Annu. Rev. Nutr. 2000. 20:105–27

REGULATION OF METABOLISM AND BODY FAT MASS BY LEPTIN

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Key Words energy balance, adipose tissue, obesity, food intake, nutrient flux

■ **Abstract** The relative stability of body weight over the long term and under a variety of environmental conditions that alter short-term energy intake and expenditure provides strong evidence for the regulation of body energy content. The lipostatic theory of energy balance regulation proposed 40 years ago that circulating factors, generated in proportion to body fat stores, acted as signals to the brain, eliciting changes in energy intake and expenditure. The discovery of leptin and its receptors has now provided a molecular basis for this theory. Leptin functions as much more than an adipocyte-derived signal of lipid stores, however. Although suppression of food intake is an important centrally mediated effect of leptin, considerable evidence indicates that leptin also functions both directly and indirectly, via the brain, to orchestrate complex metabolic changes in a number of organs and tissues, altering nutrient flux to favor energy expenditure over energy storage.

CONTENTS

OVERVIEW

The identification and sequencing of leptin, the product of the OB gene, heralded a renaissance in research on the regulation of energy balance. Leptin, synthesized and secreted by adipocytes in proportion to the amount of lipid stored, appears to act as a signal to the brain of body energy stores, thus meeting the basic criteria of the lipostatic factor postulated by Kennedy (67). Experiments with parabiotic pairs of genetically obese (db/db or ob/ob) and lean mice provided the first critical clues leading to the eventual discovery of the genes responsible for development of obesity in these strains of mice (23). The products of these genes, leptin (OB protein) and leptin receptors (OB-R), were identified in 1994 and 1995 (132, 151). Over the past 5 years, a considerable body of knowledge in this field has been accumulated, leading to much better understanding of the mechanisms involved in the regulation of body weight and fat and energy metabolism.

Leptin, a 146–amino acid cytokine-like peptide, is expressed primarily in adipose tissue. Obesity is associated with increased leptin synthesis and secretion, whereas fasting and weight loss are associated with decreased leptin synthesis and secretion (80). Leptin appears to have a variety of functions, many of which are related to body energy homeostasis. Leptin acts through both central and peripheral mechanisms to affect feeding behavior, lipid and glucose metabolism, thermogenesis, reproductive and endocrine functions, and cardiovascular and immune functions (44). The extent of leptin's actions can be seen in ob/ob mice, which have a mutation in the ob gene that results in synthesis of a defective leptin molecule that is degraded intracellularly before being secreted. These mice are obese, diabetic, and sterile and exhibit reduced activity, metabolism, and body temperature. With daily administration of leptin, however, (*a*) food intake, body weight, and body fat decrease, (*b*) serum levels of glucose and insulin normalize, (*c*) fertility is restored, and (*d*) metabolic rate, body temperature, and activity increase to levels similar to those of lean littermates (97).

The central pathways involved in leptin's effects on food intake and body weight are beginning to be defined. A number of neuropeptide systems implicated in the control the central nervous system (CNS) has on food intake and regulation of energy balance, such as neuropeptide Y (NPY), corticotropin releasing hormone, proopiomelanocortin (POMC), somatostatin, and melanin-concentrating hormone (MCH) (48), are affected by leptin. Although some of leptin's effects on adipose tissue are mediated centrally, others are a result of leptin acting directly on adipocytes. Leptin alters the transcription of several adipose-specific genes involved in lipogenesis, lipolysis, and energy metabolism, and more interesting, it appears to trigger apoptosis in white adipose tissue (103). In this review, we discuss in greater detail these and other important aspects of leptin's role in the regulation of metabolism and body fat mass.

LEPTIN RECEPTORS

The ob receptor gene is expressed in the choroid plexus, hypothalamus, and many peripheral tissues, including pancreatic β-cells and adipose tissue. Leptin receptors belong to the class I cytokine receptor family, which acts through Janus Kinases (JAK) and signal transducers and activators of transcription (STAT). Several alternatively spliced OB-R variants have been identified (74). OB-Rb, the long form of the receptor, is preferentially expressed in the hypothalamus, whereas the predominant short form of the receptor (OB-Ra) is expressed in many tissues, but mainly in kidney, lung, and choroid plexus (41). OB-Re is thought to be a soluble form of the receptor, which may act as a binding protein (74). In obese db/db mice, a mutation within the leptin receptor gene causes truncation of the intracellular domain of OB-Rb, replacing it with OB-Ra. These mice do not respond to leptin, thus demonstrating that OB-Rb is responsible for mediating leptin's effects on food intake and energy balance (21). In Zucker fatty rats (fa/fa), a missense mutation in OB-Rb causes a single amino acid substitution in the extracellular domain that is common to all receptor isoforms (98), resulting in reduced cell surface expression, leptin binding affinity, and signaling capacity (29). Obese fa/fa rats can respond to leptin, but only high doses (143).

