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LEPTIN AND THE REGULATION OF BODY WEIGHT

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I. INTRODUCTION

The assimilation, storage, and utilization of nutrient energy constitute a complex homeostatic system essential for life. In vertebrates, and especially among land-dwelling mammalian species, the ability to store sufficient quantities of energy-dense triglyceride in adipose tissue permits survival during the frequent periods of food deprivation often encountered during evolution. However, the presence of excess adipose tissue is maladaptive and renders obese organisms more susceptible to predation and less effective at hunting. In addition, the accumulation of excess adipose tissue in humans increases the risk for developing hypertension, diabetes, and cardiovascular disease, all of which are major causes of morbidity. In order to regulate fuel stores and energy balance at an optimum level, a complex physiologic system has evolved.

A number of lines of evidence support the hypothesis that body weight is regulated by a robust physiologic system. Twin studies, analyses of familial aggregation, adoption studies, and studies of animal models of obesity all indicate that obesity is to a significant extent the result of genetic factors [1–3]. Moreover, there is abundant evidence that weight is extremely stable in lean and obese individuals [4]. This is despite the fact that a large proportion of the population actively practices some form of weight control. Still, dieting is not generally successful for long-term maintenance of a reduced body weight and the majority of reduced obese individuals eventually regain the lost weight [5].

The relative stability of weight among most individuals has suggested that energy balance in mammals is controlled by a feedback loop that

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maintains constancy of total body energy stores. This system is composed of three components: afferent signals that report nutritional information, a controller in the hypothalamus, and efferent signals that coordinately regulate food intake and metabolism [6]. Thus it has been suggested that signals that report nutritional state are sensed by the brain, specifically the hypothalamus, which in turn modulates food intake and energy expenditure [7–9]. However, the identification of these signals proved difficult and, indeed, for many decades their existence was widely questioned. In recent years, several of the key components of the system regulating body weight have been identified, in particular, leptin and the leptin receptor [10]. These genes have human homologues and their functions are conserved among mammalian species.

II. Afferent Signals

In the 1970s, a critical clue regarding the identity of a key nutritional signal was suggested by studies of mutant obese (*ob/ob*) and diabetic (*db/db*) mice. Recessive mutations in the mouse *ob* and *db* genes result in obesity and diabetes in a syndrome resembling morbid human obesity [3, 11]. Affected *ob/ob* and *db/db* mice have identical phenotypes, each mutant weighing three times that of normal mice with a fivefold increase in body fat content. Data from cross-circulation (parabiosis) experiments suggested that the *ob* gene encoded (or was responsible for the generation of) a circulating factor that regulated nutritional state and that the *db* gene encoded the receptor for this factor [3].

The successful cloning and characterization of the *ob* gene in 1994 has confirmed that it encodes a novel adipocyte-derived hormone. As the wild-type *ob* gene is required to prevent obesity, its protein product was named "leptin" from the Greek word *leptos*, meaning thin. Further studies of leptin have led to the conclusion that body fat content is regulated by a negative feedback loop centered in the hypothalamus and elsewhere [12, 13] (Fig. 1). The data further suggest that weight is regulated by a set point mechanism and that weight is set at different levels among different individuals. When at the set point, a state of energy balance in which food intake equals energy expenditure is maintained. Increasing adipose tissue mass leads to an increase in leptin level. This in turn induces a state of negative energy balance until weight returns to the set point. A decreased adipose tissue mass is associated with a decrease in leptin level, which in turn induces a

state of positive energy balance. This system thus acts to maintain adipose tissue mass within a relatively narrow range. In addition, this system links changes in nutritional state to other physiologic systems (see below).

Leptin circulates as a 16-kDa protein in mouse and human plasma but is undetectable in plasma from C57BL/6J *ob/ob* mice [14]. Leptin is not modified post-translationally as the molecular mass of the native protein is identical to that predicted by the primary sequence (without the signal sequence) [15]. The plasma levels of leptin is highly correlated with adipose tissue mass and falls in both humans and mice after weight loss [16].

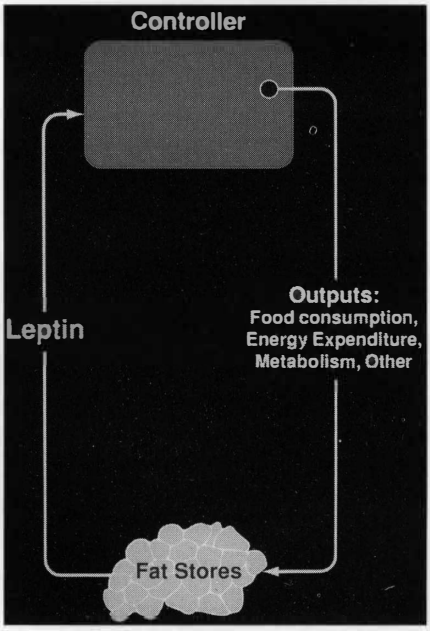

Fig. 1. Leptin and the regulation of adipose tissue mass. The cloning of the *ob* gene and the characterization of leptin has suggested that body fat content is under homeostatic control. The available data suggest that leptin is the afferent signal in a negative feedback loop regulating adipose tissue mass. The level of leptin is positively correlated with differences in body fat. Increasing leptin levels result in negative energy balance (energy expenditure < food intake) whereas decreasing levels lead to positive energy balance (food intake > energy expenditure). These effects maintain constancy of fat cell mass. The available data also suggest that the hypothalamus is an important site of leptin action.

