The Function of Leptin in Nutrition, Weight, and Physiology
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Recent advances indicate that a robust physiologic system acts to maintain relative constancy of weight in mammals. A key component of this system is leptin. Leptin is an adipocyte hormone that functions as the afferent signal in a negative feedback loop regulating body weight. In addition, leptin functions as a key link between nutrition and the function of most, if not all other physiologic systems. When at their set point, individuals produce a given amount of leptin and in turn maintain a state of energy balance. Weight gain results in an increased plasma leptin level, which elicits a biologic response characterized in part by a state of negative energy balance. Weight loss among both lean and obese subjects results in decreased plasma levels of leptin, which lead to a state of positive energy balance and a number of other physiologic responses. In humans, both the intrinsic sensitivity to leptin and its rate of production vary and both appear to contribute to differences in weight. Further studies of leptin, its receptor, and the molecular components of this system are likely to have a major impact on our understanding of obesity and the interplay between nutrition and physiology.

Key Words: leptin, adipocyte hormone, regulating body weight, energy balance, obesity

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Introduction
In recent years, several components of the physiologic system that regulate body weight and links between nutrition and physiology have been identified. Research in this area is at the center of several issues that are of general importance including: obesity, a pressing, some consider the most pressing, health problem in Western and developing countries; the ways in which alterations of nutritional state affect physiology; the role of genes and environment in determining human characteristics; and the molecular basis of behavior.

An alternative view suggests that body weight is physiologically controlled and that deviations in weight in either direction elicit a potent physiologic response that resists that change. Implicit in this view is the notion that biologic factors determine each individual’s weight, be they lean or obese, and that this weight is then defended. The effectiveness of this homeostatic system is illustrated by a few simple calculations. Over a decade the weight of an average adult individual tends to increase slightly. Approximately 10 million kilocalories are consumed over this time. To account for this modest change in weight (assuming the excess weight is deposited as adipose tissue), food intake must precisely match energy output within 0.17% per decade. This extraordinary level of precision suggests that a robust biologic system balances energy intake (food consumption) and energy expenditure.

The proposition that obesity is to a large extent the result of biologic rather than psychologic factors is supported by a plethora of genetic and physiologic data. Twin studies, analyses of familial aggregation and adoption studies all indicate that obesity is the result of major contributions from genetic factors. Indeed, the heritability of obesity is roughly equivalent to that of height and exceeds that of many disorders that are generally considered to have a genetic basis. The identity of several of these genes is now known, and in general, they encode the molecular components of the physiologic system that regulates body weight. Two of the key elements of this system are leptin and its receptor.

Leptin
Leptin is an adipocyte hormone that functions as the afferent signal in a negative feedback loop regulating
body weight (Figure 1). This hormone reports nutritional information (i.e., the size of the organism’s energy stores) to the brain and other sites. Leptin circulates as a 16-kD protein in mouse and human plasma but is undetectable in plasma from C57BL/6J ob/ob mice. In the absence of leptin, these mutant animals (as well as leptin-deficient humans) never receive the signal that there are adequate fat stores and in turn become hyperphagic and obese.

Leptin is not modified post-translationally because the molecular mass of the native protein is identical to that predicted by the primary sequence (without the signal sequence). The plasma level of leptin is highly correlated with adipose tissue mass and decreases in both humans and mice after weight loss. The levels of protein are increased in several genetic and environmentally induced forms of rodent obesity and in obese humans. Administration of recombinant leptin either by injection or as a constant subcutaneous infusion to wild-type mice results in a dose-dependent decrease in body weight at increments of plasma leptin levels within the physiologic range.

In aggregate, these data establish leptin’s role as hormonal signal in a feedback loop modulating the size of adipose tissue mass and indicate that body weight to a significant extent is under endocrine control. However, leptin is not the only afferent signal that regulates food intake and body weight. The systems that control feeding behavior and energy balance appear to be comprised of a short-term and a long-term system. The short-term system regulates meal pattern and feeding throughout the day. Previous work indicates that changes in plasma glucose concentration, body temperature, plasma amino acids, cholecystokinin, and other hormones can all modulate meal patterns. The long-term system balances food intake and energy expenditure and thus plays a dominant role in ultimately regulating the size of the body’s energy stores.