OB-Rb is located primarily in specific areas of the brain, but it is also found in several other tissues, including adipose, ovaries, testis, placenta, adrenal medulla, liver, pancreatic β-cells, lung, jejunum, heart, and skeletal muscle (31, 92). In the brain, the long form of the leptin receptor, OB-Rb, is located in high levels, primarily in the supraoptic nucleus, arcuate (ARC), dorsomedial nucleus (DMN), ventromedial hypothalamic nucleus (VMN), and ventral premamillary nuclei. Moderate levels of OB-Rb were observed in the paraventricular hypothalamic nucleus (PVN), the lateral hypothalamic area (LH), the medial mammillary nucleus, the posterior hypothalamic nucleus, the nucleus of the lateral olfactory tract, and the substantia nigra (36, 41, 48). OB-Rb is present only at very low levels in the choroid plexus and brain microvessels (10). In contrast, the short form of the receptor (OB-Ra) is present in low levels in the hypothalamus, but there are very high levels in brain microvessels and choroid plexus (10). This pattern of distribution of the leptin isoforms is consistent with previous suggestions that OB-Ra is involved in transport of leptin across the blood-brain barrier, whereas OB-Rb mediates leptin's effects on food intake, body weight, and metabolism.

REGULATION OF LEPTIN EXPRESSION AND SECRETION

White adipocytes are the primary cells that synthesize and secrete leptin, although leptin has also been shown to be produced in muscle cells, gastric epithelium, and placenta (5, 55, 141). There are considerable differences among adipose depot sites in the level of leptin gene expression (90). In humans, subcutaneous adipose tissue has higher levels of leptin mRNA than does omental adipose tissue, whereas

in adult rats, leptin gene expression is higher in gonadal and perirenal adipose than in subcutaneous adipose.

Over the long term, regulation of leptin synthesis and secretion is related primarily to the degree of adiposity; however, both circadian and ultradian variations in leptin gene expression and plasma leptin levels occur (76). In both humans and rats, leptin concentrations are maximal at night. The relative diurnal amplitude is higher in lean than obese subjects, and in men compared with women, even after controlling for adiposity. The diurnal amplitude decreases with age (111). The periodicity of the ultradian rhythm of leptin secretion is similar in men and women, but in obese compared with lean subjects, pulse frequency is decreased. Pulse amplitude is higher in women than men and correlates with body mass index (76, 111).

Feeding and fasting have also been shown to alter both leptin gene expression and plasma leptin levels (5). Leptin levels increase following a meal and begin to decrease several hours after a meal. These changes occur independently of circadian variations (28) and may be due in part to secretion of leptin from gastric epithelium (5). In rats, however, the increases in leptin mRNA and serum leptin concentration were similar during enteral and parenteral feeding, which suggests that gastric leptin may not be a significant contributor to the feeding-related increase in plasma leptin concentration (75).

The sympathetic nervous system (SNS) plays an important role both in the regulation of energy expenditure and in adipose tissue lipolysis (7). Although the importance of sympathetic innervation of brown adipose tissue (BAT) is well recognized, conclusive evidence of sympathetic innervation of white adipose tissue (WAT) has been demonstrated only recently (7). Increased sympathetic activity in WAT occurs during fasting and cold exposure, conditions also associated with decreased leptin synthesis and secretion (51, 135). Catecholamines and beta-adrenoceptor agonists have been shown to inhibit leptin production in both in vitro and in vivo studies (51, 135). This effect appears to be mediated by beta 3-adrenoceptor agonists because selective beta 3-agonists have a potent suppressive effect on leptin gene expression and decrease circulating leptin concentration (134). This and other evidence has led to the proposal that the SNS plays an important physiological role in regulation of leptin expression and secretion.

A number of hormonal and metabolic factors have been shown to alter leptin gene expression and secretion. Insulin and glucocorticoids act directly on adipocytes to increase leptin synthesis and secretion and may function as long-term regulators of leptin expression (110, 140). Glucocorticoid effects on leptin synthesis and secretion have been demonstrated in rodents and humans, both in vivo and in vitro. Administration of physiologically relevant amounts of glucocorticoids has been shown to stimulate leptin secretion (34). Conditions in which elevated circulating glucocorticoid levels occur (Cushing's syndrome, pituitary or adrenal adenoma) are associated with increased blood leptin levels, whereas adrenalectomy results in decreased leptin levels (64, 73).

Increased plasma insulin is also associated with increased plasma leptin, and injection of insulin increases both plasma leptin and adipose tissue leptin mRNA

levels (113). In streptozotocin-diabetic rats, plasma leptin and leptin mRNA levels in epididymal fat tissue were low and were unaffected by feeding or fasting, but treatment with insulin restored plasma leptin levels (96). Increased glucose transport and metabolism, rather than a direct effect of insulin, may be responsible for insulin's effects on leptin synthesis and secretion because inhibitors of glucose transport and glycolysis caused concentration-dependent inhibition of leptin secretion from rat adipocytes in the presence of insulin (93). The flux of glucose in the hexosamine biosynthetic pathway, which has been suggested to be a cellular "sensor" of energy availability, is also linked to the stimulation of leptin gene expression and secretion in both adipose tissue and muscle (141).