The levels of protein are increased in several genetic and environmentally induced forms of rodent obesity and in obese humans [13, 16–18].

Administration of leptin either by injection or as a constant subcutaneous infusion results in a dose-dependent decrease in body weight at incremental increases of plasma leptin levels within the physiologic range [19–24]. The dramatic effect of leptin vs. saline treatment of *ob* mice is shown (Fig. 2). The potency of leptin is much higher when delivered as a constant infusion vs. bolus injections. This indicates that the mode of delivery may ultimately be a critical determinant of leptin's utility as a therapeutic agent. In both *ob/ob* and wild-type mice, leptin induced weight loss is restricted to adipose tissue with sparing of lean body mass [14].

In aggregate, these data establish leptin's role as an afferent signal in a feedback loop modulating the size of adipose tissue mass. However,

Fig. 2. Both of these mice have a defect in the *obese* (*ob*) gene. This mutation usually results in a marked increase in the amount of fat. Administration of the protein encoded by the *ob* gene, called leptin, reduced the body weight of the *ob* mice. After 4.5 weeks, the *ob* mouse on the left, which did not receive leptin, weighed approximately 67 g, whereas the mouse on the right, who received daily injections of leptin, weighed 35 g. Normal mice weigh approximately 24 g, a weight equivalent to that of an orange. Daily injections of leptin to *ob* mice reduced body weight via effects on food intake and energy expenditure, meaning the treated animals ate less and also burned more calories.

leptin is not the only afferent signal that regulates food intake and body weight. The systems that control feeding behavior and energy balance seem to be comprised of a short-term and long-term system. The short-term system regulates meal pattern and feeding throughout the day. Previous work has indicated that changes in plasma glucose concentration, body temperature, plasma amino acids, cholecystokinin, and other hormones can all modulate meal patterns [25, 26]. 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 levels do not increase significantly after a meal and it does not, by itself, lead to the termination of a meal [16, 18]. These results suggest that leptin is not a classic satiety factor. However, leptin and the other components of the long-term system are likely to 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.

III. Regulation of Leptin Production

The physiologic importance of quantitative changes in leptin concentration suggests that regulation of the *ob* gene is an important control point. The level of *ob* gene expression per cell is highly correlated with the lipid content and the corresponding size of individual adipocytes [13]. Previous studies have suggested that differences in fat cell size can have an important impact on the weight of an organism [27]. The signal transduction system by which fat cells sense their lipid content (possibly size) and in turn adjust the level of *ob* expression is unknown. Extrinsic factors also modulate the expression of leptin. Leptin levels increase ~30% at night and, based on sampling of levels at 5-minute intervals, also seem to oscillate each hour [18, 28]. Although feeding does not seem to increase leptin expression, an extended fast acutely decreases plasma leptin concentration [16]. Other hormones also modulate *ob* gene expression including insulin, glucocorticoids, tumor nerosis factor (TNF), and inter-Leukin-1 (IL-1) [29–33]. The *ob* promoter seems to be responsive to several transcription factors including cEBP and PPARγ [31, 34–36]. It is not known which other factors regulate the expression of *ob* RNA, nor is

it known whether leptin is released from adipocytes from storage vesicles or via the constitutive pathway of protein secretion.

IV. A Broader Role for Leptin

Leptin-deficient (*ob/ob*) mice manifest myriad endocrine and metabolic abnormalities [3]. Many of these derangements, which include decreased body temperature, hyperphagia, decreased energy expenditure (including activity), and infertility are also observed in starved animals. This has suggested that in the absence of leptin, *ob/ob* mice exist in a state of perceived starvation and thus exhibit a constellation of signs that are characteristic of the starved state. Indeed, in circumstances where food is readily available, this biologic response would be expected to lead to the massive obesity evident in *ob/ob* mice. As predicted by such a model, replacement of leptin was found to correct all of the aforementioned abnormalities of mutant *ob/ob* mice [14, 21, 37].