Leptin appears to function largely within the long-term system and influences the quantity of food consumed relative to the amount of energy that is expended. Leptin level does not increase significantly after a meal and does not, by itself, lead to the termination of a meal. These results suggest that leptin is not a classic satiety factor. However, leptin and the other components of the long-term system interact extensively with the components of the short-term system by modulating the amount of food that is consumed during a meal and/or the likelihood that an organism will miss a meal.

A Broader Role for Leptin

Quantitative changes in plasma leptin concentration elicit a potent biologic response. Decreases in plasma leptin level activate what can be termed a “response to starvation,” whereas increasing leptin levels elicit a “response to obesity” (Figure 2). The presence of low plasma levels of leptin indicates that there are inadequate amounts of fat (stored energy) and that an adaptive response that would lead to the replenishment of those stores needs to be effected. Several clues concerning the nature of this “response to starvation” have been provided by detailed analyses of the phenotype of ob mice. Leptin-deficient (ob/ob) mice manifest myriad endocrine and metabolic abnormalities. Many of these derangements, which include decreased body temperature, hyperphagia, decreased energy expenditure (including activity), immune defects, and infertility, are also observed in starved animals. This has suggested that in the ab-
The possibility that falling plasma leptin levels signal nutrient deprivation is further suggested by the observation that exogenous leptin attenuates the neuroendocrine responses to food restriction. Fasted wild-type mice receiving leptin continue to ovulate, whereas fasted controls given PBS experience an ovulatory delay of several days. Leptin treatment blunts the changes in circulating thyroid hormone and corticosterone levels that are normally associated with food deprivation. Starvation is also associated with decreased immune function and leptin corrects these abnormalities as well. In treated ob/ob mice, leptin stimulates proliferation of CD4+ T cells and increases production of cytokines by T-helper-1 cells. These results indicate that leptin may also be a key link between nutritional state and the immune system.

Leptin is also important in regulating the onset of puberty. Extremely thin women often stop ovulating and abnormally thin adolescent women enter puberty later than their heavier counterparts. These observations have suggested that reproductive potential in women is suppressed in the absence of adequate nutritional stores. These findings have further suggested that fat tissue may produce a signal that regulates reproduction. This factor appears to be leptin. Treatment of mice with leptin accelerates the maturation of the female reproductive tract and leads to an earlier onset of the estrous cycle and reproductive capacity. In humans, a surge in plasma

Figure 2. Biologic response to high versus low leptin levels. Leptin allows the body to maintain constant stores of fat. A loss of body fat (starvation) leads to a decrease in leptin, which in turn leads to a state of positive energy balance wherein food intake exceeds energy expenditure. This biologic response also includes a pleiotype set of effects on the hypothalamic pituitary axis. Other effects include decreased temperature and an increase of parasympathetic tone. Conversely an increase in adiposity leads to an increase in levels of leptin. This response also includes a set of novel effects on fat and glucose metabolism and activation of the sympathetic nervous system. CRH = corticotrophin-releasing hormone, GnRH = gonadotrophin-releasing hormone, GH = growth hormone, SRIF = somatotrophin release–inhibiting factor, GHRH = growth hormone–releasing factor.
leptin concentration is seen in prepubertal males. The evidence suggests that sufficient levels of leptin are necessary but not sufficient for the onset of puberty. These studies suggest that leptin modulates reproductive function and provides a direct link between reproduction and the nutritional status of an organism.

Leptin’s physiologic role in preventing weight gain has also been confirmed in a number of studies. Lean mice given chronic infusions of leptin lose adipose mass in a dose-dependent fashion at leptin levels within the physiologic range. These data indicate that increasing leptin levels elicits a “response to obesity” and that dynamic changes in plasma leptin concentration act to resist weight change in either direction. The precise nature of this response to obesity is less well understood but is under intense investigation.

The Leptin Receptor and Leptin’s Sites of Action

The leptin receptor is a member of the cytokine family of receptors. These receptors have a single transmembrane domain and are generally expressed as monomers or dimers on the cell surface. Ligand binding induces dimerization and/or activation of the receptor and activates signal transduction. Ob-R is predicted to have two separate leptin-binding regions and binds leptin with nanomolar affinity. Five splice forms of the leptin receptor that differ at the carboxyl terminus have been identified. Four of these receptor forms are membrane bound, whereas Ob-Re encodes a secreted form of the receptor that circulates in plasma.