Conflicting findings have been published regarding the response of adipose tissue to the interactive effects of glucocorticoids and insulin on leptin synthesis and secretion. In one study, the combination of insulin and dexamethasone increased leptin mRNA and leptin secretion in adipose (110), whereas in another study, insulin inhibited dexamethazone-stimulated leptin synthesis and secretion (25).

There is a significant gender effect on serum leptin levels, which does not appear to be due entirely to differences in body composition and may be a result of differences in plasma estrogen and/or testosterone levels. Leptin levels are higher in females, even before puberty, compared with boys, independent of differences in body composition (32). In rats, ovariectomy caused a decrease in leptin mRNA levels in adipose tissue and a decrease in serum leptin concentration, both of which were reversed by administration of estradiol (121). Estrogen and testosterone modulate leptin synthesis and secretion directly, apparently acting through sex steroid receptor-dependent transcriptional mechanisms (78). In male rats, dihydrotestosterone decreased adipose tissue leptin mRNA, whereas in adipose tissue from female rats, 17-β estradiol increased leptin mRNA levels.

There is some evidence that both growth hormone (GH) and thyroid hormone affect leptin synthesis and/or secretion. Evidence for a thyroid hormone effect comes primarily from studies with hypo- or hyperthyroid humans; however, the most recent evidence indicates that changes in leptin mRNA and serum levels are a result of an independent effect of thyroid hormone on adipose stores (129). There is more substantial evidence that GH has a direct effect on leptin levels. In a recent report, GH treatment of Zucker rats reduced leptin mRNA levels in epididymal fat tissue, but not in subcutaneous fat tissue. Because insulin-like growth factor-I had no effect on leptin mRNA levels, these findings suggest that GH directly interacts with visceral adipose tissue to reduce leptin gene expression (62).

Recent studies have provided information about regulation of the leptin gene at the molecular level. Important regulatory domains in the leptin promoter have been identified, including those for CCAT enhancer binding proteins (C/EBP), adipocyte determination differentiation dependent factor 1/sterol regulatory element binding protein 1 (ADD1/SREBP1), Sp1, and peroxisome proliferator activated receptor gamma (PPARγ) (45, 57, 59). In vitro studies of rat adipose cells have identified a 161-bp coding sequence at the 5′ end that retains promoter activity. This sequence contains binding sites for C/EBPα and SP1. C/EBPα, a transcription factor important in adipose cell differentiation, causes activation of the leptin promoter (57). A

region located between nucleotides −101 and −83 of the leptin gene appears to be critical for glucose/insulin stimulation of transcription. The transcription factor Sp1, which binds in this region (LP1), inactivates the leptin promoter and may be involved in the effect of insulin on leptin gene transcription (45). Likewise, activation of the leptin promoter by ADD1/SREBP1 is dependent on its binding to the region contained within the major insulin response element. Feeding and fasting and in vitro exposure to insulin induced changes in gene expression of the transcription factor ADD1/SREBP1 parallel to those of leptin gene expression. Thus, it was suggested that ADD1/SREBP1 is a key transcription factor linking changes in nutritional status and insulin levels to leptin gene expression (68).

PPARs are members of the nuclear hormone receptor superfamily. After dimerization with retinoic X receptor and ligand activation, they control the expression of genes containing specific response elements. PPARγ is a key player in adipocyte differentiation (133) and controls the expression of several crucial adipocyte genes, including those for lipoprotein lipase (LPL), acyl coenzyme A synthase, fatty acid synthase, and phosphoenol pyruvate carboxykinase, all of which are involved in coordinating fatty acid uptake and storage (39). Activators of PPARγ, such as thiazolidinediones and 15-deoxy-delta (12, 14) prostaglandin J2, have been shown to down-regulate leptin expression, possibly by antagonizing the effects of C/EBPα, which contributes further to the adipogenic effect of PPARγ (59, 124, 149).

Tumor necrosis factor-α (TNFα) is a potent inhibitor of adipocyte differentiation, and it has been suggested that it plays a role in leptin-induced apoptosis (106). Exposure of 3T3-L1 adipocytes to TNFα resulted in lipid depletion, reversal of adipocyte differentiation, and adipocyte apoptosis (139, 148). There are conflicting findings on the effects of TNFα on leptin secretion, though. TNFα has been shown to stimulate secretion of leptin in mice in vivo and from mouse adipocytes in primary culture (43), but to decrease secretion of leptin from mouse parametrial and human subcutaneous adipocytes in vitro (146).