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 [38]. 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 [38]. Starvation is also associated with decreased immune function and leptin corrects these abnormalities [39]. Leptin stimulates proliferation of $CD4^+$ T cells and increases production of cytokines by T-helper-1 cells [39]. 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 capability in woman is suppressed in the absence of adequate nutritional stores. These findings have further suggested that fat tissue may produce a signal that regulates reproduction [40]. This factor may be leptin. Treatment of prepubertal female mice with leptin accelerates the maturation of the female reproductive tract and leads to an earlier onset of the oestrous cycle and reproductive capacity [41, 42]. In humans, a surge in plasma leptin concentration is seen in prepubertal females [43]. 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 animal.

These observations led to speculation that leptin's primary physiological role is to signal nutritional status during periods of food deprivation [26, 44]. However, leptin's physiologic role in preventing weight gain has recently received significant experimental support. As mentioned, lean mice given chronic infusions of leptin lose adipose mass in a dose-dependent fashion at leptin levels within the physiologic range [19, 45]. These data indicate that dynamic changes in plasma leptin concentration act to resist weight change in either direction.

V. 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 [46]. The stoichiometry of binding is not yet known, although the related granulocyte colony-stimulating factor (G-CSF) receptor also has two binding domains and binds to G-CSF with a ligand:receptor ratio of 2:2 [47].

There are several different forms of the leptin receptor, one expressed at a high level in choroid plexus and other tissues, known as Ob-Ra, and another expressed in hypothalamus and elsewhere, known as Ob-Rb. Ob-Rb (also referred to as Ob-R-L) has a long cytoplasmic region with several motifs required for signal transduction [46, 48–51]. The other splice variants, ObRc-e, have also been identified. Ob-Ra is missing motifs generally required for signal transduction by this class of receptors.

Mutations that disrupt the leptin receptor have been identified in each of the available alleles of genetically obese *db* mice as well as *fa* rats, the rat homologue of *db* [52]. In the C57BL/Ks *db*/*db* mutation, the Ob-Rb transcript is specifically mutant whereas the other splice variants are normal [48, 53]. This *db* mutation is the result of abnormal splicing leading to the insertion of a 106-bp fragment including a premature stop codin into the 3′ end of Ob-Rb RNA. The insertion is derived from the Ob-Ra 3′ end and, as a consequence, the Ob-Ra protein is expressed in

place of Ob-Rb in this mouse strain. The phenotype of these C57BLKs *db/db* mice is indistinguishable from that of ob mice. As ObRb is the only form of the receptor that is deranged in these mutant mice, these data establish that Ob-Rb is absolutely necessary for leptin to exert its weight reducing effects. Ob-Rb is normally expressed at a high level in the hypothalamus and other brain regions, T cells, and vascular endothelial cells and at a lower level in many other tissues [39, 48, 49, 54, 55].

The high level of Ob-Rb in hypothalamus suggests that this brain region is an important site of leptin action [48, 49]. *In situ* hybridization has identified the arcuate, DMH, paraventricular nucleus (PVN), VMH, and LH hypothalamic nuclei as the principal sites of Ob-Rb expression [52, 56, 57]. These hypothalamic nuclei have all been shown to play a role in regulating food intake and body weight [8]. Leptin is also expressed in other brain regions including the cortex, thalamus, and the limbic lobe [55]. Further evidence in support of a hypothalamic site of action for leptin was suggested by the observation that leptin induces a dose-dependent activation of the STAT3 transcription factor in the hypothalamus of mice within 15 minutes of a single intraperitional injection [58]. STAT3 activation was not observed in any other tissues. Leptin also increases the level of expression of fos, a STAT3 target, and several other genes in the hypothalamus [59].

Defects in leptin signal transduction may 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 [49–51, 60]. The introduction of mutations into Ob-Rb have identified several sequences in the carboxy terminus that are required for activation of different signal transduction pathways [50, 51]. These 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 [61, 62]. Thus, SHP-2 may play a role in shutting off the leptin signal transduction pathway. SOCS-3, a suppressor of JAK signaling, has also been implicated in leptin signal transduction and may also play a role in down-regulating the response to leptin [63]. Other components of the leptin signal transduction pathway have not yet been identified.

The synaptic transmission of neurons from the arcuate nucleus is altered by leptin [64]. Leptin leads to the hyperpolarization of some hypothalamic

neurons and replicates the effect of glucose on these cells [65]. This effect is dependent on a K^{ATP} channel as tolbutamide inhibits the effect [65]. These electrophysiologic effects are rapid and are unlikely to require new transcription via activation of STAT or other proteins. The components of the leptin signal transduction that alter electrical activity in neurons are not yet known and their identification could provide new therapeutic targets.