Mutations that disrupt the leptin receptor have been identified in each of the available strains of diabetic (db/db) mice. Db/db mice are also genetically obese and manifest a phenotype that is identical to that evident in leptin-deficient ob/ob mice. DNA sequence analyses of the available db strains have implicated the Ob-Rb form of the receptor as mediating many, if not all of leptin’s weight-reducing effects. The Ob-Rb form is widely expressed but is enriched in the hypothalamus. These data have suggested that the brain is an important target of leptin action. This possibility is supported by the high potency of leptin when delivered directly into the central nervous system (CNS). In addition, leptin modulates the electrical activity of neurons in culture and in slice preparations.

Leptin also acts on some peripheral cell types and has direct mitogenic effects on CD4+ human T cells (Figure 3). It affects endothelial cells directly and increases angiogenesis, although high doses are required. Leptin modulates pancreatic cell function in vivo and has direct effects on other cell types in vitro including liver, bone, and platelets. The leptin receptor is widely expressed, though the Ob-Ra (short) form of the receptor predominates in many of these tissues. Although the potency of i.c.v. leptin indicates that direct peripheral effects are not required for weight loss, the full spectrum of leptin’s sites of action is not known and could include sites that have not been mentioned.

The function of the short forms of the leptin receptor (Ob-Ra, c, d) is unclear, although it has been suggested that they play a role in mediating the uptake of leptin into the CNS as well as regulating its turnover.

The Neural Circuit Regulating Weight

The available data suggest that the concentration of leptin as well as glucose and other afferent signals are sensed by groups of neurons in the hypothalamus and other brain regions. During starvation leptin levels fall, thus activating a behavioral, hormonal, and metabolic response that is adaptive when energy stores are reduced. Weight gain increases plasma leptin concentration and elicits a different response leading to a state of negative energy balance. It appears likely that different neurons respond to increasing and/or decreasing leptin levels. In addition, the spectrum of leptin’s effects is likely to be complex, as recent studies have indicated that different thresholds exist for several of leptin’s effects.

A number of hypothalamic nuclei have been previously implicated in the control of food intake. Thus lesions of the arcuate nucleus, the ventromedial hypothalamus (VMH), DMH, and LH perturb body weight and each plays a role in the regulation of food intake or body weight. Leptin receptors have been localized to each of these hypothalamic nuclei. The LH and VMH project both within and outside the hypothalamus and modulate activity of the parasympathetic and sympathetic nervous system, respectively. The DMH also has inputs to the parasympathetic nervous system and has been implicated in integrating information among the VMH, LH, and PVN. The PVN controls secretion of peptides from both the posterior and anterior pituitary and projects to nuclei with sympathetic or parasympathetic afferents. The PVN also projects to numerous sites outside of the hypothalamus including higher centers known to modulate motivational behaviors.

These hypothalamic nuclei express one or more brain neuropeptides and neurotransmitters that regulate food intake and/or body weight. Several lines of evidence have implicated these neuropeptides as playing a role in the response to leptin and other nutritional signals. The data are consistent with the possibility that neuropeptide Y (NPY) and Agouti-related transcript (ART, also known as AgRP) play a role in the response to absent (and possibly low) leptin levels, whereas centrally expressed α-melanocyte-stimulating hormone (αMSH) and its MC-4 receptor play a role in the response to an
increased plasma leptin concentration (Figure 4). What follows is a review of these and other neuropeptides and neurotransmitters.

When administered intrathecally, NPY is the most potent orexigenic agent that is known. NPY RNA is increased in ob/ob mice and its levels decrease after leptin treatment. An NPY knockout attenuates the obesity and other features of ob/ob mice indicating that it plays a role in the response to absent leptin. Data from other knockout mice indicate that both the Y2 and possible Y5 receptors play a role in mediating some of NPY’s effects on food intake and body weight.

