triglyceride during periods of energy surfeit, and released as free fatty acids and glycerol during periods of energy need. The discovery that absence of the adipocyte hormone leptin produces severe obesity established the adipocyte as a key endocrine gland as well (Ahima and Flier, 2000) (Figure 2). Many discoveries over the past several years have extended this concept, and reveal additional mechanisms by which altered adipocyte function can translate into changes in systemic energy balance, or affect the state of diverse physiologic pathways.

Acrp30/adiponectin is an adipocyte-secreted protein that circulates at high concentration (Scherer et al., 1995). Levels of adiponectin are reduced in obesity, and the suppression correlates with insulin resistance in obesity and related disorders (Weyer et al., 2001). Replacement of deficient adiponectin has a variety of salutary effects, including reducing glucose and lipid levels, increasing lipid oxidation rates, and reducing vascular thickening (Berg et al., 2002). Adiponectin appears to act in part by activating the enzyme AMP kinase (Yamauchi et al., 2002a). Levels are also induced by treatment with antidiabetic thiazoladinediones (Yu et al., 2002). The cognate receptor for this molecule has recently been identified (Yamauchi et al., 2003), and the physiologic role of this adipocyte hormonal system is receiving great attention. Resistin is another adipoctye-secreted protein that was identified as the product of a gene whose expression in fat was suppressed by treatment with antidiabetic thiazoladinediones (Steppan et al., 2001). Initial studies suggested that resistin was induced in obesity and might be in part responsible for systemic insulin resistance. An effect of recombinant resistin to induce hepatic insulin resistance has been confirmed (Rajala et al., 2003), although data on its expression in obesity are conflicting (Way et al., 2001), and its cellular mechanism of action remains unknown. IL-6 is an immune modulating cytokine that is also expressed in fat, and adipocyte expression is increased in obesity. IL-6 deficient mice develop late onset obesity that can be prevented by low dose infusion of IL-6 into the brain (Wallenius et al., 2002). The extent to which fat-derived IL-6 exerts a normal restraint on body fat is unclear at this time. In most cases of human obesity, adipocyte expression of cytokines such as IL-6 and TNF-α is increased. The identity of the upstream pathways responsible for this "inflammatory state" within adipose tissue is a major unanswered question. It seems likely that additional adipocyte-secreted factors with systemic impact on body weight or obesity complications remain to be identified.

Several murine models in which adipocyte function is selectively altered by changing expression of transcription factors, enzymes, or other proteins are characterized by leanness and/or resistance to obesity. Adipose selective knockout of the insulin receptor gene produces lean mice that resist obesity, suggesting that insulin signaling in adipocytes is necessary for obesity to develop (Blüher et al., 2002). Foxc2 is a winged helix/forkhead transcription factor whose expression in fat is induced by feeding high fat diets. Transgenic overexpression of Foxc2 in fat leads to a lean and insulin sensitive phenotype (Cederberg et al., 2001). Genetic variability at this locus may influence the development of the metabolic syndrome (Ridderstrale et al., 2002). Mice heterozygous for deletion of the PPAR γ (Yamauchi et al., 2001; Miles et al., 2000) and CBP (cAMP response element binding protein) (Yamauchi et al., 2002b) genes are also resistant to the development of obesity and have increased insulin sensitivity. Since activation of PPAR γ by antidiabetic thiazoladinedione drugs improves insulin sensitivity, the observation that global PPAR γ haploinsufficiency improves insulin sensitivity is counterintuitive, and so far unexplained.

Two additional models suggest that alterations of adi-

pocyte lipid metabolism can produce systemic changes in metabolic rate that resist obesity. Acyl coenzyme A: diacylglycerol acyltransferase 1 (DGAT1) is one of two known enzymes that catalyze the final step in mammalian triglyceride synthesis. DGAT1-deficient mice have increased insulin and leptin sensitivity, and increased energy expenditure, likely accounting for their protection against diet-induced obesity and insulin resistance (Chen et al., 2002). The leptin-sensitizing effect of DGAT1 deficiency is present in both leptin-resistant and leptin-deficient genetic models of obesity and may occur in part by enhancing the effects of leptin in peripheral tissues. The mechanism for this enhancement is unknown. Perilipin coats adipocyte lipid droplets and was postulated to modulate hydrolysis of triacylglycerol. Mice with deletion of perilipin are lean with constitutive activation of adipocyte lipolysis, and they resist obesity due to high fat diets (Martinez-Botas et al., 2000; Tansey et al., 2001). This resistance to obesity is due to increased metabolic rate, which is currently unexplained.

