LEPTIN—MUCH MORE THAN A SATIETY SIGNAL

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■ **Abstract** Much attention has focused on the effects of leptin as a central satiety agent. There is now a significant amount of evidence that leptin is active in the periphery. This review focuses on the ability of leptin to modify insulin sensitivity, tissue metabolism, stress responses, and reproductive function. Leptin's effect on several of these systems is mediated via the hypothalamic-pituitary axis. Therefore, although in vitro studies provide evidence for direct effects on specific tissues and metabolic pathways, it is essential to consider the interactions between leptin and other regulatory factors in vivo. Little is known about the regulation of peripheral receptor expression or the production of binding proteins. Both of these factors determine the bioactivity of circulating leptin and have the potential to induce a peripheral resistance to leptin, similar to the central "leptin resistance" observed in obese subjects. Future research will clarify which of the endocrine and metabolic actions of peripheral leptin are of physiological relevance and which should be considered a pharmacological manipulation.

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INTRODUCTION

In 1953, Kennedy (116) proposed that body energy stores were regulated by a fat-derived, lipostatic, negative feedback signal acting centrally to inhibit feeding. The presence of such a factor was confirmed by parabiosis when the normal partners of hypothalamically lesioned, obese rats stopped eating and lost weight (99). Parabiotic partners share a common blood supply, and manipulation of one parabiont causes a response in the partner if circulating factors are an essential component of the activated pathway. Parabiosis identified mouse mutations that abolished the signal (ob/ob mice) or caused insensitivity to the signal (db/db mice) (53, 54). In 1994, leptin was identified as the mutated protein in ob/ob mice (240). The leptin receptor has multiple isoforms but only the subtype with a long intra-cellular domain (Ob-Rb) was considered to be capable of signal transduction (42). In db/db mice, an early stop codon renders Ob-Rb unresponsive (42). Many investigators have focused on leptin's function as a satiety signal. It is clear, however, that leptin can modulate, or regulate, multiple systems.

Leptin is expressed in the stomach (6), placenta (138), and muscle (230) but adipose tissue is the primary source of circulating leptin (240). The positive correlation between percentage of body fat and circulating leptin (57) and the profound anorexia caused by centrally administered protein (232a) support leptin's role as a lipostatic factor. In contrast, increasing peripheral leptin, by infusion or adenovirus-mediated expression, causes only a transient reduction in food intake (95, 127, 140), and high levels of leptin in obesity fail to inhibit food intake (57). Obese ob/ob mice are an exception, showing a sustained sensitivity to leptin (95, 140, 175). The dissociation between circulating leptin and satiety in other forms of obesity has been attributed to rate-limited transport of leptin across the blood brain barrier preventing activation of hypothalamic Ob-Rb receptors (35). However, short-form leptin receptors (Ob-Ra), putative transport proteins at the blood-brain barrier, increase with high-fat feeding (19) even though diet-induced obesity leads to "leptin resistance." Regulation of receptor expression (16) may be an important determinant of leptin sensitivity, as it has been reported that leptin is expressed in the brain (159). Resistance has been attributed to a postreceptor inhibitor of signaling (SOCS3), but leptin elevates this inhibitor in ob/ob mice (18), which do not become resistant (95, 140, 175).

Because leptin is secreted by adipocytes and the satiety response to peripheral leptin is transient (95, 140), the protein may have primary functions unrelated to satiety. This review focuses on leptin action in the periphery, examining its relationship with insulin, nutrient utilization, the stress response, and reproductive function.

THE RELATIONSHIP BETWEEN LEPTIN, INSULIN, AND INSULIN SENSITIVITY

The Effect of Leptin on Insulin Secretion

Leptin expression and secretion correlate with percentage of body fat (57). Obesity increases risk for insulin resistance and subsequent non-insulin-dependent diabetes. There is also substantial evidence for a direct interaction between leptin and insulin. Pancreatic islets express both Ob-Rb and Ob-Ra receptors (74, 104), with Ob-Ra accounting for 75%–90% of the population (104, 172). The receptors are present on insulin secreting β-cells and somatostatin δ-cells but not glucagon-containing α-cells (117). Investigation of the effect of leptin on insulin secretion in vitro has produced variable results. One study found that physiological concentrations of leptin stimulated basal insulin release from rat islets and a β-cell pancreatic cell line but had no effect on glucose-stimulated release (212). Others found no change in basal or glucose-stimulated insulin secretion from perfused rat pancreas (134, 179) or from islets from ob/ob mice exposed to higher concentrations of leptin (45). In contrast, a majority of reports demonstrate inhibition of insulin secretion by leptin. In human islets and a mouse insulinoma cell line, low concentrations of leptin rapidly inhibited basal insulin release (129). However, rat islets only responded in hyperglycemic conditions. There was a U-shaped response, with inhibition of insulin release by 10 nM leptin but not by 1 or 100 nM leptin (172). In genetically obese rodents, leptin inhibited insulin release from islets and pancreas of ob/ob mice but not db/db mice or the leptin-insensitive fa/fa Zucker rat, implicating Ob-Rb in the response (74, 117). Despite the presence of receptors on δ-cells, leptin had no effect on somatostatin release (117).

The discrepancies between studies may be attributed to different leptin preparations or involvement of multiple signaling pathways controlling Ca^{2+} flux. Islets from ob/ob mice are hypersensitive to acetylcholine (43) and glucose and are hyperpolarized because of abnormal regulation of ATP-sensitive K^+ channels (186). In these islets, leptin activated K^+ channels to increase membrane conductance (117) by an insulin-sensitive phosphatidylinositol-3 kinase (PI-3K)–dependent mechanism (96) that may be specific for ob/ob mice, as there is no effect of leptin on K^+ channels in rat islets (171, 179). Leptin also reversed the hypersensitivity to acetylcholine (43) by inhibiting activation of protein kinase C (44). Protein kinase C and cAMP-protein kinase A are also inhibited in rat islets (171, 179). In islets from neonatal rats and a pancreatic cell line, leptin inhibited glucose-stimulated insulin secretion, increased phosphodiesterase 3B activity, and increased PI-3K activity fivefold. The inhibition of insulin secretion was blocked by either phosphodiesterase or PI-3K inhibitors (241).