αMSH and MSH agonists decrease food intake. Reduced signaling of αMSH in genetically obese Aγ or MC-4R knockout mice results in obesity and leptin resistance. Leptin modulates the expression of pro-opiomelanocortin (POMC), the precursor of αMSH. A subset of neurons express both Ob-R and POMC and αMSH antagonists blunts the response to exogenous leptin. Finally, leptin depolarizes GFP-labeled POMC neurons in a slice preparation.

AgRP, an endogenous antagonist of melanocortin signaling, is also implicated in the regulation of weight because transgenic mice that overexpress AgRP are markedly obese. In addition, mRNA for this hypothalamic peptide is increased eightfold in ob/ob mice. In total, these results suggest that NPY and AgRP may play a role in mediating the response to absent leptin, whereas increased leptin levels stimulate melanocortin-signaling pathways. It is important to note, however, that these molecules are likely to respond to other signals and that these complex neural circuits are likely to interact with one another.

Melanin-concentrating hormone (MCH), a neuropeptide expressed in the lateral hypothalamus, is increased in ob/ob mice and injections of it increase food intake.
intake in mice. Mice with mutations of MCH are hypophagic and lean thus confirming a role for this peptide in the maintenance of body weight. MCH-expressing neurons receive inputs from NPY neurons in the arcuate nucleus and appear to relay NPY’s orexigenic signal to a number of sites throughout the brain. These neurons project to many of the same brain regions as αMSH and these molecules have opposite effects on food intake. Both act via distinct receptors and MSH increases, whereas MCH decreases cAMP signaling. Thus the plasma leptin concentration influences the relative activity of these anorexigenic and orexigenic circuits at multiple levels.

Many other neurotransmitters and neuropeptides are likely to function in this homeostatic system. It is likely that leptin, glucose levels, and other signals potentiate the action of some of these anorexigenic agents and antagonize the orexigenic (i.e., stimulate food intake) and activity of others. These are briefly reviewed here. Cholecystokinin (CCK) was the first neuropeptide suggested to play a role in regulating food intake. Injections of CCK increase satiety in food-deprived rats via afferent vagal nerves. Recently, CCK was reported to potentiate the anorectic effect of leptin.

Cocaine-amphetamine-regulated transcript (CART), a hypothalamic peptide, is implicated in the response to leptin. CART decreases food intake, CART antibodies increase food intake, and CART mRNA is increased in ob/ob mice. In addition, CART is co-localized with αMSH and Ob-R in some hypothalamic neurons.

Bombesin also reduces food intake and induced mutations of the bombesin 3-receptor result in mild obesity. A growing body of evidence indicates that insulin acts on the hypothalamus to decrease food intake. Two recently identified neuropeptides, orexin-a and orexin-b, that regulate the state of an animal’s arousal may also modulate food intake.

Corticotropin-releasing factor (CRF) is another factor that is likely to mediate some of leptin’s effects. CRF is expressed at high levels in the PVN and in the amygdala, which projects to the LH. Additionally, the DMH has CRF-containing neurons. CRF regulates

Figure 4. The neural circuit activated by leptin. In the arcuate nucleus of the hypothalamus the leptin receptor is expressed in at least two different classes of neurons. One class expresses NYP and AGRP, two neuropeptides that increase food intake. Another class expresses POMC, the precursor of αMSH and CART. Both CART and αMSH decrease food intake. The evidence suggests that leptin suppresses the activity of NPY/AGRP neurons and stimulates the activity of POMC/CART neurons. Thus in the absence of leptin the NPY/AGRP neurons are maximally active and food intake is stimulated. In the presence of increased leptin levels the POMC/CART neurons are maximally active and food intake is reduced. When an individual is at their stable weight the activity of these pathways is balanced. The neural mechanisms by which these neurons change food intake is not known. Figure credit: Lex von der Plueg, Merck Pharmaceuticals.
pituitary ACTH release and adrenal glucocorticoid secretion. Delivery of CRF to the PVN also results in reduced food intake and increased energy expenditure in lean and obese rodents.\textsuperscript{56,57} Leptin has been shown to increase CRH mRNA in the PVN and to stimulate release of CRF from perfusion slices of both amygdala and the PVN.\textsuperscript{58,59} Leptin may also inhibit the increase in CRH evident during a stress response.\textsuperscript{60}