Increased systemic exposure to glucocorticoids induces obesity, but most obese individuals have no such systemic overexposure. The enzyme 11 β Hydroxysteroid dehydrogenase-1 (HSD-1) reactivates glucocorticoids locally from inactive metabolites, and this enzyme is expressed within adipose tissue, and is overexpressed in fat in obesity (Rask et al., 2001). Transgenic overexpression of the enzyme in fat produces obesity, with preponderance of the more dangerous visceral fat and a full range of metabolic complications, suggesting a possible role for this enzyme in obesity and its complications (Masuzaki et al., 2001).

Gut Signals Also Regulate Energy Balance
Well before the discovery of leptin as a peripheral signal of energy stores and energy balance, the gut was known as a source of signals that influenced appetite, in particular providing signals to limit individual meals. Since leptin levels do not rise after meals, it was clear that leptin is not such a signal. On the other hand, leptin deficiency nullifies the efficacy of meal-related signals, since individuals lacking leptin have little or no satiety in response to meals. In addition to sensors for stretch that send signals directly to the brain via afferent nerves, endocrine signals from the gut can regulate appetite. Cholecystokinin (CCK) is the most venerable of these. CCK is released from the small intestine into the circulation in response to luminal nutrients such as fatty acids, and influences satiety by actions on CCK receptors located on peripheral vagal afferent terminals, which transmit neural signals to the brainstem (Moran, 2000). A physiologic role for CCK in the control of meal size has been demonstrated by studies with antagonists, and in Otsuka Long Evans Tokushima Fatty rats, which lack the CCK-A receptor and are obese (Moran et al., 1998).

Three other gut peptides that are released postprandially are GIP, GLP-1, and PYY, and recent studies with each have caused renewed interest in gut factors in energy balance. PYY is released from the intestine postprandially in proportion to meal size. Data from rodents and humans suggest that this peptide reduces food intake postprandially and may do so by actions on inhibi-
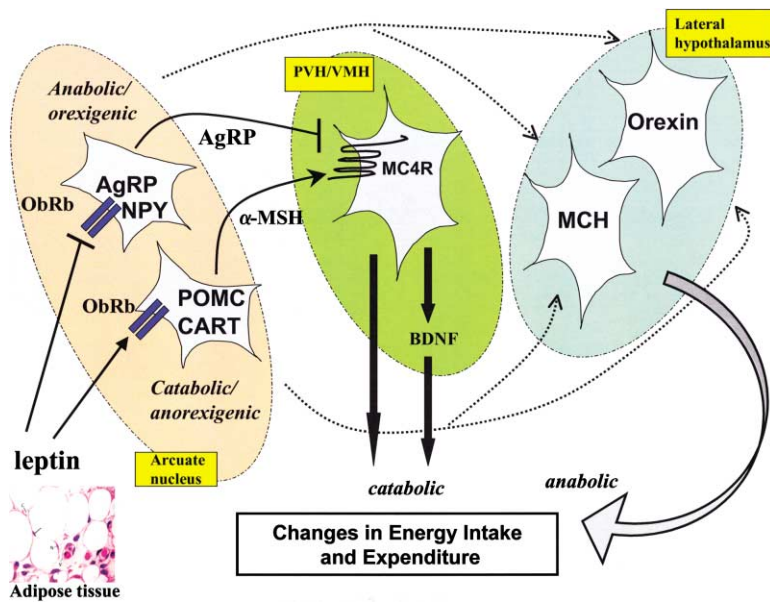
Figure 3. Leptin-Regulated Hypothalamic Circuits Are Important for Energy Balance