The relevance of in vitro observations to the in vivo situation remains to be determined. Leptin reverses hyperinsulinemia in ob/ob mice, but at higher concentrations than are required for normoglycemia (175), which suggests improved insulin sensitivity. We demonstrated an inhibition of glucose-stimulated insulin

release in leptin-treated mice (94), but others have shown that depletion of intracellular triglycerides in hyperleptinemic rats prevents normal islet function (127, 198). Therefore, inhibition of insulin release in vivo may be secondary to the metabolic state of the islets, rather than a direct action of leptin. The in vitro studies examined acute responses, when insulin secretion was determined by intracellular calcium. Extended exposure to leptin has been reported to inhibit insulin mRNA expression (172); however, there is no evidence for insulin insufficiency in vivo. Twelve months of leptin treatment caused weight loss but did not correct hyperinsulinemia in a human subject with congenital leptin deficiency (79), and in normoglycemic subjects, there was a positive correlation between fasting concentrations of leptin and insulin (90, 243), independent of body mass index (BMI) or waist-to-hip ratio.

The Effect of Insulin on Leptin Expression and Secretion

In rats, there is a diurnal cycle of leptin expression, with a nocturnal peak that is abolished by food deprivation but is restored within 4 h of refeeding (191). The stimulation of leptin is insulin dependent, as food deprivation does not change already low levels of leptin expression in insulin-deficient streptozotocin-treated rats (174), but insulin replacement rapidly restores leptin to normal (146). In fasted mice and rats, injections of glucose or insulin rapidly stimulate leptin expression (157, 191), but there is no effect in hyperinsulinemic mice (157). In humans, the nocturnal rise in circulating leptin shifts when meal times are delayed (193), even though food intake has no immediate effect on leptin concentrations (57, 147).

In vitro, pharmacological concentrations of insulin caused a rapid, but transient, stimulation of leptin expression and secretion by rat adipocytes (10). Hyperinsulinemia increased leptin mRNA expression in human adipocytes after 72 h, but this was reversed by 96 h even though leptin release remained elevated (124). Others reported an increase in leptin expression in human adipocytes exposed to insulin, and the response was substantially augmented by the presence of cortisol, which suggests that the two hormones could act synergistically during the development of obesity (227). Insulin promotes transcription of the leptin gene (139) and relocation of intracellular protein from the endoplasmic reticulum toward the cell membrane (10), but the delayed response may be secondary to a tropic effect of insulin on adipocytes, providing an in vitro model of overfeeding (124). This concept is supported by observations that the increase in leptin expression was prevented by blocking glucose transport (163).

In vivo, the time course of insulin stimulation of leptin is dose dependent. During a hyperinsulinemic, euglycemic clamp, using a pharmacological level of insulin, leptin concentrations in rats increased within 2 h. When insulin represented only three times the basal concentration, leptin expression was doubled after 2 days in one experiment (62) and in another plasma leptin was not increased until day 5 (126). In human subjects with a wide range of body fat content and insulin sensitivity, hyperinsulinemic, euglycemic clamps of short duration (2–5 h) show no effect of insulin on leptin (65, 124, 132). An acute response was initiated only

when circulating insulin increased from 32 to 823 pmol/liter (217). In two studies with 9-h infusions, serum leptin progressively declined in a saline control group, whereas infusions that tripled fasting insulin and inhibited lipolysis prevented the fall in leptin, and high concentrations of insulin increased leptin (148, 189). This effect could not be attributed to a change in free fatty acids (FFA), as there was no response to a FFA infusion with normal levels of insulin (20). Moderate amounts of insulin stimulate leptin when the hyperinsulinemia is maintained for several days (20, 124), indicating that, under physiological conditions, insulin does not have a direct effect on leptin expression and secretion. It is more likely that hyperinsulinemia promotes fat deposition, which subsequently increases leptin expression. However, one piece of in vivo evidence supports a chronic regulatory effect of insulin on leptin production. Insulinoma patients were hyperinsulinemic, hypoglycemic, and hyperleptinemic compared with BMI-matched controls, until their tumors were removed, when insulin and leptin returned to normal levels (64).

The Relationship Between Leptin and Insulin Sensitivity

During an extended hyperinsulinemic clamp, the diurnal rhythmicity of leptin release was exaggerated and cyclic changes in leptin and insulin resistance were synchronized but opposite, implying that leptin caused insulin resistance (20). Additionally, in healthy children and adults (90, 111, 132, 243), obese individuals (110, 111), or those who have insulin-dependent or non-insulin-dependent diabetes (65), there is a positive correlation between fasting leptin and insulin concentrations, independent of body fat content. Two studies (3, 132) demonstrated a significant correlation between fasting leptin and insulin resistance in healthy postmenopausal women after controlling for body fat content. Leptin correlates with islet secretion of insulin and glucagon in fasting conditions, in response to arginine, and with insulin released in response to a glucose challenge. In contrast, there is no correlation with secretion of a third islet protein, pancreatic polypeptide, or with glucagon secreted during hyperglycemia. These results led to the suggestion that leptin may be a signal from adipocytes to islets to hypersecrete insulin when fat content is increased and insulin sensitivity is lowered (132). The relationship between leptin and islet secretory function is lost in subjects in whom islet compensation for insulin insensitivity has failed (132), and leptin is inappropriately low in subjects with poorly controlled diabetes and deterioration of β-cell function (48).