CENTRAL PATHWAYS INVOLVED IN LEPTIN EFFECTS

Brain Sites of Action

A study showing that intracerebroventricular (i.c.v.) administration of leptin reduced food intake and body weight and altered metabolism in ob/ob and lean mice provided the first evidence that leptin had a central site of action (14). Over the past few years, a variety of techniques have been used to determine possible direct sites of leptin action in the brain. The mediobasal hypothalamus, particularly the arcuate nucleus (ARC) and ventromedial hypothalamic nucleus (VMN), contains neurons that coexpress leptin receptors and neuropeptides thought to be essential in the control of food intake and regulation of energy balance (44). Microinjection of leptin directly into the VMN decreased food intake in rats, whereas the same dose had no effect when injected i.c.v. or into the dorsal raphe nucleus (63). However, bilateral microinjections of leptin into the ARC were more effective than VMN

injection in decreasing food intake and body weight gain (115). Rats with bilateral VMN lesions did not respond to either intravenous (i.v.) or i.c.v. injection of leptin (116). Likewise, lesion of the ARC by neonatal MSG treatment also eliminated response to i.c.v. injection of leptin (130).

Feeding- and fasting-related changes in OB-R expression in the brain provide another source of information about leptin's possible sites of action. OB-R mRNA expression in the VMN and ARC increases after fasting in wild-type rats and mice. However, in ob/ob mice, which are leptin deficient, fasting had no effect, but systemic administration of leptin decreased OB-Rb mRNA levels in the VMN and ARC (8). The leptin receptor is a member of the gp130 family of cytokine receptors, which stimulate gene transcription through activation of cytosolic STAT proteins. This information has been used as another tool to investigate possible leptin sites of action in the brain. STAT3 immunoreactivity has been localized to OB-R– containing neurons in the parvocellular PVN, ARC, periventricular nucleus, and LH in rats (50). In ob/ob and wild-type mice, but not in db/db mice (which lack active leptin receptors), leptin injection activated STAT3 only in the hypothalamus, providing further evidence that the hypothalamus is a direct target of leptin action (137).

Expression of c-fos protein, a marker of cellular activation, has been used to show leptin effects in specific hypothalamic sites. After i.v. injection of leptin in rats, increased c-fos–like immunoreactivity (c-FLI) was detected in the VMN, the DMN, the ventral premamillary hypothalamic nuclei, the parvocellular PVN, and the lateral parabrachial subnucleus (35). Third ventricular injection of leptin in rats increased c-FLI in the PVN, DMH, and central amydgala (138).

Neuropeptides Involved in Leptin-Mediated Effects

There is considerable evidence that leptin's effects are mediated through the synthesis and release of neuropeptide effector molecules in specific areas of the brain. A number of these neuropeptide pathways have been identified, including NPY, melanin concentrating hormone (MCH), α-melanocyte stimulating hormone (αMSH), agouti-related protein, corticotropin releasing hormone, galanin, glucagon-like peptide 1, neurotensin, and cocaine- and amphetamine-regulated transcript (CART) (48, 71, 84, 119).

Neuropeptide Y NPY, a potent stimulator of feeding, is thought to play a physiological role in the control of food intake and regulation of energy balance. NPY-containing neurons in the hypothalamus are located primarily in the ARC and project to other hypothalamic sites, such as the PVN. In rats, NPY secretion in the PVN increased during fasting and decreased during feeding (112).

Evidence from several studies suggests an important interaction between leptin and NPY. NPY and OB-R mRNA are coexpressed in ARC neurons that are activated during fasting (49). NPY synthesis in ARC was decreased by i.c.v. leptin injection, whereas leptin-induced inhibition of feeding was blocked by microinjection of NPY into the PVN (69). In ob/ob mice, which have increased hypothalamic

NPY mRNA levels, intraperitoneal injection of leptin reduced NPY mRNA levels in the ARC (118, 128). Although these findings suggest the importance of the leptin-NPY link, NPY is not the only important neuromodulator of leptin's effects on food intake and body weight, because NPY gene knockout in ob/ob mice did not fully reverse their obesity (38).

Corticotropin Releasing Factor The involvement of the hypothalamic-pituitary-adrenal system in body weight regulation and in leptin synthesis, secretion, and action is well accepted; however, the interactions are complex and not yet clearly defined. Corticotropin releasing factor (CRF) appears to be an important downstream effector of leptin's anorexigenic effect. CRF is present in the PVN and VMN and has potent inhibitory effects on feeding, energy expenditure, and body weight gain (46, 54), and both synthesis and secretion of hypothalamic CRF are increased by leptin (26, 119). Leptin injected i.c.v. in rats decreased food intake, increased CRF mRNA levels in the PVN, and increased CRF type 2 receptor mRNA (CRF-R2) in the VMN, but not in the PVN (95, 119). These effects were inhibited by simultaneous injection with a CRF antagonist, thus providing evidence that CRF is involved in leptin's effects on food intake (136).

CRF-R2 receptors are believed to mediate the anorexigenic effect of CRF (126), and CRF-R2 in the VMN also appear to have some role in leptin-mediated effects, because continuous systemic infusion of leptin in rats increased CRF-R2 mRNA in the VMN but not in the PVN (95). However, VMN lesion did not eliminate the suppression of food intake by i.c.v. injection of CRF (3), whereas VMN lesion did eliminate the response to leptin. Thus, VMN CRF-R2 may mediate a nonfeeding-related leptin action.