The importance of the central nervous system (CNS) as a site of leptin action has also been assessed in comparisons between peripherally and centrally administered leptin. A single injection of leptin ICV reduces food intake at doses that have no effect when delivered peripherally [19, 22, 23, 66]. Chronic infusion of leptin ICV at a dose of 3 ng/hr results in total depletion of body adipose stores whereas peripheral administration requires doses greater than 500 ng/hr to achieve the same effect [19]. The effect of a 3 ng/hr ICV dose seems to be at the peak of the dose response curve as higher doses (50 and 500 ng/hr) have an identical effect. To date, all of the weight-reducing and anorectic effects of peripheral leptin are reproduced by low-dose leptin infused into the III ventricle as are the effects of leptin on glucose and fat metabolism [19, 67, unpublished observation]. These data establish leptin as the most potent weight-reducing peptide known when delivered into the CSF and suggest that the CNS is a major site of leptin action.

Leptin also acts on peripheral cell types and has direct mitogenic effects on $CD4^+$ human T cells [39] (Fig. 3). Leptin has direct effects on endothelial cells directly and increases angiogenesis, although high doses are required [54]. Leptin modulates pancreatic cell function in vivo and has direct effects on other cell types in vitro [68]. The leptin receptor is widely expressed, although the Ob-Ra (short) form of the receptor predominates in many of these tissues [48, 49, 57]. Although the potency of i.c.v. leptin indicates that direct peripheral effects are not absolutely required for weight loss, the full spectrum of its actions is not known. It is thus possible that leptin acts on a variety of tissues and thus functions in other physiological systems.

The importance of Ob-Ra expression or the other forms of the receptor in a particular tissue is not clear. Ob-Ra could function in the transport of leptin across the blood brain barrier or in clearance of the peptide from the CSF [46, 69]. At present, however, the mechanism of leptin transport into the CNS has not been elucidated. Ob-Ra may also serve functions distinct from Ob-Rb and could even form complexes with other cell surface proteins that in turn respond to leptin. A soluble form of the leptin receptor, Ob-Re, has also been identified in mouse and human plasma

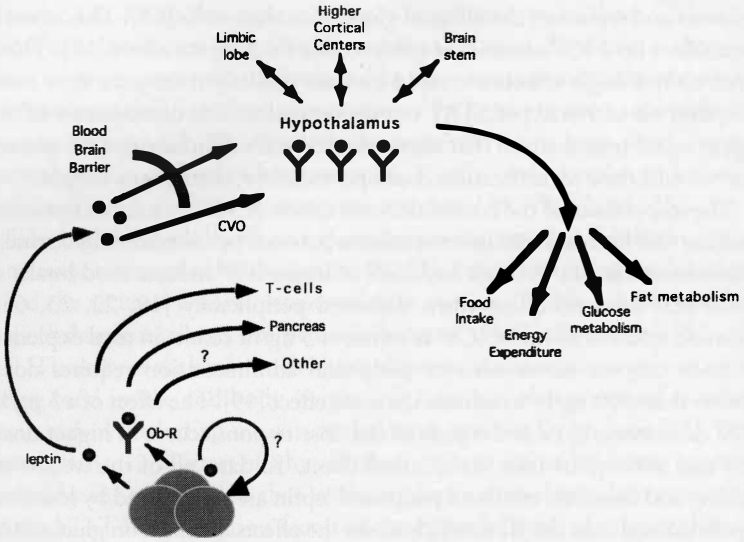

Fig. 3. Leptin is secreted from adipocytes either as a 16 kDa protein or bound to a soluble form of its receptor. The level of leptin is positively correlated with differences in body fat. Increasing leptin levels result in negative energy balance (energy expenditure < food intake) whereas decreasing levels lead to positive energy balance (food intake > energy expenditure). Leptin acts predominately on the hypothalamus. Extensive connections exist between the hypothalamus and other brain regions. Leptin acts centrally to decrease food intake and modulate glucose and fat metabolism. Peripheral effects on T cells, pancreatic islets, and other tissues have also been demonstrated. CVO, circumventricular organ.

[62, 70]. The function of Ob-Re is unknown and experiments that test the effects of recombinant Ob-Re are necessary. In most cases, such as with the growth hormone and interferon, soluble receptors chelate the ligand and have inhibitory effects but in the case of CNTF, the soluble receptor can activate signal transduction [71–73].

VI. The Neural Circuit Regulating Weight

The available data suggest that the concentration of leptin is 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 food is unavailable. Weight gain increases plasma leptin concentration and elicits a different response leading to a state of negative energy balance. It is as yet unclear whether the same (or different) neurons respond to increasing vs. 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 [74].

As mentioned, various nuclei in the hypothalamus have been implicated in the control of food intake and their role with respect to leptin action has recently been examined. Leptin receptors have been localized to several of these hypothalamic nuclei including the arcuate, VMH, LH, DMH, and PVN [8, 52, 56, 57]. The LH and VMH project both within and outside the hypothalamus and modulate activity of the parasympathetic and sympathetic nervous system, respectively [75]. 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 efferents [75]. The PVN also projects to numerous sites outside of the hypothalamus including higher centers known to modulate motivational behaviors.