The positive correlation between leptin and insulin secretion in vivo is in direct opposition to evidence of inhibition of insulin secretion in vitro, which suggests an overriding influence of additional regulatory factors in vivo. The relationship between leptin and insulin is further confused by the effect of exogenous leptin on insulin sensitivity in experimental animals. We demonstrated that insulin release in response to a glucose challenge was exaggerated 2 h after a leptin injection but was blunted in mice infused with leptin for 5 days (94). In ob/ob mice, which are

hypersensitive to leptin (95, 140), leptin treatment reverses hyperglycemia before there is any change in insulin or body weight, indicating either improved insulin sensitivity or that leptin has insulin-like activity (175). In support of the latter concept, high-dose leptin infusions in insulin-depleted and insulin-resistant streptozotocin rats prevented weight loss, normalized fasting glucose, and increased whole body glucose utilization during a hyperinsulinemic clamp (46). Lower doses of leptin partially normalized blood glucose and prevented hyperphagia (203). Obviously, a great deal more work is needed to clarify the relationship between leptin and insulin sensitivity, in both pharmacological and physiological conditions.

LEPTIN AS A GROWTH FACTOR

Leptin and Cell Proliferation

A majority of immune and blood cells are derived from undifferentiated hematopoetic stem cells that are found in the yolk sac, spleen, and bone marrow of the fetus. In adults, they are only in bone marrow, which also contains adipocytes. Primary culture of human bone marrow stromal cells showed that the adipocytes express exceptionally high levels of leptin (130), which plays a direct role in hematopoesis. The adipocytes require glucocorticoids to differentiate but do not respond to insulin and do not express either β3-adrenergic receptors or UCP1 (130). Leptin receptors Ob-Ra, Ob-Rb, and a novel leptin receptor, with an intermediate-length cytoplasmic domain, are found in fetal stem cells (14, 47). In cell culture, leptin stimulated proliferation of stem cells, acting synergistically with other growth factors (14). An essential role for leptin in cell proliferation in vivo was demonstrated by abnormal erythropoesis and lymphopoesis in db/db mice (14), and by overexpression of leptin receptors in cells from patients with myeloid leukemia but not lymphoid leukemia. In these cells, leptin enhanced proliferative activity of other cytokines, such as IL-3, and inhibited apoptosis (125).

In a line of human marrow stromal cells, leptin did not change proliferation but promoted differentiation of osteoblasts and increased mineralization of matrix proteins with no effect on adipocyte differentiation (215). In vivo, there was a positive correlation between leptin and periosteal envelope expansion in premenarchal girls (154) and an inverse relationship between adipocytes and osteoblasts in osteoporosis (215); however, there was no correlation with markers for bone formation or resorption in adult women (184). This suggests that leptin has a role in bone formation during growth but is less important in maturity. In addition to its effect on hematopoetic cells, leptin stimulated proliferation of pituitary cells (109) of a pancreatic β-cell line (211) and in a mouse embryonic cell line (208). Leptin has been reported to promote angiogenesis (201) and may also be necessary for normal brain development (1). Proliferation is associated phosphorylation of mitogen-activated protein kinase (208, 211). Thus, the proliferative activity of leptin is not limited to a particular cell type and is mediated by postreceptor proteins that are also responsible for insulin's mitogenic activity.

The Relationship Between Leptin and Growth Hormone

Pituitary-derived growth hormone (GH) plays a key role in determining body composition. Pulsatile release of GH from the hypothalamus is stimulated by growth hormone releasing hormone (GHRH) and inhibited by somatostatin (SRIH). Many effects of GH are mediated by circulating insulin-like growth factor-1 (IGF-1), which is secreted from the liver under control of GH. IGF-1 inhibits hypothalamic GH release, and its bioactivity is regulated by circulating binding factors 1–6. In obesity, GH is suppressed and the response to pharmacological interventions that promote GH release is also blunted (192).

The inverse relationship between adiposity and GH implies that adipose-derived factors regulate GH secretion. Leptin receptors colocalize with GHRH-containing neurons in the arcuate nucleus of the hypothalamus (91) and have been identified in pituitary tissue (109, 199). However, any effect that leptin has on GH secretion appears to be mediated by hypothalamic neuropeptide Y (NPY). In vitro, high concentrations of leptin did not change basal or GHRH-stimulated GH release from anterior pituitary cells (50), whereas in vivo, central [intracerebroventricular (i.c.v.)] administration of a leptin antibody decreased the amplitude of pulsatile GH release in fed rats, and leptin restored GH release, but not IGF-1 expression, in fasted animals (36, 225). In fed rats, i.c.v. leptin stimulated pituitary GH and hypothalamic GHRH mRNA expression, inhibited hypothalamic expression of SRIH and NPY (50, 225), enhanced responsiveness to GHRH, and augmented GH secretion (213). It has been shown that NPY stimulates SRIH and inhibits GH release (185); therefore, leptin stimulation of GH is probably mediated by a down-regulation of NPY (225).

When considering the influence of GH on leptin secretion, GH infusions had no effect on leptin expression in hypophysectomized rats, although IGF-1 suppressed already-low levels of expression (22). In contrast, leptin expression was inhibited, without any change in fat mass, in fa/fa rats injected with GH for 7 days (106). This response could not be replicated by infusing IGF-1 (105), although high doses of IGF-1 reduced fat pad weight and leptin in normal rats (23). These studies indicate that GH acts indirectly, possibly through IGF-1, or by influencing insulin release and cell metabolism. The indirect relationship is also evident in human subjects. In prepubertal children given a GH stimulation test, leptin was inversely related to peak GH release, independent of body fat content (60). In both young and adult GH-deficient subjects, 4 weeks of GH replacement did not change leptin (169), which was elevated in parallel with body fat content. Despite the lack of response to sustained treatment, GH acutely stimulated leptin in GH-deficient subjects (169) and critically ill patients (218), but the change in leptin correlated with insulin or IGF-1, which has insulin-like activity (218). In contrast, GH treatment of obese men with IGF-1 deficiency temporarily stimulated energy expenditure and suppressed leptin (114). Therefore, there is little evidence that GH has a direct effect on leptin expression or secretion, although, via inhibition of hypothalamic NPY expression, leptin increases the tone of the GH axis. Thus, in the obese or

"leptin-resistant" state, inhibition of hypothalamic leptin receptor activity could suppress GH secretion. It has been reported that chronic exposure to high levels of GH increases pituitary expression of Ob-Rb (32); therefore, inhibition of GH in obesity could produce a reciprocal insensitivity to leptin.