Glucocorticoids have long been known to play a role in regulating body weight. Increased fat deposition is a feature of Cushings syndrome and increased glucocorticoids result in obesity in mice.\textsuperscript{61} High levels of glucocorticoids are also observed in most strains of genetically obese mice. Moreover, adrenalectomy and glucocorticoid antagonists blunt the obesity evident in \textit{ob/ob}, \textit{db/db}, and other obese mice.\textsuperscript{62,63} Low-dose glucocorticoid replacement restores the obese phenotype of adrenalectomized \textit{ob/ob} mice indicating that they play a permissive role in its development.\textsuperscript{63} It is unclear if the requirement for glucocorticoids in the development of the full \textit{ob/ob} phenotype depends on the suppression of CRH, a known effect of glucocorticoids, or if another mechanism is operative.

More recent data have implicated endocannabinoids and the muscarinic 3 receptor in the response to leptin and/or feeding in general. Endocannabinoids play a role in the response to decreased leptin levels, whereas an MC3 knockout leads to hypophagia and decreased weight.\textsuperscript{64,65} Ghrelin, a peptide that is synthesized in stomach and brain, increases food intake by interacting with a hypothalamic G protein–coupled receptor.\textsuperscript{66}

A more full understanding of the system that controls weight will also require the identification of the upstream and downstream components of these key hypothalamic neurons. Recently a novel system that employs an engineered pseudorabies virus to trace neural circuits was developed.\textsuperscript{67} This vector was successfully used to identify inputs to leptin-responsive neurons in the hypothalamus. Results using this system have shown that a number of brain regions including pyriform cortex (which receives olfactory information), the amygdala (known to regulate emotional behavior), and cortical regions (known to control higher brain functions) can modulate leptin signaling in hypothalamus. Studies aimed at understanding the ways in which these multiple relevant inputs are integrated may illuminate the interplay between conscious and unconscious (and other) factors in regulating food intake and body weight. These studies may also have general implications for our understanding of the regulation of a complex behavior.

**Efferent Pathways Regulating Metabolism**

The evidence indicating that leptin can act centrally to modulate body weight raises the following question: How does the CNS regulate peripheral metabolism in response to differences in leptin and other signals? The effects of leptin on metabolism are distinct from those that are seen after food restriction. Whereas increasing leptin levels lead to fatty acid oxidation and a reduction in adipose tissue mass, leptin deficiency, evident in \textit{ob/ob} mice or mice receiving a leptin antagonist, is associated with an increase in fat deposition.\textsuperscript{6,68} In \textit{ob/ob} mice the rate of lipogenesis is markedly increased as is the abundance of the RNAs encoding the enzymes that are rate limiting in fatty acid synthesis.\textsuperscript{8,69} It is yet unclear whether the metabolic derangements of \textit{ob/ob} mice are solely the result of increased food intake or if other factors are also important.

The mechanism by which centrally administered leptin leads to lipolysis and the loss of adipose tissue mass is similarly unclear. It is important to note that increased fatty acid oxidation is not restricted to adipose tissue and that leptin treatment also results in the loss of intracellular lipid in other cell types.\textsuperscript{70} In this and other respects, the available data show that the metabolic response to leptin is markedly different from the response to reduced food intake. Whereas food restriction (i.e., dieting) leads to the loss of both lean body mass and adipose tissue mass, leptin-induced weight loss is specific for the adipose tissue mass.\textsuperscript{6,11,71} Leptin also prevents the reduced energy expenditure normally associated with a decreased food intake.\textsuperscript{11} Hyperleptinemic animals undergoing a rapid period of weight loss fail to show any rise in serum free fatty acids or ketones.\textsuperscript{70} This contrasts with food-restricted (pair-fed) animals, which show a marked rise in serum free fatty acids. Indeed, despite the fact that the respiratory quotient falls after leptin treatment (indicative of fatty acid oxidation), the metabolic fate of stored triglycerides in adipose tissue is unknown.\textsuperscript{11} Finally, data using DNA microarrays have shown that leptin has novel (indirect) effects on gene expression in adipose tissue.\textsuperscript{72}