Leptin acts directly on arcuate nucleus neurons coexpressing NPY and AgRP, and POMC and CART, via the ObRb form of the leptin receptor expressed on these cells. The former neurons stimulate anabolic and orexigenic effects and are suppressed by leptin, and the latter neurons stimulate catabolic and anorexic actions that promote weight loss, and are activated by leptin. A key downstream target of these neurons are neurons expressing melanocortin 4 receptors (MC4R) that are activated by the POMC product α MSH, and inhibited by the neuropeptide AgRP. Activation of these neurons promotes catabolism by reducing food intake and increasing energy expenditure. Neurons expressing BDNF in the ventromedial hypothalamus may be downstream of the MC4R neurons. Neurons in the lateral hypothalamus expressing melanin concentrating hormone (MCH) receive projections from leptin responsive arcuate neurons, and activation of these widely projecting neurons promotes feeding, suppresses energy expenditure, and promotes weight gain. These circuits integrate additional information not illustrated here.

tory presynaptic NPY Y2 receptors present on arcuate nucleus NPY neurons (Batterham et al., 2002). Since PYY levels appear to be reduced in obese individuals after meals and replacement doses of PYY reduce appetite in the obese, therapeutic implications are substantial (Batterham et al., 2003). GLP-1 is another gut-derived peptide, also expressed within the brain, that is released postprandially and produces anorectic effects as well as symptoms of visceral illness, and activates the sympathetic nervous system (Yamamoto et al., 2002). GIP is released from duodenum, predominantly in response to ingested fat, and mice fed a high fat diet have increased GIP levels and obesity. Mice lacking the GIP receptor are protected against high fat diet-induced obesity, and Gipr$^{-/-}$ mice bred to ob/ob mice are substantially less obese (Miyawaki et al., 2002). Thus, GIP may promote obesity in response to fat.

Ghrelin is a peptide recently found to be made in the stomach, which acts on a previously identified orphan receptor (growth hormone secretagogue receptor), whose activation in hypothalamus causes growth hormone release from the pituitary gland (Kojima et al., 1999). This peptide may be more important for regulating food intake and body weight through actions in the arcuate nucleus, than for growth hormone regulation. Ghrelin is unique among gut hormones in stimulating food intake, and its chronic administration to rats causes obesity (Tschop et al., 2000). It has been suggested that ghrelin levels rise just before spontaneous feeding and may in part account for meal initiation. The fall in ghrelin levels after gastric bypass surgery for obesity has been hypothesized to in part account for the suppression of appetite commonly seen after such operations (Cummings et al., 2002), although interventional studies replacing ghrelin in postsurgical patients are needed to confirm this. In addition to stomach-derived ghrelin, a population of hypothalamic ghrelin neurons that contact

other feeding-related neurons has been described (Cowley et al., 2003). The functional implications of these cells are unknown.

CNS Integration of Afferent Signals

The hypothalamus is the primary locus for integration of signals that influence energy balance (Figure 3). The results of genetic studies have revealed the outlines of several critical hypothalamic circuits. The best defined of these is the melanocortin pathway (Fan et al., 1997). This pathway involves neurons within the arcuate nucleus that express proopiomelanocortin (POMC), from which the peptide α-MSH is cleaved. Leptin directly depolarizes and increases POMC expression in these neurons (Cowley et al., 2001). α-MSH is an agonist for a key receptor expressed within the CNS, the melanocortin 4 receptor (MC4R) and administration of α-MSH or its analogs centrally suppresses feeding. Complete loss of function of this G protein-coupled receptor (GPCR) produces obesity in mouse and humans (Huszar et al., 1997), (Yeo et al., 1998). The pivotal nature of this pathway is indicated by the fact that haploinsufficiency at this locus also causes substantial obesity (Huszar et al., 1997). Remarkably, the MC4R receives not only the agonist ligand, α-MSH, whose expression is induced by leptin, but also an antagonistic ligand that promotes feeding (AgRP) (Ollmann et al., 1997) whose expression in a nearby but distinct population of arcuate neurons (that coexpress AgRP and NPY) is diminished by leptin. Although this pathway is clearly a major leptin-regulated pathway whose output affects appetite, energy expenditure, and metabolic actions in the periphery, the melanocortin pathway is not the only pathway downstream of leptin, and leptin is not the only factor that regulates the melanocortin pathway. Regarding the latter point, MC4R pathways appear to be involved in the regulation of appetite and body weight mediated through serotonin

5HT2C receptors (Heisler et al., 2002), activation of which causes weight loss and deletion of which causes a syndrome of adult onset obesity in mice. The serotonin and melanocortin systems may also be relevant to cachexia (Marks et al., 2001) and perhaps eating disorders such as anorexia nervosa.