Tissue-Specific Effects of Leptin on Energy Utilization

Weight loss in leptin-treated animals is almost exclusively from loss of adipose tissue, and correction of leptin deficiency reduces fat and causes a small decrease in body water but retains protein (79, 140, 175). This contrasts with loss of lean mass and preservation of fat during food restriction or stress (242). Little is known about the mechanisms protecting muscle mass. In a cell line of C_2C_{12} myotubules, which express only Ob-Ra receptors (17), leptin had no effect on protein synthesis but caused a small inhibition of protein breakdown (182). In the same cells, leptin produced a temporary protein kinase C– and PI 3K–dependent increase in basal glucose uptake and a sustained PI-3K–dependent increase in glycogen synthesis (17). Examination of proteins common to signal transduction for both insulin and cytokines indicated association of PI-3K with insulin receptor substrate-2 (IRS-2) (115) but not IRS-1 (17). There was also increased binding of PI-3K with janus kinase 2 (JAK2) but not JAK1. Therefore, leptin may augment, or mimic, insulin signaling downstream of PI-3K (115).

Cultured skeletal muscle cells express Ob-Rb receptors (165) and do not respond in the same manner as C_2C_{12} myotubules (183). Leptin stimulated glycogen synthesis in muscle from ob/ob mice (143) but had no effect in muscle from lean mice or rats (143, 165). High concentrations of leptin inhibited triglyceride synthesis and stimulated fatty acid oxidation, whereas physiological concentrations caused only small changes in lipid metabolism (165). Despite this minimal response in vitro, i.c.v. or peripheral leptin infusions increased muscle glucose uptake and glycogen content in mice and rats (63, 112). These responses were mediated centrally, as denervation blunted the response in oxidative muscle and abolished it in glycolytic muscle (112). Recently, it was reported that glucosamine, the product of muscle hexosamine synthesis and an index of energy availability, or feeding a high-fat diet (231) induced leptin expression in skeletal muscle, stimulated adipose leptin expression, doubled circulating leptin, and induced peripheral insulin resistance (230). Therefore, if leptin stimulates fatty acid oxidation, it may regulate muscle metabolism to match nutrient availability.

The specific loss of fat in leptin-treated animals is caused by inhibition of lipogenesis and promotion of fatty acid oxidation. Experiments involving gold thioglucose–lesioned mice or transplantation of fat into hyperleptinemic rats indicate that neural input is not essential for leptin action on adipocytes (30, 234), which express both Ob-Rb and Ob-Ra receptors (118). Several investigators found no effect of leptin on glucose uptake or lipolysis in adipocytes or 3T3-L1 cells (155, 183). In contrast, Muller et al (164) reported that a 6-h exposure to leptin inhibited insulin action in cultured adipocytes. Inhibition was reversible and

specific, as there was no change in vanadate stimulated lipogenesis. The inhibition of glucose transport (239) and lipogenic enzymes (232) has been confirmed in adipocytes and in a preadipocyte cell line transfected with the leptin gene (7). The response to leptin is faster in pieces of adipose tissue (200) and in vivo (30, 84) than in vitro, and the acute response to leptin in isolated adipocytes is different from that in cells from leptin-treated mice (94), implying mediation by intermediary paracrine factors. Glycerol release from leptin-treated adipocytes has been interpreted as a stimulation of lipolysis (164); however, circulating FFAs are not elevated in hyperleptinemic rats (198) because they are oxidized and not released from adipocytes (232). Leptin does not stimulate glycerol release in adipocytes from db/db mice (84) or fa/fa rats (232), implicating Ob-Rb in the response. Some of the fat loss can be attributed to uncoupling proteins (UCP), which dissociate oxidative metabolism from ATP synthesis, as discussed elsewhere (127a). UCPs were decreased in fat, muscle, liver, and brown adipose tissue of mice pair fed to leptin-treated animals, but i.c.v. leptin prevented the down regulation in all tissues, favoring energy expenditure in a situation of reduced energy intake (63).

Whole-body glucose turnover is stimulated by leptin, primarily through regulation of hepatic glucose metabolism. Infusion (i.c.v. or peripheral) of leptin into conscious mice increased glucose turnover, promoted glucose uptake into muscle and brown fat, reduced liver glycogen content, and doubled whole-body glycolysis (112). In a similar study, low concentrations of leptin-infused i.c.v. did not change insulin-stimulated glucose clearance or glycolysis, but it inhibited hepatic glycogen synthesis and stimulated glucose production by stimulating phosphoenolpyruvate carboxykinase (PEPCK), the rate-limiting enzyme in gluconeogenesis. The ratio of glycolysis to gluconeogenesis determines the malonyl CoA concentration, which is rate limiting for triglyceride reesterification; therefore, leptin would promote fatty acid oxidation by inhibiting triglyceride formation (187). Identical responses were obtained with a β3-adrenergic receptor antagonist, implying that the sympathetic nervous system mediated the changes in hepatic glucose production (142). Peripheral leptin produced similar effects (187), possibly by acting centrally. In contrast, leptin inhibited PEPCK expression in ob/ob mice (31). This may have been secondary to inhibition of arcuate nucleus NPY expression (195), which would have the same end result (153).

The studies described above infused leptin for periods of hours, but extended treatment produces a different hepatic response. Eight days of peripheral leptin infusion reduced adiposity and enhanced insulin action, compared with pair-fed rats. Whole-body and liver glycogen synthesis were increased, as was hepatic glycolysis, but glucose production was reduced because of almost total inhibition of glycogenolysis despite increased PEPCK expression and gluconeogenesis (12). In a second study, which compared rats that lost weight during food restriction or during infusion of either leptin or a β3-adrenergic receptor agonist for 8 days, only leptin stimulated glycolysis, gluconeogenesis, and PEPCK expression (11). In addition, glycogen was preserved longer in fasted hyperleptinemic rats than in control rats, and glycogen accumulated faster following glucose injection

(170). Thus, chronic treatment with exogenous leptin promotes glucose oxidation and maintenance of glycogen stores simultaneously, while stimulating fatty acid oxidation and inhibiting lipid accumulation in adipose tissue.