Each of these hypothalamic nuclei express one or more neuropeptides and neurotransmitters that regulate food intake and/or body weight (Table I). Genetic evidence has implicated several of 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 melanin-concentrating hormone (MCH) play a role in the response to absent (and possibly low) leptin levels, whereas centrally expressed αmelanocyte stimulating hormone (αMSH), its MC-4 receptor, and the Agouti related transcript (ART also known as AGRP) play a role in the response to an increased plasma leptin concentration (Fig. 4) [76]. Differential effects of leptin on neurons expressing either NPY or POMC have been reported [77]. Each of these molecules is expressed in the hypothalamus and in most instances other brain regions as well. What follows is a brief description of the functional properties of these and other neuropeptides and neurotransmitters that have been suggested as being components of the neural circuit that regulates food intake and/or body weight.

When administered intrathecally, NPY is the most potent orexigenic agent that is known. NPY RNA is increased in *ob/ob* mice and its levels

TABLE I. HYPOTHALAMIC MODULATORS OF FOOD INTAKE

Increase food intake	Decrease food intake
NPY	α-MSH
AGRP	CART
MCH	CCK
Galanin	CRH
Orexin a and b	Insulin
Peptide YY	GLP-1
Ghrelin	Bombesin
Moradrenaline (α2 receptor)	Urocortin 1
	Urocortin 2
	Serotonin

decrease after leptin treatment [23]. 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 possibly Y5 receptors play a role in mediating some of NPY's effects on food intake and body weight [78–80].

αMSH as well as MSH agonists decrease food intake. Reduced signaling of MSH in genetically obese A^y or MC-4R knockout mice results in obesity and leptin resistance [19, 81, 82]. Leptin modulates POMC gene expression (POMC is the precursor of MSH) [83]. In addition, a subset of neurons express both Ob-R and POMC [84]. ART, an endogenous antagonist of melancortin signaling, is also implicated in the regulation of weight as transgenic mice overexpressing AGRP are markedly obese [85]. In addition, mRNA for this hypothalamic peptide is increased eightfold in *ob/ob* mice [86]. In aggregate, these results suggest that NPY may play a role in mediating the response to starvation, whereas melanocortin functions in the pathways that prevent obesity [23]. 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.

MCH, a neuropeptide expressed in the lateral hypothalamus, is increased in *ob/ob* mice and injections of it increase food intake in mice [87]. Mice with mutations of MCH are hypophagic and lean, thus confirming a role for this peptide in the maintenance of body weight [88]. MCH-

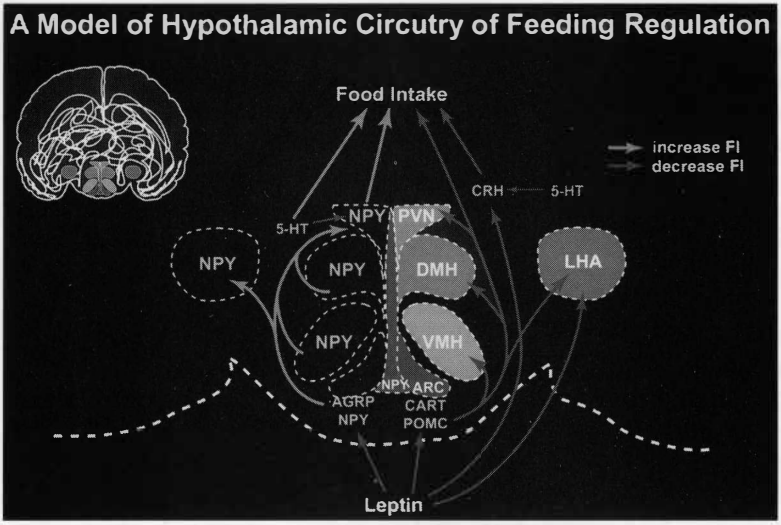

Fig. 4. The neural circuit activated by leptin. In the arcuate nucleus of the hypothalamus, the leptin receptor is expressed in at least two different classes of neurons. One class expresses NYP and AGRP, two neuropeptides that increase food intake. Another class expresses POMC, the precursor of αMSH 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.

expressing neurons receive inputs from NPY neurons in the arcuate nucleus and appear to relay NPY's orexigenic signal. MCH and MSH positive neurons in turn project to many of the same brain regions as αMSH and these molecules have opposite effects in 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 anorexigenic and orexigenic circuits.

Many other neurotransmitters and neuropeptides are likely to function in this homeostatic system (Table I) [10]. These molecules respond to a

number of nutritional signals, and it is likely that leptin, glucose levels, and other signals potentiate the action of some of these anorexogenic agents and antagonizes the orexigenic (i.e., stimulates food intake) of others. Indeed, many leptin receptor–expressing neurons also express one or more of these mediators [84]. These are briefly reviewed here.

