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MINIREVIEW

The Role of Leptin in Human Physiology and Pathophysiology

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Summary

This review focuses on current knowledge of leptin biology and the role of leptin in various physiological and pathophysiological states. Leptin is involved in the regulation of body weight. Serum leptin can probably be considered as one of the best biological markers reflecting total body fat in both animals and humans. Obesity in man is accompanied by increased circulating leptin concentrations. Gender differences clearly exist. Leptin is not only correlated to a series of endocrine parameters such as insulin, glucocorticoids, thyroid hormones, testosterone, but it also seems to be involved in mediating some endocrine mechanisms (onset of puberty, insulin secretion) and diseases (obesity, polycystic ovary syndrome). It has also been suggested that leptin can act as a growth factor in the fetus and the neonate.

Key words

Leptin • Obesity • Puberty • Body mass index • Hormones • Pregnancy

Introduction

Leptin is attracting the attention of many scientists in the world. Only six years have passed since the discovery of leptin and more than 2000 published articles have appeared in the MEDLINE database. This review is a short summary of the recent advances in understanding both physiology and pathophysiology of leptin.

Fifty years ago, a genetic defect that proceeds in massive obesity was identified in inbred obese mice by Ingalls *et al*. (1950). The genetic defect in the obese or *ob/ob* mouse, which is a recessively inherited disease manifested early in life, is associated with diabetes type II and infertility. The *ob/ob* mice eat continuously and weigh three times more than normal mice. Parabiosis experiments in which the circulation of obese mutant homozygous *ob/ob* mice was cross-connected with that of normal wild-type mice resulted in decreased food intake and weight loss of the obese mice. These findings suggested that *ob/ob* mice were lacking a blood-borne factor that regulated nutrient intake and metabolism (Coleman 1978). It was confirmed later that the obesity and type II diabetes in inbred *ob/ob* mice were due to mutations in the *ob* gene (Friedman *et al*. 1991). The structure of the mouse obese (*ob*) gene and its human homologue by positional cloning was described by Zhang

et al (1994). The product of the *ob* gene was named leptin (from the Greek word λεπτοσ, *leptos*, meaning thin).

Leptin receptors

The leptin receptor (ob-R) was first isolated from the mouse choroid plexus using expression cloning (Tartaglia *et al.* 1993). This receptor was identified as a member of the cytokine family of receptors. The ob-R gene was found to encode at least five alternatively spliced forms (Lee *et al.* 1996). One of the splice variants, ob-Rb, is expressed at high levels in the hypothalamus and at a lower level in other tissues (Ghilardi *et al.* 1996). The high level of ob-Rb in the hypothalamus suggests that this brain region is an important site of leptin action (Satoh *et al.* 1997). Ob-Ra could function in the transport of leptin across the blood brain barrier or in its clearance from the cerebrospinal fluid (De Vos *et al.* 1996). The mechanism of leptin transport into the central nervous system (CNS) has not been elucidated. This issue is of great importance as it has been suggested that the entry of leptin into the cerebrospinal fluid may be a limiting factor in some obese subjects (Schwartz *et al*. 1996).

The leptin receptor is also expressed in hematopoietic stem cells (Konopleva *et al*. 1999). Leptin is secreted from bone marrow adipocytes and stimulates normal myeloid and erythroid development. Leukemic cells of some patients also express the leptin receptor. Leptin alone and in combination with other cytokines enhances the proliferation of leukemic cells. These findings suggest that leptin may play a role in the pathophysiology of leukemia (Hino *et al.* 2000). On the contrary, leptin levels in patients with sideropenic and pernicious anemia are not changed after the treatment (Marková *et al.* 2000).

The biology of leptin

Leptin is the product of *ob* gene coding for a 167 amino acid protein with a 21 amino acid signal peptide (Zhang *et al.* 1994). Its crystal structure indicates that leptin is a member of the cytokine family and has four or possibly five helical segments (Madej *et al.* 1995). The human *ob* gene displays 84 % homology with the mouse gene. *Ob* gene is localized on chromosome 7 (Zhang *et al.* 1994). In adult animals, leptin mRNA is primarily detected in white adipose tissue (Masuzaki *et al.* 1996), and brown adipose tissue (Maffei *et al*. 1995). A number of nonadipocyte tissues have been shown to synthesize and secrete leptin. These include the gastric mucosa (Bado *et al*. 1998), mammary epithelial cells (Smith-Kirwin *et al.* 1998), myocytes (Wang *et al.* 1998) and the placenta (Senaris *et al.* 1997). Its expression has also been reported in the testes, ovary and hair follicles (Hoggard *et al.* 1997).