Although changes in liver metabolism may be initiated, in part, in the brain, there is evidence for a direct effect on hepatocytes. In human hepatoma cells that express Ob-Rb, leptin rapidly inhibited phosphorylation of IRS-1 but increased its association with PI-3K. In a rat hepatoma cell line, leptin stimulated PEPCK expression and partially reversed insulin inhibition, similar to the response in vivo (51). In a different study, using rat cells transfected with Ob-Rb cDNA, leptin did not phosphorylate IRS-1 or IRS-2 or increase association of PI-3K with IRS-1, but it did increase PI-3K binding to IRS-2 and activated STAT3. These responses were not replicated in human hepatoma cells transfected with Ob-Rb cDNA (233), illustrating the limitations of cell lines that do not contain all of the signaling machinery present in hepatocytes. In an isolated liver preparation, perfusion with insulin or leptin inhibited glucose production, but, in glycogen-depleted livers, leptin increased glycogen synthesis and increased lactate uptake (167). Cohen et al (52) compared livers from leptin-treated ob/ob mice with those exposed to leptin in vitro. In vivo treatment caused a much greater increase in glycogen synthesis and inhibited fatty acid synthesis, whereas fatty acid synthesis was stimulated in vitro. These results demonstrate that leptin influences hepatic metabolism directly, by modifying tissue enzymes, and indirectly, by changing neural and hormonal input to the organ.

LEPTIN AND STRESS

Leptin and the Hypothalamic-Pituitary-Adrenal Axis

Stress responses are initiated by activating corticotrophin releasing factor (CRF), which recruits endocrine, immune, neural, and neurochemical systems. The type and degree of stress determines the outcome, but consistent responses are stimulation of the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system (SNS). Leptin has been shown to modulate HPA axis activity (97), SNS activity (55), and release of proinflammatory cytokines (81), implying that it has the potential to modify the stress response (24, 97). However, evidence for this is inconsistent. Third ventricle infusion of leptin at the start of the dark period, when leptin is low and corticosterone is rising, caused a delayed but extended stimulation of corticosterone, insulin, and leptin (219). In contrast, a peripheral injection of leptin blunted the corticosterone and adrenocorticotropic hormone (ACTH) response to food deprivation or immobilization stress (2, 97), but this was not replicated with infusion of smaller quantities of leptin (103). In vitro, leptin inhibited CRF release from hypoglycemic hypothalamic tissue (97), but CRF release from perfused tissue was stimulated within 15 min (181) and ACTH release from perfused pituitary tissue increased after 45 min (181). These conflicting results may be attributed to experimental conditions, or they may demonstrate that leptin

inhibits stress-induced release of CRF and corticosterone but maintains tone of the HPA axis in nonstress conditions.

Arcuate nucleus proopiomelanocortin (POMC) expression is suppressed in ob/ob and db/db mice and is stimulated by leptin in ob/ob mice and in fasted rats (158, 196). POMC is the precursor for several peptides, including β-endorphin, which inhibits hypothalamic CRF release, and α-melanocyte–stimulating hormone, which contributes to the regulation of food intake, immune function, and aspects of reproduction. Leptin increases POMC expression but it is not known whether posttranscriptional control promotes production of specific peptides. Wand et al (229) proposed that leptin inhibited the HPA axis via release of β-endorphin, which inhibits hypothalamic CRF secretion. Thus, chronic exposure to leptin would down-regulate the response to stress by increasing opioid tone.

In contrast to stimulation of CRF and ACTH release, leptin inhibits glucocorticoid release from the adrenal cortex in vitro. Leptin receptors, a majority of which are Ob-Ra (33), have been identified in adrenal medulla and cortex (33, 86). Autoradiographs show dense leptin binding to the medulla but not the cortex, correlating with receptor distribution (86). Leptin produces a dose-dependent inhibition of basal and ACTH-stimulated cortisol secretion in cultured adrenal cells (26, 86), by inhibiting enzymes in the steroid synthetic pathway (86). Leptin exerts an equal, or greater, inhibition of aldosterone but has no effect on catecholamine release from cultured adrenal chromaffin cells in either basal or ACTH-stimulated conditions (86), except when excessive concentrations of leptin are used (209). Stimulation of CRF and ACTH secretion with an inhibition of glucocorticoids would permit leptin to accentuate central actions of CRF independent of an HPA endocrine response. Additionally, the balance between SNS and glucocorticoid activity within the adrenal gland determines the concentrations of neurotransmitters, cytokines, and growth factors that modulate hormone release (27).

Glucocorticoids are more potent regulators of leptin than either CRF or ACTH. In vitro, dexamethasone and cortisol stimulated leptin expression in adipocytes (56, 204, 227). In incubated adipose tissue, leptin secretion correlates with adipocyte size and is greater in subcutaneous than omental fat (220) and in fat from women than from men (37). Fat from obese subjects gives an exaggerated response to dexamethasone because of increased sensitivity rather than increased receptor binding (92). In one study, both dexamethasone and insulin stimulated leptin expression in visceral and subcutaneous fat (92). The response was partially prevented by inhibiting translation or by hyperinsulinemia and was totally blocked by inhibiting protein synthesis (92). Another study (188) found depot-specific responses in fat from obese subjects. Leptin mRNA expression was transiently stimulated by dexamethasone in both subcutaneous and omental fat and was augmented by insulin but blocked by a transcription inhibitor. However, leptin secretion increased only in subcutaneous tissue. Thus, insulin and glucocorticoids may work in concert to promote leptin release in conditions such as obesity, but the magnitude of response may be determined by the size of specific fat depots.