Leptin also has novel effects on glucose metabolism. The possibility that leptin modulates glucose metabolism was first suggested in studies of \textit{ob/ob} mice treated with leptin. \textit{Ob/ob} mice are diabetic and the severity of the diabetes is dependent on the background strain carrying the mutation.\textsuperscript{8} In one study, leptin normalized the hyperglycemia and hyperinsulinemia evident in \textit{C57BL/6J ob/ob} mice at doses that did not decrease weight.\textsuperscript{73} Anti-diabetic effects have also been observed in insulin-deficient rats.\textsuperscript{74}

Leptin also improves the insulin resistance and hyperglycemia evident in a diabetic lipodystrophic transgenic mouse line.\textsuperscript{75} The anti-diabetic effects of leptin in these animals appear to come from leptin’s ability to stimulate lipolysis and fatty acid oxidation in liver, skeletal muscle, and other peripheral tissues. Previous
studies have indicated that insulin signaling is adversely affected by excess intracellular lipid. For example, a selective increase of lipid uptake by liver or muscle leads to decreased insulin action only in that organ. Thus leptin appears to improve insulin signaling by reducing intracellular lipid levels. The precise mechanism by which intracellular lipid decreases insulin signaling is not precisely known but may be the result of effects on PKC theta and IRS1 (personal communication). These results could in part explain the frequent association of obesity with insulin resistance because obesity is associated with increased circulating leptin, analogous to the increased insulin levels seen with insulin-resistant diabetes. In general, high plasma leptin levels are evident in obese rodents and humans. In a subset of cases, obesity is associated with normal levels of leptin. Differences in leptin production and leptin sensitivity could be the result of genetic, environmental, and psychologic factors.

Pathogenesis of Obesity

In principle, alterations in body weight could be the result of abnormalities in the production of leptin, the cells that receive leptin’s signal, or the efferent pathways that effect changes in weight. Thus the pathogenesis of obesity can be inferred in a general way by measurement of the plasma leptin levels (Figure 5). An increase in plasma levels suggests that obesity is the result of leptin resistance. A low or normal plasma concentration of leptin in the context of obesity suggests decreased production of leptin. This interpretation is similar to that relating insulin to the pathogenesis of diabetes. However, this designation is quite general because a great number of hormones as well as genetic, environmental factors, and even psychologic factors are likely to influence leptin sensitivity and production.

Plasma leptin levels have been measured in rodents and in humans using both RIA and ELISA. In all forms of rodent obesity studied, the obese animals have a higher leptin level than controls (not including ob/ob mice). The data suggest that these forms of animal obesity are the result of leptin resistance. In each of three cases that have been tested, obese animals that are hyperleptinemic can be shown to be completely or partially resistant to exogenous leptin.

Diet-induced obese mice (DIO) become obese only after exposure to a high-fat diet. For example, Akr mice (and some other strains) remain lean when fed a standard chow diet but become obese only when fed a high-fat diet. By contrast, other mouse strains do not become obese when exposed to an identical diet. DIO animals develop hyperleptinemia and exhibit a partial response to exogenous leptin. The leptin resistance observed in such mice emphasizes the fact that environmental factors can...
modulate leptin sensitivity. This indicates that the pathogenesis of DIO and leptin resistance is the result of an interaction between genetic and environmental factors.

A fuller understanding of the mechanisms by which fat content in the diet modulates weight is likely to be relevant to human obesity. The incidence of obesity increases in many populations when exposed to a high-fat “Western” diet. How might genes interact with environmental factors to cause obesity? As most people have experienced first hand, exposure to a highly palatable diet often leads to transient weight gain. In most cases, the gained weight is eventually lost. However, it is possible that in other cases, the induced increase in endogenous leptin level (which accompanies weight gain) leads to a down-regulation of the leptin response and a failure to return to the starting weight. If tachyphylaxis to increased levels of endogenous leptin is influenced by genetic factors, one might predict that a subset of individuals (and some populations) would be especially susceptible to DIO. The observation that animals that express constitutive levels of leptin are insensitive to a high-fat diet supports this possibility (unpublished). However, alternative explanations are possible and additional studies are required. Studies to identify the AKR alleles that predispose to DIO may illuminate the underlying mechanism.