POMC/αMSH The melanocortin system plays an important role in the control of food intake and regulation of body weight, and it is thought to mediate some of the central responses to leptin. The melanocortin system includes peptides processed from proopiomelanocortin (POMC), such as αMSH, agouti-related protein (ARP) (an endogenous antagonist of αMSH), and melanocortin receptors (MC-R). There is evidence that hypothalamic MC4-R and its peptide ligand αMSH are involved in feeding inhibition. Central injection of αMSH or an analog markedly inhibited feeding in rats, whereas ARP increased feeding and antagonized the effects of αMSH (13, 109). In both fa/fa rats, which are insensitive to leptin, and food-restricted rats, MC4-R binding was increased in the VMN, ARC, median eminence, and DMN. Rats with diet-induced obesity had decreased MC4-R binding in VMN, ARC, and median eminence (53).

Overproduction of ARP (lethal yellow mutation—A^y/a), which inhibits binding of αMSH to MC4-R, results in obesity in mice (61). Genetically obese ob/ob and db/db mice have increased levels of ARP mRNA and reduced levels of POMC mRNA in the hypothalamus (122). Treatment with leptin, however, decreased ARP mRNA levels and increased POMC mRNA levels (87, 88). Leptin has been

shown to increase hypothalamic POMC mRNA expression specifically in the ARC, a site in which leptin receptor mRNA is highly colocalized with POMC-containing neurons (22). Although these findings suggest that the melanocortin system may mediate some of the effects of leptin, a recent study of mice with a double mutation (ob and A^y) found that the effects of leptin deficiency and increased ARP production on food intake and body weight were independent (11). Other studies indicate that the melanocortin and NPY systems interact directly, because defective signaling in the melanocortin system is associated with increased NPY mRNA expression in the DMH (47). The nature of the interaction between leptin and either of these neuropeptide systems remains to be determined.

Other Peptides Several other peptides have been implicated in leptin's central effects on feeding and body weight. The two most likely candidates are CART and MCH. CART is a neuropeptide with potent anorexic effects after i.c.v. injection (72). It is expressed in specific hypothalamic areas, including the ARC, PVN, and DMN, and in many extrahypothalamic areas. Centrally administered anti-CART antisera increased food intake in rats, thus providing evidence that this peptide is an endogenous inhibitor of feeding (72). Expression of CART mRNA in the ARC is reduced during fasting and is lower in ob/ob mice and fa/fa rats than in lean mice and rats, but infusion of leptin can restore CART mRNA levels in ob/ob mice (71). These findings support the proposal that CART is involved in leptin's central effects on feeding.

MCH is primarily expressed in the LH. Like NPY, it is a potent stimulant of food intake after i.c.v. injection. MCH mRNA levels increased during fasting, and expression of MCH was increased in ob/ob compared with lean mice (87). Treatment with leptin decreased hypothalamic MCH mRNA levels (60). In contrast to mice with genetic NPY deficiency, which have normal body weight and food intake, mice with genetic MCH deficiency are hypophagic and lean and have an increased metabolic rate, in spite of having decreased leptin levels. These findings suggest that MCH functions downstream of leptin as a stimulant of feeding.

Sympathetic Nervous System A number of leptin's actions have been attributed to increased sympathetic activity. Leptin increased sympathetic nerve activity to BAT, kidney, hindlimb, and adrenal gland in Sprague Dawley rats, but not in fa/fa rats (56). Leptin-mediated increased uncoupling protein (UCP) 1 gene expression in BAT was shown to be dependent on sympathetic innervation, whereas the increase in LPL gene expression was not (117). Microinjection of leptin into the VMN increased glucose uptake in BAT through activation of the SNS, but did not affect glucose uptake in WAT (85). Moreover, in hyperleptinemic rats, transplanted epididymal adipocytes with no sympathetic innervation were nevertheless depleted of fat (145). Thus, sympathetic activation by leptin may be responsible for increased thermogenesis, but it does not appear to be necessary for leptin-induced fat mobilization.

Neuroendocrine Effects of Leptin

Gonadotropin Secretion Normal reproductive function is dependent on adequate nutrition and body energy stores. Evidence from a number of studies indicates that leptin may function to relay nutritional and metabolic information to the reproductive endocrine system. In ob/ob mice, leptin restored fertility, and treated females were able to become pregnant and deliver viable young (6, 20). Leptin also accelerated the onset of puberty in normal female mice (1) and prevented inhibition of gonadotropin secretion caused by food deprivation (2). Leptin has been shown to stimulate LH-RH release from hypothalamic explants in vitro and to stimulate both LH and FSH secretion directly from the anterior pituitary (147), and i.c.v. administration of leptin antiserum decreased LH pulsatility and impaired reproductive function in female rats (15). Thus, leptin may act on both the hypothalamus and the pituitary to control gonadotropin secretion.