Cholecystokinin (CCK) was the first neuropeptide suggested to play a role in regulating food intake [89]. Injections of CCK increase satiety in food deprived rats via afferent vagal nerves [90]. Recently, CCK was reported to potentiate the anorectic effect of leptin [91].

CART, a hypothalamic peptide, is also implicated as playing a role in the response to leptin [92]. CART decreases food intake, its antibodies increase food intake, and its mRNA is increased in *ob/ob* mice. In addition, CART co-localized with αMSH in some hypothalamic neurons.

Bombesin also reduces food intake and induced mutations of the bombesin 3 receptor result in mild obesity [93]. A growing body of evidence indicates that insulin acts on the hypothalamus to decrease food intake [94]. Two recently identified neuropeptides, orexin-a and orexin-b, also regulate the state of an animal's arousal but may also modulate food intake [95, 96].

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 [97, 98]. Additionally, the DMH has CRF-containing neurons [99]. CRF regulates pituitary adrenocorhicotropic hormone (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 [100, 101]. 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 [102, 103]. Leptin may also inhibit the increase in CRH evident during a stress response [104].

Glucocorticoids have long been known to play a role in regulating body weight. Increased fat deposition is a feature of Cushings syndrome, and increased glucorticoids result in obesity in mice [105]. High levels of glucocorticoids are also observed in most strains of genetically obese mice. Moreover, adrenalectomy and glucocorticoid antagonists blunt the obesity evident in *ob/ob*, *db/db*, and other obese mice [106, 107]. Low-dose glucorticoid replacement restores the obese phenotype of adrenalectomized *ob/ob* mice, indicating that they play a permissive role in the development of the obese phenotype [107]. It is unclear whether the requirement for

glucocorticoids in the development of the full *ob/ob* phenotype depends on the suppression of CRH, a known effect of glucocorticoids, or if another mechanism is operative.

The functional relationship of these orexigenic and anorexigenic agents is likely to have therapeutic implications. Antagonists of NPY and its receptors are currently under development as are agonists of the MC4 receptor [108]. Serotonin, which reduces food intake, and norepinephrine, which is orexigenic, are both targets for known weight-reducing agents such as fen-fen (dexfenfluramine and phentermine). A knockout of the HT2c form of the serotonin receptor leads to a form of mild obesity [109]. Future opportunities for the development of antiobesity agents may depend on progress toward understanding the functional relationship among these and other components of this neural circuit that regulates weight.

VII. 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 concentration? Whereas increasing leptin levels lead to fatty acid oxidation and a reduction in adipose tissue mass, leptin deficiency, evident in *ob/ob* mice or mice receiving a leptin anatgonist, is associated with an increase in fat deposition [3, 110]. In *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 [3, 111]. It as yet unclear whether the metabolic derangements of *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. The available evidence suggests 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 [14, 19, 20]. Leptin also prevents the reduced energy expenditure normally associated with a decreased food intake [19]. Finally, hyperleptinemic animals undergoing a rapid period of weight loss fail to show any rise in serum-free fatty acids or ketones [112]. This is in contrast to 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 [19].

Leptin also has novel effects on glucose metabolism. The possibility that leptin modulates glucose metabolism was first suggested in studies of *ob/ob* mice treated with leptin. *ob/ob* mice are diabetic, and the severity of the diabetes is dependent on the background strain carrying the mutation [3]. In one study, leptin normalized the hyperglycemia and hyperinsulinemia evident in C57BL/6J *ob/ob* mice at doses that did not decrease weight [21]. Antidiabetic effects have also been observed in insulin-deficient rats [113]. Leptin also corrects the insulin resistance and hyperglycemia evident in a lipodystrophic transgenic mouse line [114]. These data confirm that leptin has novel and clinically relevant effects on glucose metabolism.

The mechanisms by which the CNS regulates fat and glucose metabolism in response to leptin are also unknown. Given the dense concentration of leptin receptor in the hypothalamus, there are two general possibilities. One is that a releasing hormone is secreted into the hypophyseal portal system and acts to stimulate secretion of a pituitary hormone (known or unknown). Alternatively, leptin may activate a neural effector pathway(s) that modulates fat and glucose metabolism. The former seems unlikely as hypophysectomized and adrenalectomized rats still lose copious amounts of weight after administration of centrally infused leptin [33; unpublished data]. Furthermore, corticosterone and thyroid hormone levels do not change in hyperleptinemic animals (unpublished data). Direct measurement of nerve activity has indicated that infusion of leptin increases sympathetic activity to brown adipose tissue, kidney, hind limb, and adrenal gland [115, 116]. It is not yet known whether blockade of beta adrenergic receptors attenuates (or blocks) leptin-induced weight loss. Thus, although ICV leptin seems to modulate the activity of the SNS, the role of the SNS in mediating the weight reducing effect of leptin is not established.