NPY is another neuropeptide whose expression within the arcuate nucleus (coexpressed with AGRP) is linked to energy balance. Its expression at these sites is negatively regulated by leptin and insulin, and central NPY administration induces feeding and suppresses energy expenditure (Billington and Levine, 1992). The numerous subtypes of NPY receptors, of which there are at least 5, and the complex phenotypes observed when antagonizing this system either pharmacologically or through gene knockout (Wieland et al., 2000), has led to some confusion about the physiology of this system. Although deletion of the NPY gene partially suppresses the obesity of ob/ob mice (Erickson et al., 1996), normal mice with combined deletion of both NPY and AgRP suffer (Qian et al., 2002) at most very limited deficits in feeding or body weight.

Melanin-Concentrating Hormone

In contrast to NPY, melanin-concentrating hormone (MCH) is a key player in another hypothalamic circuit for weight regulation that does not readily tolerate disruption (Figure 3). This peptide is expressed exclusively in large neurons whose cell bodies reside within the lateral hypothalamus and zona incerta, an area implicated through lesion studies as a "feeding center." These neurons make broad monosynaptic projections throughout the neuraxis, and MCH receptors are similarly widespread. Deletion of the MCH gene produces a lean and hypophagic mouse with increased energy expenditure (Shimada et al., 1998), and eutopic overexpression of MCH produces obesity (Ludwig et al., 2001) and susceptibility to a high fat diet. MCH binds to a GPCR that is expressed in the brain, and targeted deletion of this receptor (MCHR1) produces a lean mouse due largely to increased energy expenditure, rather than suppression of feeding (Marsh et al., 2002). Administration to mice of an antagonist of this receptor suppresses food intake and obesity that develops on a palatable diet, and has effects as well to reduce behaviors interpreted as mouse "anxiety" (Borowsky et al., 2002). A second MCHR is found in human but not rodent brain, and its function is unknown. Neuroanatomical studies reveal a connection between arcuate neurons expressing POMC and AGRP/NPY and MCH neurons in the lateral hypothalamus (Elias et al., 1999). The potential functional relationship between these circuits is being evaluated through genetic cross breeding. Deletion of the MCH gene substantially suppresses many aspects of the ob/ob phenotype (Segal-Lieberman et al., 2003). The orexins are two peptides expressed in lateral hypothalamic cells distinct from MCH cells. Although these peptides have effects on feeding, the main role of orexins appears to be related to sleep, as deficiency of orexin or its receptors causes narcolepsy (Mieda and Yanagisawa, 2002).

Additional CNS Pathways Exist

Several additional hypothalamic pathways that may regulate energy balance have emerged over the past several years. Bombesin-like peptides are widely distributed in the CNS and gastrointestinal tract and bind to several GPCRs. Mice lacking bombesin receptor subtype-3 develop obesity, hyperphagia, and reduced metabolic rate, as well as impaired glucose metabolism, indicating a role for this receptor in central control of energy balance (Ohki-Hamazaki et al., 1997). Brain derived neurotrophic factor (BDNF) was initially studied for its role in sensory neuron development. BDNF and its receptor, TrkB, have been identified in hypothalamic neurons associated with satiety. Mice heterozygous for BDNF deficiency are obese and hyperphagic (Kernie et al., 2000), and this can be reversed with central injection of BDNF. Obesity is also seen after conditional deletion of BDNF in brain, suggesting that this neurotrophin may have regulatory roles in the central energy balance circuits (Rios et al., 2001). BDNF is highly expressed in the ventromedial nucleus (VMN), where its expression is regulated by nutrition and MC4R signaling. BDNF may be an important effector of MC4R signaling (Xu et al., 2003). Brain histamine has also been implicated in energy balance circuits, with central histamine administration suppressing food intake and fat accumulation. Leptin has also been shown to modulate central histamine turnover, which is reduced in leptin deficient or resistant mice (Yoshimatsu et al., 1999). Leptin suppression of hypothalamic endocannabinoids anandamide and 2-arachidonoyl glycerol has also been implicated in regulated energy balance (Di Marzo et al., 2001). Acting through CB1 receptors, these endocannabinoids may mimic the effect of marijuana to stimulate appetite, a conclusion supported by the phenotype of CB1 receptor knockout mice (Cota et al., 2003). VGF is a neuropeptide expressed in hypothalamus, deficiency of which produces a lean hypermetabolic mouse (Hahm et al., 1999). Genetic crosses suggest that VGF, which is in part coexpressed in POMC neurons in the arcuate nucleus, is downstream of melanocortin 4 receptors that regulate energy expenditure pathways (Hahm et al., 2002).