Growth Hormone GH secretion is closely coupled to nutritional status, and because genetically obese rodents (ob/ob mice, fa/fa rats) are known to be deficient in GH, leptin's role in the control of GH secretion has been of interest. Leptin infused i.c.v. for 7 days increased basal and GH-releasing factor (GHRH)-stimulated GH secretion (131), whereas leptin antiserum administered i.c.v. decreased plasma GH levels in normal fed rats (16). Although i.c.v. leptin injection had no acute effect on GH secretion in fed rats, it reversed the inhibitory effect of food deprivation on GH secretion in fasted rats (16).

GH secretion is regulated at the hypothalamic level by the inhibitory action of somatostatin (SRIF) and the stimulatory action of GHRH. The presence of leptin receptors in several hypothalamic nuclei containing these peptides suggests that leptin may act via either one or both of these peptides to regulate GH secretion. In a recent study, leptin-induced GH secretion in fasted rats was completely blocked by i.v. administration of anti-GHRH serum, whereas anti-SRIF serum significantly increased leptin-induced GH secretion (17). Furthermore, i.c.v. leptin administration in hypophysectomized food-deprived rats reversed both the decrease in GHRH mRNA levels and the increase in SRIF mRNA levels in the hypothalamus (17). Although it is not clear whether these effects were a result of direct action of leptin or whether intermediary neuropeptides might be involved, these findings demonstrate that leptin's effects on GH secretion are mediated at the hypothalamic level by both GHRH and SRIF.

Hypothalamic-Pituitary-Adrenal Axis Adrenalectomy has been reported to eliminate many of the phenotypic differences between obese (fa/fa) and lean Zucker rats and obese (ob/ob) and lean mice (18, 42), which suggests an interaction between leptin and the hypothalamic-pituitary-adrenal axis. Because of a number of conflicting findings, however, the nature of the interaction is not yet clearly defined. As discussed above, glucocorticoids stimulate synthesis and secretion of leptin from adipocytes, and CRF appears to be an important hypothalamic

mediator of leptin's effect on food intake. In some studies, leptin has also been shown to have direct effects on the adrenal gland. In both human and rat primary adrenocortical cells, leptin inhibited ACTH-stimulated secretion of corticosterone in a dose-dependent manner (100). However, in adrenocortical cells from mice, leptin increased basal corticosterone secretion and had no effect on ACTH-stimulated corticosterone secretion (83).

Because OB-R mRNA has been detected in the human anterior pituitary (33), it has been suggested that leptin directly affects ACTH secretion. Although injection of leptin stimulated ACTH secretion in rats (82), leptin did not alter secretion of ACTH in vitro from rat primary pituitary cells (58); thus, leptin's in vivo effect on ACTH secretion may have been secondary to other changes. The finding that the presence of adrenal glands was not a requirement for leptin's effects on energy balance (4) adds to the complexity. Undoubtedly, further studies will be needed to unravel this complicated picture.

Interaction of Leptin- and Glucose-Sensitive Cells in the Brain Parenteral administration of gold thioglucose (GTG) results in destruction of neurons in the VMN and development of obesity (30). GTG-induced lesions and the subsequent hyperphagia and obesity can be prevented by administration of glucose analogs or by insulin deficiency (30). Moreover, GTG treatment eliminates the feeding response to glucoprivation induced by 2-deoxyglucose (9). Thus, it has been suggested that "glucose-sensitive" neurons in the VMN are involved not only in feeding behavior, but also in body weight regulation.

GTG-lesioned mice have a reduction in the number of leptin receptors in the hypothalamus (41), and both GTG-lesioned and db/db mice show a 20-fold increase in leptin mRNA in adipose tissue (79). In recent studies utilizing patch clamp recording techniques, leptin was shown to hyperpolarize glucose-sensitive neurons in the ARC and VMN (127) and to depolarize neurons in the PVN (99). These findings suggest that there is an important interaction between leptin- and glucose-sensitive hypothalamic neurons and support the concept of a central integration of information from short-term signals of metabolic activity (glucose) and long-term signals of energy stores (leptin).

EFFECTS OF LEPTIN ON NUTRIENT FLUX

The extreme depletion of adipose depots in rats or mice treated chronically with leptin or in rats made hyperleptinemic by administration of a leptin-adenovirus construct (114, 145), the loss of adipose as opposed to muscle tissue in leptin-treated animals (65), and the prolonged recovery time for repletion of fat stores following long-term increased central or peripheral levels of leptin in rats (144, 153) illustrate the profound effects of leptin on nutrient flux. Leptin has complex effects on the storage and metabolism of fats and carbohydrates. These are mediated both directly, through actions on specific tissues, and indirectly, through CNS endocrine and neural mechanisms. Adipose tissue is both the primary site of leptin

production and a major effector organ for many of leptin's actions. However, there are marked differences in response to leptin between brown and white adipose tissues, as well as among white adipose depots. Leptin has been shown to alter glucose and fatty acid uptake and metabolic pathways involved in lipid oxidation and synthesis. Chronically elevated levels of leptin, either peripherally or centrally, result in activation of nuclear transduction pathways that trigger a cascade of events leading to reversal of adipocyte maturity and even cell death by apoptosis.