VIII. Pathogenesis of Obesity

In principle, alterations in body weight could be the result of abnormalities in the generation of the aforementioned afferent signals, the cells that receive the signal or the efferent pathways that effect changes in weight. Thus, the pathogenesis of obesity can be inferred in a general way by mea-

surement of the plasma leptin levels (Fig. 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, as a great number of hormones as well as genetic, environmental, and even psychologic factors influence leptin sensitivity and production.

Plasma leptin levels have been measured in rodents and humans using both radio-immunoassay (RIA) and enzyme-linked immunoassay (ELISA) [16, 18]. In all forms of rodent obesity studied, the obese animals have higher leptin levels than controls (not including *ob/ob* mice) [16, 17]. The data suggest that these forms of animal obesity are associated with leptin resistance. In each of three cases that have been tested, obese animals that are hyperleptinemic are indeed resistant to exogenous leptin (complete or partial), suggesting that their obesity is the result of leptin resistance [19]. Diet-induced obese (DIO) mice are only partially leptin resistant and lose moderate amounts of weight at high doses. A^y mice are completely resistant to high ICV doses of leptin and thus appear to have a defect in the neural circuit that is activated by leptin. Indeed, the insensitivity of A^y mice to leptin strongly suggests that normal function of the MC-4 receptor is required for the response to exogenous leptin. NZO mice are resistant to peripherally administrated leptin but respond normally to centrally administered leptin. Thus, in NZO (and possibly DIO) mice, leptin resistance may be the result of decreased transport of leptin into the brain [19, 117]. This raises the possibility that differences in the access of leptin to the brain could be important in the pathogenesis of some forms of obesity. Consistent with this possibility, the levels of CSF leptin in obese humans seems to plateau at high plasma leptin concentrations [118, 119].

Mutant *fat* and *tubby* mice are also hyperleptinemic [16]. The basis for the apparent leptin resistance in these mice is unclear. Carboxypeptidase E (CPE), the gene product of the *fat* locus, alters post-translational processing of many peptides including insulin, neurotensin, POMC, and MCH [120, 121]. The identity of the substrates of CPE that regulate weight and lead to obesity when these enzymes are defective is not known. The *tub* gene product is expressed at high levels in the PVN of the hypothalamus [122, 123]. As mentioned, neurons in the arcuate nucleus, LH, and VMH (sites of Ob-Rb expression), are known to project to the PVN

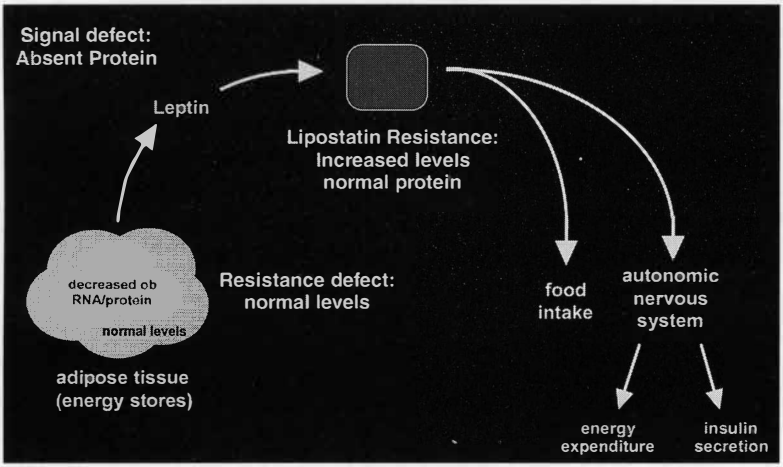

Fig. 5. Pathogenesis of obesity. There are three general ways in which alterations of the leptin regulatory loop could lead to obesity. (a) Failure to produce leptin, as occurs in *ob/ob* mice, would result in obesity, as would (b) inappropriately low leptin secretion for a given fat mass. In the latter case, the fat mass would expand until "normal" leptin levels are reached, resulting in obesity (also see Fig. ?). (c) Finally, obesity could result from relative or absolute insensitivity to leptin at its site of action. Such resistance would be 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 psychological factors.

[75]. Further studies may reveal the link between this gene product and the leptin response.

The leptin resistance observed in DIO mice emphasizes the fact that environmental factors can modulate leptin sensitivity [19, 117]. Akr mice (and some other strains) remain lean when fed a standard chow diet but become obese when fed a high-fat diet. However, other mouse strains do not become obese when exposed to an identical diet. This indicates that the pathogenesis of diet-induced obesity 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 as the incidence of obesity increases in many

populations in proportion when exposed to a high-fat, "Western" diet [124]. How might genes interact with environmental factors to cause obesity? As travelers to France well know, exposure to a highly palatable diet often leads to transient weight gain. In most cases, the gained weight is rapidly lost. However, it is possible that in some 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 leptin is influenced by genetic factors, one might predict that a subset of individuals (and some populations) would be especially susceptible to diet-induced obesity. The observation that animals that express constitutive levels of leptin are insensitive to a high-fat diet supports this possibility. However, alternative explanations are possible and additional studies are required. Studies to identify the AKR alleles that predispose to diet-induced obesity may illuminate the underlying mechanism [125, 126].