In vivo, daily injection of glucocorticoids, at doses that inhibited food intake and weight gain, increased leptin expression fourfold in rats (68), but a 3-week infusion caused weight loss and inhibited expression, indicating that fat mass is the primary determinant of leptin expression. Replacement of corticosterone in adrenalectomized rats prevented a drop in leptin expression but had no effect in sham-operated rats (210). In contrast, dexamethasone doubled leptin expression and circulating leptin in humans (66, 156, 173). Although these experiments were criticized for using pharmacological doses of dexamethasone (216), others have shown a 50%–100% increase in circulating leptin within 24 h of administration of a physiological dose of cortisol (168) or dexamethasone (73, 173). With continued administration, leptin returned to baseline (168), which suggests that it facilitates the early stages of a chronic stress response, whereas the 24-h delay implies that acute stress would have no effect on leptin release.

Despite experimental evidence, the in vivo leptin response to stress is minimal. A variety of situations that activate the HPA axis, or chronically elevate cortisol, show no correlation between leptin and glucocorticoid concentrations. There is no evidence of inappropriate levels of leptin in Cushings patients (141). Similarly, activation of the HPA axis by CRF (141), by CRF and arginine vasopressin (223), or by opioid blockade (229) has no effect on leptin, despite substantial elevation of ACTH and corticosterone. Additionally, an ACTH challenge had no effect on leptin (229), indicating that the inverse relationship between diurnal rhythms of ACTH and leptin release were not due to ACTH inhibiting leptin secretion (141). Torpy et al (216) suggest that leptin secretion responds only to inappropriately high doses of synthetic glucocorticoids. However, glucocorticoid release is normally pulsatile and any event that elevates glucocorticoids also activates other systems, such as the SNS, that may antagonize stimulation of leptin expression and secretion.

Leptin and the Inflammatory Response

The inflammatory response includes activation of the HPA axis, cytokine mediated fever, protein catabolism, hypoglycemia, and hypoinsulinemia. Involvement of leptin is not surprising, as it has the tertiary structure of a long-chain helical cytokine (238), and Ob-Rb belongs to the family of class I cytokine receptors (214). However, signal transduction by Ob-Rb is distinct, as it does not oligomerise with gp130 or leukemia inhibitory factor receptor (166). In HepG2 cells transfected with the Ob-Rb gene, leptin caused phosphorylation of signal transducer and activation of transcription (STAT) factors and induced gene expression through interleukin-6 (IL-6) response elements (13). These results demonstrate that leptin has cytokine activity in cells that express appropriate postreceptor signaling proteins and response elements.

Impaired T-cell immunity in ob/ob and db/db mice provides evidence for leptin's involvement in immune function (39, 80). In both genotypes, there is a diminished in vivo response to cell-mediated and humoral challenges (39, 80). Leptin may also provide the link between immunosuppression and malnutrition, as it

reversed starvation-induced suppression of cellular immune responses (145). In the cell-mediated immune response, antigens are presented to T-cells, which release lymphokines that activate macrophages. T-cells, rather than antigen presenting cells, responded to leptin by showing an increased rate of proliferation and production of proinflammatory cytokines (145). In addition to this indirect activation of macrophages, leptin acted directly via Ob-Rb receptors to increase the number of macrophages showing phagocytic activity (85, 144). It did not stimulate cytokine production directly, but enhanced endotoxin-induced production of tumor necrosis factor α (TNFα), IL-6, and IL-12 by a posttranscriptional mechanism (144).

In vivo, leptin normalized the hypersensitivity of ob/ob mice to the endotoxin lipopolysaccharide (LPS) but did not increase resistance in lean mice. The protective effect must be mediated by Ob-Ra, as db/db mice have a normal sensitivity to LPS (76). fa/fa rats are insensitive to leptin and showed a suppressed cytokine response to an LPS challenge; however, there was increased liver damage, attributed to cytokines that sensitize hepatocytes to TNFα and to decreased phagocytic activity by Kupffer cells (236). Many responses to LPS are mediated by TNFα, which is lethal for ob/ob and db/db mice at doses lower than that for lean mice. Leptin normalized sensitivity in ob/ob mice but did not increase resistance in lean mice (207). The protective effect of leptin may be dependent on stimulation of POMC and subsequent release of α-melanocyte–stimulating hormone and glucocorticoids, both of which inhibit TNFα activity (207). Alternatively, it may be attributed to uncoupling proteins. LPS inhibited UCP-2 expression and doubled superoxide anion production in peritoneal macrophages (59), affecting the binding of oxidative-sensitive transcription factors to DNA. Macrophages from ob/ob mice had low levels of UCP-2 expression, high rates of superoxide anion production, and increased binding between the oxidative-sensitive transcription factor C/EBP-β and DNA.

In addition to leptin promoting secretion of inflammatory cytokines, LPS stimulates leptin expression and secretion, despite a simultaneous inhibition of food intake (89). The leptin surge that followed LPS injection initially correlated with inflammation and peaked at 12 h but was reversed within 48 h (160). Two proinflammatory cytokines, TNFα and IL-1β, mediated this response. TNFα stimulated leptin expression and secretion from adipocytes in vitro and increased circulating leptin in vivo (81). Adipocytes from TNFα knockout mice have increased levels of leptin expression but reduced circulating concentrations of protein, implying that TNFα actively stimulates leptin secretion (119). The stimulatory effect of IL-1β has been demonstrated in vivo using turpentine inflammation, which promotes secretion of IL-1β and IL-6 but not TNFα or IL-1α. Turpentine and LPS induced adipose leptin expression and increased serum leptin concentrations in wild-type and IL-6 knockout mice but had no effect in IL-1β knockouts (75). These results suggest that TNFα is an intermediary, promoting IL-1β, which is responsible for the stimulation of leptin. Cytokine-induced expression of leptin in humans has to be confirmed. Leptin was elevated in patients with acute sepsis, and survivors had

higher average levels of leptin than those who did not survive (25), but leptin was normal in patients who had been septic for at least 14 days (34). In cancer patients given daily infusions of IL-1α (107) or TNFα (244), leptin stimulation was transient. Therefore, the positive feedback loop between leptin and inflammatory cytokines may augment early responses to infection, but leptin is not important in the sustained immune response.