In addition to signals from neuropeptides and neurocytokines, hypothalamic centers involved in energy homeostasis can also be influenced by metabolic substrates. Neurons that receive signals from regulators such as leptin may also sense changes in the levels of glucose and free fatty acids. Hypothalamic neurons that sense both low and high levels of glucose have been identified (Dunn-Meynell et al., 2002). Low glucose (hypoglycemia) is well known to induce hunger acutely, and the circuitry underlying this is uncertain. Several lines of evidence combine to suggest that fatty acids and pathways related to fatty acid metabolism may influence hunger and metabolic pathways through actions in the brain. Central administration of an inhibitor of fatty acid synthase (FAS) reduces food intake and body weight (Loftus et al., 2000), and it was proposed that buildup of the FAS substrate malonyl CoA might mediate the effect, perhaps by inhibiting the mitochondrial enzyme carnitine palmitoyl transferase I (CPT I) which would raise cellular levels of long chain acyl CoA derivatives. Consistent with this, mice with global deficiency of the enzyme acetyl CoA carboxylase 2 (ACC2) have de-

creased levels of malonyl CoA, the product of the enzyme, in several tissues, and display increased rates of lipid oxidation and hyperphagia, likely due to the ability of reduced malonyl CoA to disinhibit CPT I, thereby reducing long chain fatty acyl CoA derivatives, and increasing lipid oxidation rates (Abu-Elheiga et al., 2001). Finally, intracerebroventricular administration of the long chain fatty acid oleic acid suppresses food intake acutely (Obici et al., 2002a), and genetic or biochemical inhibition of hypothalamic CPT I decreases food intake (Obici et al., 2003). It seems likely that key neurons in the hypothalamus, most likely in the arcuate nucleus, integrate endocrine signals such as leptin with metabolic signals such as glucose and free fatty acids to establish a coherent representation of the state of energy balance.

It is evident that our knowledge of the hypothalamic signals that influence energy balance is large and growing, and that the circuitry with the CNS that mediates this system is more complex than initially envisioned. Rather than viewing these hypothalamic pathways as a simple linear cascade, a systems approach will be needed to fully comprehend the complex regulatory networks involved.

Hedonic Mechanisms for Regulating Feeding

Most of the foregoing discussion has focused upon the homeostatic regulation of energy balance, in which the control of hunger and satiety promote the unconscious maintenance of adequate stores of energy for short-term health and long-term survival. Although this homeostatic system deserves attention, feeding is also powerfully influenced by pleasure and reward. The pathways for energy homeostasis must intersect robustly with these circuits for pleasure and reward (Saper et al., 2002). The neurobiology of taste and smell has seen tremendous advances, but as yet few insights have emerged to link these sensory inputs to the pathways regulating hunger and satiety. Given the powerful and rapid ability of odors and tastes to stimulate or suppress our urge to eat, and the increasing ability of the food industry to provide products that stimulate these urges, the significance and opportunity represented by this gap in our knowledge can barely be overestimated. In addition to taste and smell, brain reward mechanisms, studied most intensively in the context of drug addiction, are highly relevant to feeding behavior, and ultimately, the state of energy balance. Interactions between dopaminergic, serotonergic, and opioid systems may underlie this circuitry, which involves the nucleus accumbens, and very likely, direct and indirect relays to the lateral hypothalamus (Saper et al., 2002). MCH neurons in the lateral hypothalamus are well placed to be involved in these reward pathways. Further evidence that homeostatic and hedonic aspects of feeding intersect come from recent observations that food restriction and leptin have opposite effects to influence reward behavior in a well-established model of intracranial self stimulation (Fulton et al., 2000), and that leptin inhibits sweet sensitive taste cells in the tongue (Kawai et al., 2000). It seems evident that future studies of the circuitry of energy balance that seek to fully explain behavior will need to incorporate assessment of reward circuitry, and the mechanisms by which taste and smell affect feeding behavior.