IX. 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 [16, 18]. These data suggest that, in most cases, human obesity is likely to be associated with insensitivity to leptin. However, 5–10% of obese human subjects have relatively low levels of leptin [16, 18]. Low leptin levels also predispose to weight gain in preobese Pima Indians [127]. 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 and/or leptin transport into the CNS. It has been suggested that entry of leptin into the CSF may be limiting in some obese subjects [118, 119]. If true, the development of morbid obesity later in life could result when the plasma leptin levels exceed the capacity of the transport system. Treatment with recombinant leptin leads to an increase in CSF leptin concentrations in humans [128]. Leptin uptake has been demonstrated in the capillary endothelium of mouse and human brain and is decreased in preobese animals [69, 129,

130]. It has thus been proposed that transport across the 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 [69]. Moreover, defects anywhere in this transport pathway could lead to the development of obesity.

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. Factors that directly modulate energy expenditure or activate adipogenesis and lipogenesis could also result in apparent leptin resistance. Finally, leptin's actions are likely to be influenced by psychological factors via connections between the higher cortical centers that modulate an animals motivational state and neural circuits within the hypothalamus. The neuroanatomic and functional relationships between these brain regions are not well understood.

X. Mutations Associated with Human Obesity

In almost all cases, obese subjects express at least some leptin, an observation that suggested that human *ob* gene mutations are likely to be rare. Recently, two cousins born from an extended family have been found to be homozygous for a frameshift mutation in the leptin gene. These individuals do not have any circulating leptin [131]. In these two subjects, the mutation is associated with profound obesity, suggesting that leptin is of critical importance for the control of body weight in humans. Affected members of a Turkish kindred with a missense mutation in the leptin gene also manifest extreme obesity and amenorrhea, which further suggests that leptin also plays a role in modulating reproductive function [132]. Similar conclusions were reached in studies of three massively obese members of a French family carrying mutations in the leptin receptor [133]. These three studies also indicate that, apart from severe obesity and abnormalities of reproductive function, the other abnormalities identifiable in *ob* and *db* mice such as hypercortisolemia, cold intolerance, and severe diabetes are not necessarily apparent. The heterozygous members of these families are of normal weight.

The association of massive obesity with mutations in leptin and its receptor in humans confirms its importance in regulating body weight. This assertion is supported by data from early clinical trials in humans (see

below). However, mutations in these genes and other human genes including POMC, PC-1, and the MC4 receptor are relatively rare [10]. Thus, the pathogenesis of most human obesity is largely unknown. It is likely that some of the genes responsible for human obesity in the general population will modulate either leptin secretion or leptin sensitivity. Both genetic and physiologic studies will be required to confirm this prediction in humans.

XI. Prospects for New Treatments for Human Obesity

One important issue relevant for any treatment for obesity concerns the therapeutic endpoint. The health risk of obesity is greatly diminished when even modest amounts of weight (i.e., 5% of total weight) are lost [134, 135]. This is the result of a marked improvement in the diabetic, hypertensive, and cardiovascular status of obese subjects affected by these conditions [134]. Dieting by itself is only rarely effective for the long-term maintenance of weight loss, emphasizing the need for additional therapies [5]. Clearly, an important indication for leptin treatment would be for the management of the co-morbiditys associated with obesity. A recent prospective epidemiologic study of more than one million individuals has confirmed that obesity is an independent risk factor for increased mortality [136]. This finding amplifies the need for efficacious and safe means for treating this disorder.

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 [137]. In addition to reducing food intake, weight, and body fat content, the hormone also stimulated cycling of gonadotropins 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 also establishes a link between leptin signaling and reproductive capacity [137].

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 ($p < .02$) (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 vs. 1.7 kg in a group receiving 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 obese subjects, although studies of more patients are clearly required [138].

Further studies to determine the possible utility of leptin for the treatment of Type II diabetes are also indicated, as animal studies suggest that leptin can increase glucose metabolism independent of weight [21, 67, 139]. 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 [16]. This provides a possible explanation for the high failure rate of dieting, as a low leptin level is likely to be a potent stimulus for weight gain. Thus, leptin treatment after a very low calorie diet (VLCD) could, in principle, reduce the 95% failure rate of diets for long-term maintenance of weight loss. Further studies should reveal whether leptin is of therapeutic value in some obese subjects.

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