Energy Metabolism

The increase in UCP expression in adipose tissue and muscle that occurs during leptin treatment may account for leptin's ability to prevent the decrease in energy expenditure that typically occurs during a reduction in food intake. Activation of UCP uncouples ATP synthesis from oxygen consumption in mitochondria, allowing high rates of substrate oxidation and heat production without phosphorylation of ADP. Stimulation of sympathetically innervated β_3-adrenergic receptors (β_3-AR) induces synthesis of UCP1 and activates BAT mitochondrial UCP1 (152). Chronic peripheral administration of leptin in both wild-type and ob/ob mice and in rats has been shown to increase UCP1 and UCP2 expression in BAT and WAT and UCP3 expression in BAT and skeletal muscle (24, 114, 117). Leptin treatment also prevented fasting-induced decreases in UCP1 and UCP3 mRNA levels in BAT (125).

Recent findings indicate that leptin's effects on UCP gene expression are mediated by the brain. Leptin had no effect on UCP1 expression in brown adipocytes in vitro or in denervated BAT pads in vivo (117, 123), whereas i.c.v. administration of leptin, in amounts below effective peripheral doses, increased UCP2 expression in WAT and prevented the food deprivation–induced decrease in mRNA levels of UCP1, −2, and −3 in both BAT and WAT (27, 106). Because leptin stimulates SNS activity, and sympathetically innervated β_3-AR activate UCP1 gene transcription, leptin's effect on UCP1 in BAT may be mediated by SNS activation of β_3-AR.

Lipid Metabolism

Leptin has both direct and indirect effects on mobilization and synthesis of lipid by adipocytes. In vitro, leptin increased lipolysis in mature white adipocytes from Zucker lean, but not obese, (fa/fa) rats, which suggests a direct OB-Rb–mediated effect on adipocyte lipolytic mechanisms (123). Expression of hormone-sensitive lipase, the primary regulatory enzyme in the lipolytic pathway (108), increased in WAT, but not BAT, in mice treated with leptin (114). Whether this was a direct or indirect effect has not been determined; however, because hyperleptinemia resulted in complete depletion of lipid from denervated fat pads in rats, a neural mechanism does not appear to be involved in leptin-induced lipid mobilization (145). Bilateral lesions of the VMH prevented depletion of body fat in hyperleptinemic rats, however, so leptin may trigger the release of a factor from the

brain that acts directly on adipocytes to increase lipolysis (70). CART has been suggested as a possible candidate (145).

Leptin also alters synthesis and uptake of fatty acids. In vitro, leptin inhibited expression of acetyl coenzyme A-carboxylase, the major rate limiting enzyme in fatty acid synthesis, and fatty acid synthase, which catalyzes the final reaction in fatty acid biosynthesis (114). Inhibition of acetyl coenzyme A-carboxylase leads to a reduction in malonyl-coenzyme A, an inhibitor of carnitylacyltransferase I and mitochondrial beta-oxidation, thus blocking fatty acid synthesis and increasing mitochondrial fatty acid uptake and oxidation. Leptin also increases expression of lipoprotein lipase (LPL) in brown adipocytes but has little or no effect on LPL expression in WAT (114, 117, 123).

Recent findings suggest that leptin induces a novel form of lipolysis in adipocytes. Typically, during food deprivation–induced lipid mobilization, there is an increase in both glycerol and free fatty acid (FFA) release from adipocytes, and serum FFA and ketone levels rise. However, leptin increases lipolysis without causing an increase in FFA release, both in vivo and in vitro (120, 145). Increased LPL expression along with increased mitochondrial uptake and metabolism of fatty acids might account for the lack of increase in plasma triglyceride and FFA levels despite increased lipolysis during treatment with leptin. It has been suggested that lipid mobilized from WAT is taken up and recycled by muscle and BAT to be used for mitochondrial oxidation, thus preventing the rise in plasma triglyceride levels (114).

Carbohydrate Metabolism

The finding that leptin treatment normalizes blood glucose and insulin levels in ob/ob mice suggests involvement of leptin in the regulation of glucose utilization. In vivo, chronic leptin treatment increased insulin sensitivity (66); however, in vitro exposure of skeletal muscle or adipocytes to leptin did not acutely alter glucose transport in the presence or absence of insulin (154). More recently, leptin was shown to have differential, tissue-specific effects on glucose and oxygen utilization, resulting in increased energy consumption in BAT and muscle and decreased energy storage in WAT (142). Chronic peripheral injection of leptin increased levels of the glucose transporter GLUT4 and increased glucose uptake in BAT, but decreased GLUT4 and glucose uptake in WAT. Chronic leptin treatment also increased insulin sensitivity of liver tissue and increased insulin-stimulated glycogen synthesis (52). In muscle, leptin decreased glycogen synthesis, increased fatty acid oxidation, and decreased fatty acid incorporation into triacylglycerol (94).