**Leptin and Human Obesity**

In human subjects, a highly significant correlation between body fat content and plasma leptin concentration has been observed and obese humans generally have high leptin levels. These data suggest that in most cases human obesity is likely to be associated with insensitivity to leptin. However, five to ten percent of obese human subjects have relatively low levels of leptin. Low leptin levels also predispose to weight gain in pre-obese Pima Indians. These data suggest that in some instances obesity results from a subnormal secretion rate of leptin from fat.

The basis for leptin resistance in the overwhelming majority of obese hyperleptinemic human subjects is unknown. Data from studies of animals clearly indicate that this condition is likely to be very heterogeneous and that many factors are likely to influence the activity of the neural circuit that regulates feeding behavior and body weight. It has also been suggested that entry of leptin into the cerebrospinal fluid (CSF) may be limiting in some obese subjects thus leading to leptin resistance. If true, the development of morbid obesity could result when the plasma leptin levels exceed the capacity of the transport system. Treatment of humans with recombinant leptin has been shown to result in an increase in CSF leptin concentrations. Leptin uptake has been demonstrated in the capillary endothelium of mouse and human brain and is decreased in pre-obese animals. It is yet unknown whether this increase in CSF levels after treatment with recombinant leptin is variable among different individuals. It has thus been proposed that transport across the blood brain capillary endothelium is required for leptin to find its way to its site of action in the brain interstitial space and that Ob-Ra and/or other proteins mediate leptin transport. Moreover, defects anywhere in this transport pathway could lead to the development of obesity.

Defects in leptin signal transduction could also be important in the development of leptin resistance. Studies of the function of the leptin receptor in vitro have confirmed that Ob-Rb is capable of activating signal transduction. Leptin activates the STAT transcription factor in hypothalamus within 15 minutes of a single intraperitonal injection. In vitro studies have indicated that activation of the leptin receptor is dependent on phosphorylation of the JAK2 kinase after ligand binding to an Ob-Rb homodimer. Leptin also leads to the tyrosine phosphorylation of the SHP-2, a phosphotyrosine phosphatase, which in turn decreases both the state of JAK-2 phosphorylation and transcription of a leptin-inducible reporter gene. Thus SHP-2 may play a role in shutting off the leptin signal transduction pathway. SOCS-3, a suppressor of JAK signaling has been implicated in leptin signal transduction and may also play a role in down-regulating the response to leptin. The other components of the leptin signal transduction pathway have not yet been identified.

Leptin resistance in humans is likely to be the result of a complex interplay of many factors. In principle, leptin resistance could result from altered activity of any of the aforementioned components of the leptin signal transduction pathway in the cells that respond directly on any cells downstream in this neural circuit. Leptin’s actions are also likely to be influenced by psychologic factors via connections between the higher cortical centers, which modulate an animal’s motivational state and neural circuits within the hypothalamus. The neuroanatomic and functional relationships between these brain regions are only now being elucidated.

**Mutations Associated with Human Obesity**

In almost all cases, obese subjects express at least some leptin. However, two cousins born from an extended family have been found to be homozygous for a frame shift mutation in the leptin gene. These individuals do not have any circulating leptin. In these two subjects, the mutation is associated with profound obesity confirming that leptin is of critical importance for the control of body weight in humans. Affected members of Turkish kindred with a missense mutation in the leptin gene also have reduced leptin levels and manifest extreme obesity.
and amenorrhea. These results further suggest that leptin plays a role in modulating reproductive function in humans. Similar conclusions were reached in studies of three massively obese members of a French family carrying mutations in the leptin receptor. These three studies also indicate that apart from severe obesity and abnormalities of reproductive function, the other abnormalities identifiable in ob/ob and db/db mice such as hypercortisolism, cold intolerance, and severe diabetes are not necessarily apparent.

Mutations in other genes are also associated with human obesity. Approximately 1 to 2% of morbidly obese humans have mutations in the human MC-4 receptor. In these cases, the obesity is inherited in a dominant mutation indicating that a 50% decrease in αMSH signaling can have important effects on body weight. A recent report also showed linkage of POMC and other loci on human chromosome 2 to leptin levels and to a lesser extent body mass index (BMI, kg/m²) in a population of Mexican Americans. POMC is the precursor of αMSH. Variation at this locus may contribute to leptin resistance. The importance of MSH signaling is further confirmed by the development of obesity in two individuals with mutations in the POMC gene. Obesity has also been observed in a woman with a mutation in the PC-1 gene. PC-1 is a protease that is known to cleave neuropeptide precursors including POMC and thus serves a similar function to CPE, the gene that is mutant in fat/fat mice.