Energy Expenditure

The maintenance of energy balance involves coordinated changes in energy intake and expenditure, and these two limbs of energy balance are physiologically linked, most importantly through hypothalamic circuits. In most well-identified syndromes of obesity, such as those involving defects in leptin and the melanocortin pathway, obesity results from both increased feeding and decreased energy expenditure, suggesting that the the leptin and melanocortin pathways are upstream of effector mechanisms that regulate both appetite and energy expenditure. In rodent models, energy expenditure can be directly assessed by measuring oxygen consumption or heat production. A role for defective thermogenesis can be inferred when obesity develops in one group of animals despite food intake that is maintained equivalent to a second lean group, a maneuver called pair feeding. Although several lines of evidence suggest that obesity in humans may be in part determined by reduced energy expenditure (Levine et al., 1999), such studies are far more difficult to carry out convincingly in humans, and molecular insights into the pathways for reduced energy expenditure in obesity have lagged behind those related to altered appetite.

Energy expenditure can be viewed as occurring in three major categories. The first is the obligatory energy expended on basic cellular and physiologic functions that require ATP. The second is the energy expended in physical activity. The third is referred to as adaptive thermogenesis, or that component that is stimulated in response to external stresses such as environmental cold or changes in the amount of food ingested, so-called diet-induced thermogenesis. Adaptive thermogenesis requires an uncoupling between fuel oxidation and biological work, and this could result from the operation of futile cycles, or the creation of proton leak across the mitochondrial inner membrane that dissipates energy stored in the mitochondrial protein electrochemical gradient.

Brown adipose tissue is one clear mechanism utilized by small rodents in particular to induce regulated mitochondrial uncoupling and heat production in response to both environmental cold temperature and overfeeding. Triggered by cold exposure, or changes in diet mediated in part through leptin and melanocortin pathways, central sympathetic nervous impulses innervate brown fat deposits, where norepinephrine acts via β adrenergic receptors to activate, as well as increase expression of the mitochondrial uncoupling protein UCP-1. UCP-1 knockout mice cannot maintain temperature in the cold (Enerback et al., 1997), and mice with toxigene-induced deficiency of brown adipose tissue develop obesity (Lowell et al., 1993). In adult humans, the amount of brown adipose tissue is minimal, and UCP-1 expression is thought to be insufficient to be physiologically meaningful under most circumstances. It was originally thought that the UCP-1 homologs UCP-2 and UCP-3, which are widely expressed in tissues of rodents and humans, might provide a common molecular explanation for interindividual differences in thermogenic ca-
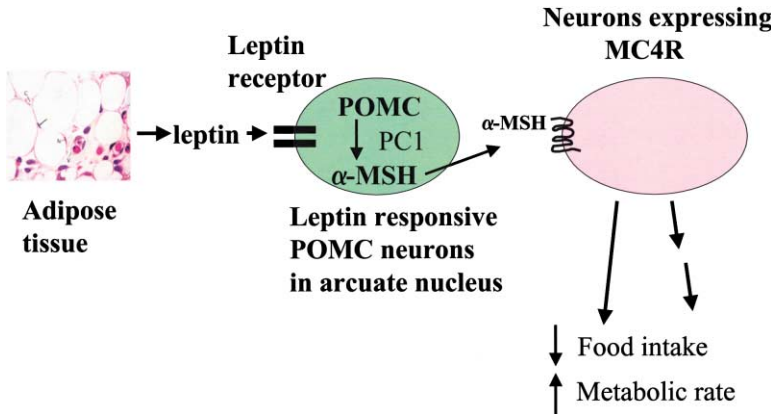
Figure 4. Human Obesity Genes Define a Pathway for Regulated Energy Balance

Several genes confirmed to play a role in human obesity define a linear biochemical pathway for the regulation of energy balance and body weight. These include leptin, the leptin receptor, proopiomelanocortin (POMC), protein convertase 1 (PC1) which is required for processing POMC to α-MSH, and the melanocortin 4 receptor (MC4R). The precise chemical identity of the MC4R expressing neurons is unclear, but their output, directly or indirectly, regulates appetite, energy expenditure, and metabolism.

pacity. Although they resemble UCP-1 in mediating mitochondrial protein leak activity, and their forced overexpression can increase energy expenditure and reduce body fat, they appear not to be critically involved in whole body energy expenditure, as energy homeostasis is normal in mice lacking either protein (Arsenijevic et al., 2000; Vidal-Puig et al., 2000).