Leptin has also been shown to decrease circulating insulin levels, independent of the decrease in food intake. A number of studies have demonstrated that leptin acts directly on pancreatic islets to inhibit insulin secretion and reduce insulin mRNA levels (37). That these effects are mediated through OB-Rb receptors on islet cells is supported by the finding that leptin activated a STAT3 signaling mechanism in rat pancreatic β-cells (91).

Leptin's centrally mediated effects on the SNS may also contribute to changes in whole body glucose turnover. Sympathectomy eliminated the leptin-induced increased glucagon secretion and decreased insulin secretion in rats (86). In intact rats, i.v. injection of leptin augmented the glucagon response to hypoglycemia, but there was no effect in sympathectomized rats. In vagotomized rats, glucose-stimulated insulin levels were decreased after leptin injection, but sympathectomy eliminated the response.

LEPTIN-INDUCED ADIPOCYTE APOPTOSIS

Following leptin treatment, there is a prolonged recovery period before body weight and fat stores return to pretreatment levels (65). The finding of increased adipocyte apoptosis following i.c.v. administration of leptin suggests that the delayed recovery of body weight is a result of loss of adipose tissue, and not just depletion of lipid stores (103). Following a 5-day treatment period with leptin injected i.c.v., there was clear untrastructural and biochemical evidence of apoptosis in adipose tissue from retroperitoneal and epididymal or parametrial fat pads from male and female Sprague Dawley rats. Morphological and cellular data demonstrated a loss of adipocytes and the appearance of adipocytes with condensed chromatin, a characteristic feature of apoptotic cells. Adipocyte size distribution data indicated a greater number of small adipocytes in fat pads from leptin-treated rats. In contrast, adipose tissue from control pair-fed rats showed no evidence of apoptosis (103).

Apoptosis of mature white adipocytes has previously been shown to occur in malignancy and in streptozotocin-induced diabetes (77) and can be induced in vitro by TNFα, growth factor deprivation, or mild heat shock (101, 102, 104). Although the cellular mechanisms that mediate this process have not been clearly defined, PPARγ and retinoic acid receptor are thought to be involved in the signal transduction pathway of adipocyte apoptosis, which involves repression of transcription. In a recent study, expression of UCP2, PPARγ, retinoid X receptor, and NFκB were increased after i.c.v. administration of leptin, (106), whereas expression of the transcription factors C/EBPα, -β, and -δ, which are specific for mature adipocytes, was decreased (105).

Leptin-induced adipocyte apoptosis appears to be a CNS-mediated effect because the i.c.v. doses used were below those that cause body fat loss when administered peripherally (103). In addition, bilateral VMH lesions have been shown to eliminate the body weight and food intake responses to leptin (116). Although the SNS has been shown to affect susceptibility of brown adipocytes to apoptosis (12), the finding that denervation did not eliminate the response of white fat pads to leptin (145) suggests that a blood-borne factor may be involved.

Ultrastructural findings from mice treated chronically with leptin demonstrated that adipocytes from WAT underwent morphological transformation from mature unilocular lipid filled cells to small lipid-depleted cells with numerous active mitochondria (114). In rats, adenovirus-induced hyperleptinemia depleted adipocyte fat and down-regulated lipogenic enzymes and their transcription factor, PPARγ,

in epididymal fat. Enzymes of fatty acid oxidation and their transcription factor, PPARα, normally low in mature adipocytes, were up-regulated. This transformation was accompanied by loss of the mature adipocyte markers, adipocyte fatty acid–binding protein 2, TNFα, and leptin and by the appearance of the preadipocyte marker Pref-1 (150). In vitro studies have shown that the increased ability of mature adipocytes to resist apoptosis is associated with expression of cell survival genes, such as neuronal apoptosis inhibitory protein and Bcl-2 (19, 81). Although the mechanism involved in leptin-mediated apoptosis of adipocytes is not known, if leptin triggers a process that leads to reversion to a less mature state, down-regulation of these important cell survival genes may make adipocytes much more susceptible to apoptosis. Thus, it is possible that the increase in adipocyte apoptosis that occurs with leptin treatment is not a specific effect of leptin, but is the end result of a cascade of metabolic and transcriptional changes.

SUMMARY

The discovery of leptin and its receptors provided a molecular basis for the lipostatic theory of body energy balance regulation (67), triggering an explosion of research in the field. Leptin's effects have been shown to cover a broad spectrum of metabolic, neuroendocrine, and behavioral systems, the functions of which are closely tied to nutritional status (44). Humans and animals with leptin deficiency due to a genetic defect exhibit a similar range of abnormalities, including marked obesity, hyperphagia, and pituitary deficiency, and administration of exogenous leptin in physiological amounts results in reduction of food intake and body weight (40, 89, 97). However, the role of leptin in obesity of nongenetic origin is unclear. Leptin synthesis and secretion increase as fat stores increase, which suggests that in non–leptin-deficient obese individuals, a state of relative leptin resistance can develop (14). Although potential commercial applications for the peptide have focused on its use in decreasing body fat stores, leptin's primary physiological role may be as a signal of sufficient, rather than excess, energy stores.

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