Although rare, the association of massive obesity with mutations in leptin and its receptor in human confirms their importance in regulating body weight. This assertion is supported by data from early clinical trials with leptin in humans. However, because the mutations in these genes, and POMC, PC-1, and the MC4 receptor are uncommon, the pathogenesis of most human obesity is unknown. It is likely that some of the genes responsible for human obesity in the general population will at some level modulate either leptin secretion or leptin sensitivity. Both genetic and physiologic studies will be required to confirm this prediction.

Prospects for New Treatments of Human Obesity

A recent prospective epidemiologic study of more than one million individuals confirmed that obesity is an independent risk factor for mortality. This finding amplifies the need for efficacious and safe means for treating this disorder. The health risk of obesity is greatly diminished when even modest amounts of weight (i.e., 5% of total weight) are lost. This is the result of a marked improvement in the diabetic, hypertensive, and cardiovascular status of obese subjects affected by these conditions. Dieting, by itself, is only rarely effective for the long-term maintenance of weight loss emphasizing the need for additional therapies. Clearly an important indication for such treatment would be for the management of the comorbidities associated with obesity.

The possible therapeutic benefit of leptin treatment in humans is now being tested in several clinical settings. It is has been demonstrated that leptin therapy has potent weight-reducing effects in the two leptin-deficient individuals that have been treated thus far. In addition to reducing food intake, weight, and body fat content, the hormone also stimulated cycling of gonadotrophins in one of the prepubescent 11-year-old children receiving it. The efficacy of treatment with exogenous leptin in these subjects confirms that leptin plays a physiologic role to regulate weight in humans and establishes a link between leptin signaling and reproductive capacity.

Recent data from early clinical trials in the general population have demonstrated that 4 weeks of leptin injections are safe and cause small but significant weight loss in lean and obese subjects compared with placebo (P <0.02) (unpublished observation. Treatment of a subset of eight obese subjects for a total of 6 months resulted in an average weight loss of 7.1 kg in a group receiving 0.3 mg/kg leptin versus a loss of 1.7 kg in a group receiving a placebo. Some of the subjects in this group lost substantial amounts of weight whereas others did not. This limited study suggests that leptin could ultimately emerge as an effective therapy for some, but not all, obese subjects although studies of more patients are clearly required.

Further studies to determine the possible utility of leptin for the treatment of type 2 diabetes are also indicated because animal studies have shown that leptin can increase glucose metabolism independently of its effects on weight and can obviate the need for insulin in lipodystrophic mice. As mentioned, this effect could be a result of leptin’s action to clear lipid from peripheral sites. It is also possible that leptin could be of therapeutic benefit for the maintenance of weight loss after a diet (rather than as a means to induce weight loss). In humans, diet-induced weight loss results in a decrease in plasma leptin concentration. This provides a possible explanation for the high failure rate of dieting; a low leptin level is likely to be a potent stimulus for weight gain. Thus leptin treatment after a very low calorie diet could, in principle, reduce the 95% failure rate of diets for long-term maintenance of weight loss.

Summary

Studies of the physiologic system that regulates weight have identified several molecules that have potential as therapeutic targets. Leptin or leptin agonists may be of use for the treatment of obesity and other disorders such
as lipodystrophy. αMSH agonists and NPY antagonists are also in development. The fact that patients with MC4R mutations exhibit an obese phenotype makes αMSH agonists particularly attractive as a potential therapeutic. MCH antagonists could be of potential benefit especially in light of the lean phenotype of MCH knockout mice. It is highly likely that as additional components of the system that regulate weight are identified, new therapeutic modalities will be developed. Whether or not new therapies emerge, the identification of leptin and its receptors has provided an intellectual framework in which to consider the regulation of food intake and body weight, the pathogenesis of obesity and other nutritional disorders, and the links between nutrition and physiology.

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