Does a regulated increase in energy expenditure mediated by sympathetic nerves in response to changes in diet, have the capacity to regulate body weight and fat stores, at least in rodents? Definitive evidence for this question was recently provided, through development of mice with deletion of the three β receptor subtypes, β1, 2, and 3. When these "betaless" mice are fed a high fat diet, they develop severe obesity despite food intake identical to wild-type controls (Bachman et al., 2002). Unlike wild-type mice, betaless mice are unable to increase energy expenditure in response to the calorically dense diet. It is not yet clear whether the site for diet induced thermogenesis in wild-type mice is limited to brown adipose tissue, or includes other sites such as muscle.

Another discovery with potential relevance to regulated energy expenditure is the identification of the coactivator protein PGC-1, a mediator of the thermogenic phenotype in brown fat cells in response to cold exposure (Puigserver et al., 1998). This protein can also induce mitochondrial biogenesis (Wu et al., 1999) and change the thermogenic state of muscle from white to red fiber type (Lin et al., 2002). A role for PGC-1 in determining the thermogenic capability of rodents and humans has not escaped investigators, but published evidence to support this idea is not yet available. However, PGC-1 dependent transcriptional pathways have recently been linked to the pathogenesis of Type 2 diabetes in humans (Mootha et al., 2003; Patti et al., 2003). Activation of the PPARδ nuclear transcription factor has been shown to stimulate fat oxidation and energy expenditure, perhaps through interaction with PGC-1 (Wang et al., 2003). Mice deficient in PPARδ are prone to obesity induced by high fat diet, and activation of PPARδ by either transgenic or pharmacologic approaches causes mice to have increased rates of fat oxidation and resistance to obesity caused by high fat diet or genetic changes (Wang et al., 2003).

In a number of rodent models, gene knockouts have produced resistance to obesity associated with increased energy expenditure of uncertain origin. Several of these knockouts involve pathways of lipid synthesis. Deletion of stearoyl CoA desaturase-1, an enzyme that desaturates C18 fatty acids, markedly reduces fat mass and body weight of $Lep^{ob/ob}$ mice without reducing their food intake, but the basis for increased energy expenditure seen in these mice is unclear (Cohen et al., 2002). Mice deficient in DGAT-1, the enzyme catalyzing the final step in mammalian triglyceride synthesis, also resist obesity through increases in energy expenditure (Smith et al., 2000), and once again, the link between the genetic defect and energy expenditure is obscure. As stated earlier, deficiency of perilipin leads to leanness with increased energy expenditure that is not yet explained. Mice deficient in the MCHR1, which binds a peptide (MCH), which stimulates feeding and induces obesity, resist obesity through increases in energy expenditure, at least in part via physical activity (Marsh et al., 2002). This or some other central pathway for influencing energy expenditure might eventually be linked to the observation that individuals resistant to obesity may engage in physical activity in the form of fidgeting that can burn calories, currently without explanation (Levine et al., 1999)

Genetic Basis for Obesity and Leanness
The genetic contribution to body fat content in humans is as strong as that for height. Although the search for genes responsible for human obesity has had some notable successes, we have not yet accounted for the genetic explanation for, or susceptibility to obesity in the vast majority of patients. In rare cases of severe childhood-onset obesity, single gene defects have been identified. These include loss of function mutations in the genes for leptin, the leptin receptor, proopiomelanocortin (POMC), prohormone convertase 1 (PC1), and the melanocortin 4 receptor. The latter may account for 1%–4% of severe early onset obesity. These genes all encode proteins that act in a central nervous system pathway for regulation of energy balance (Figure 4). The responsible genes are still unknown in >95% of severe early onset obesity, and the fraction of this population that will eventually be shown to have a Mendelian basis for obesity is uncertain. In the general population of adults with obesity, the disorder is certainly mainly polygenic, with genetic variations creating susceptibility to environmental factors. Our ability so far to identify such

obesity susceptibility genes in the general population has been limited. Genome wide scans in different ethnic populations have localized major obesity loci on chromosomes 2, 5, 10, 11, and 20 (Clement et al., 2002). It is possible that a strategy based on identifying genes that confer resistance to obesity, or leanness, might offer an advantage over a search for genes predisposing to obesity. Another potential approach is the use of model organisms, such as *C. elegans*, which lack adipose cells but store energy as lipid droplets in intestinal cells. Using RNAi, *C. elegans* genes regulating lipid stores have been identified, some of whose homologues are known participants in mammalian energy balance (e.g., serotonin pathway), and others, although conserved across animal phylogeny, have not previously been linked to energy balance, and represent interesting targets for future study (Ashrafi et al., 2003; McKay et al., 2003).