Leptin and Energetic Stress

Starvation, or energy restriction, is associated with a greater fall in leptin than can be accounted for by loss of fat mass (235), especially in women (71). During food restriction, leptin correlated negatively with a metabolic index that represented decreased glucose availability and increased lipolysis (71), which suggests that it was regulated by the metabolic state of cells. When the fall in glucose and insulin during food restriction was prevented (21), or if glucose was infused to prevent ketogenesis (123), circulating leptin concentrations did not change. On the other hand, ketone infusion did not inhibit leptin concentrations (123). Therefore, inhibition of leptin during fasting may be associated with adipocyte metabolism but is not directly caused by ketosis (123). This hypothesis is supported by observations that leptin fell rapidly during exercise in fasted subjects (122) and in type 1 diabetics during insulin withdrawal (4), situations that are metabolically stressful to adipocytes.

In other situations of negative energy balance the effects are inconsistent. Infusion of a β-agonist stimulated lipolysis and inhibited leptin, despite a significant increase in insulin. The response was rapidly reversed at the end of infusion, implicating the SNS (177). In trained rats exposed to exhaustive exercise, circulating leptin did not change but leptin mRNA expression was inhibited because of β3-adrenergic receptor activation (29), demonstrating a dissociation between leptin expression and circulating leptin. Leptin was decreased in marathon or ultramarathon runners (131, 133), but this may have been secondary to loss of fat. Exhaustive exercise had no immediate effect on leptin in trained individuals (100), and exercise training did not change leptin once body fat had been taken into account (101, 176), but 2 h after an exercise bout, glycerol and FFA were increased and leptin was decreased (72). The dissociation between leptin expression and circulating leptin may result from inhibition of leptin clearance. Alternatively, as exercise activates the SNS and β-adrenergic agonists inhibit leptin expression while promoting lipolysis in adipocytes (151), it is possible that leptin secretion is actively stimulated during exercise. Following exercise, the decline in leptin would reflect the inhibition of leptin expression.

Leptin and the Hypothalamic-Pituitary-Thyroid Axis

Thyroid hormone concentrations also decrease in response to food restriction. The hypothalamic-pituitary-thyroid (HPT) axis regulates circulating concentrations of thyroid hormones and binding proteins. The hypothalamus produces

thyroid releasing hormone (TRH) that regulates secretion of thyroid stimulating hormone (TSH) from the pituitary gland. Thyroid hormones stimulate basal metabolic rate and increase heat production, in part, through regulation of mitochondrial UCPs (87, 202). Starvation is associated with low concentrations of hypothalamic pro TRH, low or normal TSH in the pituitary, and low-circulating triiodothyronine (T3) and thyroxine (T4). The relationship between leptin and the HPT axis was first observed when leptin blunted the reduction in thyroid hormones in fasted mice (2). In a second study, with rats repeatedly treated with leptin during a 3-day fast, weight loss was not prevented, but total and free T4 and T3 and hypothalamic pro-TRH mRNA levels were maintained (135). Leptin receptor mRNA is expressed in arcuate nucleus NPY containing neurons that synapse on TRH neurons in the paraventricular nucleus of the hypothalamus (91), therefore it was hypothesized that maintenance of pro-TRH was mediated by NPY (135). This is supported by observations that rats with arcuate nucleus damage had low T4, TSH, and leptin, but high corticosterone, compared with fed, control animals. Fasting these animals did not change pro-TRH, and leptin had no effect on the thyroid axis (136). In nonstress conditions, leptin may provide tonic regulation of thyroid hormones. The small number of humans with genetic leptin deficiency or insensitivity have normal concentrations of thyroid hormones but elevated TSH, indicating abnormal hypothalamic regulation of the thyroid axis (49). In obese subjects, the TSH response to TRH is increased (70), and there is a positive correlation between body fat content and TSH levels (178).

Evidence that thyroid hormones influence leptin is weak. Leptin expression and secretion in adipocytes from hypothyroid and euthyroid rats were not different (77), although T3 and T4 inhibited leptin expression and serum leptin concentrations in hypothyroid rats (78). In humans, there were no changes in leptin after a week of T3 treatment that increased heart rate and inhibited TSH (152). Evaluation of leptin status in hypo- and hyperthyroid patients showed no change in hyperthyroid patients but significant increases in the hypothyroid patients (137). There was no relationship between leptin and thyroid status when leptin was corrected to BMI (58), but BMI underestimates body fat in hyperthyroid subjects who have low concentrations of leptin relative to body fat mass (178). As thyroid hormones potentiate the effects of sympathetic activation, the inverse relationship between leptin and thyroid status may be secondary to increased catecholamine sensitivity.

LEPTIN AND REPRODUCTIVE FUNCTION

Leptin and the Hypothalamic-Pituitary Gonadal Axis

There is an optimal range of adiposity for fertility (83), and malnutrition—including starvation, obesity, and type I diabetes—disrupts reproduction at the hypothalamic/pituitary level. Leptin provides a link between fat and the hypothalamic-gonadal axis. Leptin mutation in humans causes obesity and hypogonadism (205),

and ob/ob and db/db mice are infertile because of hypothalamic-pituitary dysfunction (206), but leptin restored fertility in ob/ob mice (9, 40, 161). The effect on sexual maturation may be effected at both the hypothalamus and the gonads. Centrally, receptors do not colocalize with gonadotrophic releasing hormone in the hypothalamus; therefore, leptin must act indirectly in the arcuate nucleus, either recruiting POMC derivatives (61) or inhibiting NPY-containing neurons (5). In vitro, leptin stimulated secretion of rat hypothalamic gonadotrophic-releasing hormone, and of pituitary gonadotropins, follicle-stimulating hormone, and luteinizing hormone (LH) (237), and in vivo, it prevented inhibition of LH pulses during fasting (82). In the periphery, leptin receptors are expressed at high levels in reproductive organs (47). In vitro, leptin augmented estradiol release from leutinized ovarian granulosa cells in basal conditions and in cells stimulated by follicle stimulating hormone and IGF-1, increasing activity of aromatase enzymes (120). There was no effect of leptin in the absence of LH (113), implying that any effect on ovarian cells requires a functional hypothalamic-pituitary axis.