Targets for Pharmaceutical Development

Existing approved drugs for treatment of obesity possess only modest efficacy. Sibutramine blocks presynaptic uptake of norepinephrine and serotonin, and potentiates the anorectic effects of these neurotransmitters. Orlistat reduces absorption of dietary fat by inhibiting intestinal lipases. Drugs with greater efficacy that are also safe are needed. Many of the CNS pathways discussed above have sparked aggressive efforts at pharmaceutical development. These include agonists for the melanocortin 4 receptor, antagonists of the MCHR1 and the ghrelin receptor, and antagonists of the endocannabinoid receptor CB1. Additional potential targets with strong rationales include inhibitors of the enzymes 11 β HSD-1, PTP1b, and SOCS3.

Although leptin has proven to be of limited efficacy in most obese subjects as a result of leptin resistance, there is substantial interest in the ability of ciliary neurotrophic factor (CNTF), a neurocytokine with a receptor homologous to the leptin receptor, to have leptin-like actions in several obesity models, including mice lacking leptin receptors and those with obesity induced by high fat diets. In rodents, systemic administration of CNTF activates the JAK STAT pathway in cells in arcuate nucleus that overlap with those activated by leptin (Gloaguen et al., 1997). A modified version of CNTF, termed Axokine, had promising results in a Phase II study in obesity, but the results of the definitive Phase III study were disappointingly limited, at least in part due to development of antibodies to the drug. A curious aspect of Axokine in animal studies is the prolonged effect of the agent after discontinuation of therapy, a result that is so far unexplained (Lambert et al., 2001).

Scientific progress in elucidating the molecular physiology of energy balance and obesity has so far failed to mitigate the increasing prevalence of the condition and this tension between expectations and reality has stimulated an increasingly vigorous public policy debate. Is it rational to pursue development of pharmaceutical antidotes to the existing environment, or should we devote all efforts to changing the "toxic" environment? This debate is sometimes pursued with a religious fervor. Several facts can be accepted by virtually all knowledgeable parties to the discussion. First, obesity

and its complications are increasing worldwide, with major adverse implications for public health. Second, the recent increase in prevalence is due to changes in the environment (availability of food, composition of diet, physical exercise, etc.) rather than changes in the genome. Third, some individuals, due to genetic inheritance, are more susceptible than others to these consequences. Seeing these facts, some observers stress the importance of changing elements of the environment that promote obesity, viewing this approach as the more natural and logical pursuit. This approach is meritorious, but limited by the fact that the precise nature of the relevant changes to be recommended is not clear, at least on the level of public policy, and even if the changes were clear and supported by data, they would be difficult to implement. For example, should the entire public be advised to ingest low fat diets, as has been recommended over recent decades, or to pursue diets low in rapidly absorbed carbohydrates, as several authorities now recommend (Ludwig, 2002)? Is any single set of nutritional recommendations appropriate to the entire population at risk? To what extent are controlled trials of nutritional recommendations achievable, and desirable? How can a desire to have people exercise more be implemented in a free society in which some people pursue fitness intensively, while many choose to exercise as little as possible? How can we reconcile discordant views within the scientific and public policy communities on some of these issues, so that a response can be offered to a general public that is starved for guidance?

Therapeutic approaches based on rational dietary and lifestyle changes must be pursued, and wherever possible, data for the effectiveness of such programs must be obtained. In the meanwhile, it seems logical to exploit our recent understanding of the systems regulating body weight to develop safe and effective drugs that will reduce the susceptibility of those most likely to develop obesity and its complications in the world in which we now find ourselves. However much the image of people being medicated to reduce the obesity epidemic may discomfort those who seek a behavioral or public policy remedy, to cease the search for safe and effective medications would be to abandon a major segment of the population to an unhealthy fate.

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