There is also evidence that gonadal steroids influence leptin release and activity. Plasma leptin is higher in women than in men for any given percentage of body fat (190). In children and adults, leptin is negatively associated with testosterone in males and positively correlated with estrogen in females, after accounting for fat mass (67, 226). In women, leptin fluctuations during the menstrual cycle correlate with estrogen, but they have no relationship with progesterone (149, 180). However, the gender difference is not a simple effect of estrogen, as leptin is the same in pre- and postmenopausal women and does not change with hormone replacement therapy (38). The gender difference is replicated in vitro. Leptin release is greater in fat from females than from males (37), and fat from females who still have ovaries is more responsive to dexamethasone and estradiol (37, 128), but in women who have undergone ovariectomies, the difference is abolished (128). In contrast, testosterone has been reported to inhibit (226), or to have no effect (128) on, leptin release. Estrogen can also influence leptin activity at the receptor level. In rats, all hypothalamic cells immunoreactive for estrogen receptors also expressed leptin receptors (69), and estrogen treatment of intact female rats down-regulated Ob-Ra receptors in all brain areas, but Ob-Rb was inhibited only in the hypothalamus. Ovariectomy did not change Ob-Rb but it increased Ob-Ra receptor expression (15). Although circulating leptin did not change during the estrous cycle, regulation of the receptor was not limited to pharmacological conditions because arcuate nucleus Ob-Rb expression was highest during estrous and metestrous and was inversely correlated with NPY expression (16), providing a potential mechanism for cyclic variations in energy intake and activity.

Leptin as a Signal for Sexual Maturation

The link between adiposity, leptin, and puberty is demonstrated by early puberty of obese children (121). In nonobese children, leptin triples during puberty in

females, whereas in males there is a prepubertal surge in leptin, which declines at the onset of puberty (150). At the end of puberty, leptin correlates negatively with LH and follicle-stimulating hormone in girls but there is no relationship with gonadotropins in boys (28). In adult males, testosterone and leptin are inversely correlated (222) and suppression of testosterone strengthens the relationship between body fat and leptin, which has been interpreted as inhibition of leptin by testosterone (222). In contrast, in androgenic women with polycystic ovary syndrome, there was no relationship between leptin and testosterone (224), which suggests a gender difference in hormone sensitivity.

Animal studies provided strong evidence that leptin is essential for sexual maturation. Leptin treatment accelerated onset of puberty and behavioral estrous in lean mice (41), although attempts to replicate these findings have not always been successful (61). In starved mice, leptin prevented a drop in LH, a delay in estrous or a fall in testosterone, and blunted hypothalamic NPY expression (2). In fed rats, leptin inhibited weight gain but prevented the delay in puberty that was apparent in pair-fed animals. In fasted rats, the delay was only partially reversed (88), and leptin treatment did not increase sexual behavior in fasted hamsters (228). Therefore, other metabolic factors, in addition to leptin, signal that body energy stores are adequate to support reproduction.

Leptin During Pregnancy

Although leptin is essential for fertility, it is not essential for maintaining pregnancy. In pregnant ob/ob mice treated with leptin prior to pregnancy, withdrawal of leptin did not prevent completion of the pregnancy (162). Placental leptin expression is similar to that in adipose tissue (138), and receptors are present in the placenta (118), although their function has not been determined. In humans, leptin is detected in cord blood at 18 weeks and increases dramatically at 34 weeks of gestation (108). It is not clear whether placental leptin enters the maternal circulation, as placental leptin expression and umbilical leptin were increased in mothers with insulin-dependent diabetes who were treated with insulin, but maternal leptin did not change (138). During pregnancy, maternal leptin is elevated before body composition changes (93, 102) and increases with gestation (98), which suggests the development of "leptin resistance." There is no correlation between maternal leptin and fetal birth weight (194), and post partum, leptin falls below baseline levels (93) possibly because of the energy cost of lactation.

It has been reported that cord blood leptin is higher for female than for male fetuses (108), indicating gender differences at a very early stage of development; however, others have not confirmed these observations (197). Cord leptin concentration correlates with birth weight (197) and with measures of fetal growth rather than with adiposity (221). This may be explained by in vitro observations that leptin stimulation of fetal pituitary tissue promoted release of GH but not ACTH, prolactin, or gonadotropins (199). Fetal leptin drops precipitously within hours of birth (108), implying that its function in utero changes at parturition.

CONCLUSIONS

There is substantial evidence that leptin activates, or modulates, a number of physiological systems independent of any effect on food intake. Many of the responses to leptin have been investigated in isolation, but multiple effects are mediated by the hypothalamus and pituitary gland; therefore, it is essential to confirm in vitro observations with in vivo studies in which simultaneous activation of divergent pathways may act synergistically or antagonistically. It has been demonstrated that obesity is associated with central "leptin resistance"; however, it is not yet clear whether in peripheral tissues resistance also develops secondarily to regulation of receptor expression or increased expression of binding proteins, which could reduce leptin bioavailability. Many observations described above were obtained from animals treated with exogenous leptin, and the responses in these animals did not always correspond with the outcome of increased endogenous leptin caused by weight gain. Therefore, future studies need to clarify the physiologic, versus pharmacologic, relevance of specific leptin activities. This research will determine whether leptin's primary function is as a satiety signal or whether it has more significant peripheral activities independent of food intake.

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