It was recently reported that leptin is expressed in the gastric mucosa (Cinti *et al.* 2000) and fundic glands of humans (Mix *et al.* 1999). A possible physiological role for the gastric effects of leptin is to activate gastric vagal afferent signals to the brain (Yuan *et al*. 1999) and to increase the potency of cholecystokinin-derived vagal afferent to the CNS (Yuan *et al.* 2000).

Leptin circulates in the plasma as a free form or bound to leptin-binding proteins (Houseknecht *et al*. 1996). These plasma leptin-binding proteins have not yet been identified, but are likely to include a soluble form of the leptin receptor (Lee *et al.* 1996). The great majority of leptin circulates in the bound form in lean individuals and in the free form in obese subjects (Sinha *et al*. 1996). Leptin levels increase at night. The diurnal rhythms in circulating leptin levels are opposite to those seen in cortisol levels in humans, peaking in the middle of the night hours, while cortisol levels peak during early morning (Sinha *et al.* 1996). Leptin slowly declines during aging. This reduction is higher in women than in men, and is independent of BMI and other age-related endocrine changes (Isidori *et al.* 2000).

Leptin provides the brain with the information about the fat stores of the body and thus acts as a part of the feedback mechanism that can function as a lipostat. In human subjects, a high correlation between the body fat content and plasma leptin concentrations has been found (Considine *et al.* 1996).

It has been suggested that when fat cells increase in number and size, the *ob* gene starts to produce leptin, which is secreted into the circulation (Klein *et al*. 1996). When leptin reaches the brain *via* regions outside the blood-brain barrier, including parts of the hypothalamus, it decreases appetite and enhances the metabolic rate. The ventromedial nucleus in the hypothalamus is believed to play a central role in the regulation of feeding behavior, and may therefore be a particularly important target organ for this protein (Zhang *et al.* 1994).

The available data indicate that the loss of body fat decreases leptin levels, which induces a state of positive energy balance and other adaptive changes

(Ahima *et al*. 1996). Conversely, an increase in adiposity leads to an increase in leptin levels and to a negative energy balance. These and other data suggest that the biological response to low plasma leptin levels (indicative of starvation) is distinct from the response to high leptin levels, which is characteristic for obesity.

Leptin may act on the brain by reducing the levels of neuropeptide Y (NPY). NPY arises from neurons in the arcuate nucleus and is released in the paraventricular nucleus (Leibowitz 1994). It is the only known peptide that can induce obesity through prolonged central administration, with or without increased food intake. Jeanrenaud and co-workers (Zakrzewska *et al*. 1997) have shown that seven-day infusion of NPY into the lateral ventricle induced metabolic aberrations associated with obesity and insulin resistance in the mouse. Decreased NPY synthesis and secretion (Stephens *et al.* 1995) produced by leptin or a change in sensitivity to NPY (Smith *et al.* 1996) could account for the decreased food intake. However, leptin is still effective in transgenic animals with a knockout of NPY, suggesting that NPY is not essential for the action of leptin (Erickson *et al.* 1996).

Other targets of leptin in the hypothalamus include the appetite-regulating neuropeptides, namely the melanocyte-stimulating hormone (MSH), orexin, corticotropin-releasing hormone (CRH), agouti-related protein (Flier and Maratos-Flier 1998), proopiomelanocortin (POMC) (Mizuno *et al.* 1998), cocaine- and amphetamine-regulated transcript (CART) (Flier and Maratos-Flier 1998, Friedman and Halaas 1998, Elmquist *et al.* 1999).

Orexins (orexin-A and orexin-B) are newly discovered neuropeptides, which are produced almost exclusively in neurons of the lateral hypothalamus and have been shown to increase food intake after intracerebroventricular injection (Rauch *et al.* 2000). A recent study has revealed that orexin and its receptors exist in the gut (Kirchgessner and Liu 1999). Orexin and leptin receptors have been localized in the lateral hypothalamic area ("a feeding center") and the ventromedial hypothalamic nucleus ("a satiety center") and arcuate nucleus ("a feeding controlling center") (Oomura *et al.* 1969, Sakurai *et al.* 1998). The results show that leptin suppresses food intake whereas orexin-A increases food intake (Lopez *et al.* 2000). These differences are associated with leptin and orexin-A modulatory effects on the glucose-responding neurons located in the lateral hypothalamic area, paraventricular nucleus and ventromedial hypothalamic nucleus (Shiraishi *et al*. 2000). Recent studies have shown that POMC-containing neurons in the arcuate nucleus are thought to function as feeding inhibitors (Huszar *et al.* 1997). On the contrary, NPY-containing neurons seem to be involved in feeding stimulation (White and Kershaw 1990). Funahashi *et al.* (2000) reported that orexin neurons project to POMC- and NPY-containing neurons and inhibit POMC-containing neurons while they activate NPY-containing neurons in the arcuate nucleus of the hypothalamus. This may suggest that orexin and leptin reciprocally regulate POMC- and NPY-containing neurons. These findings strongly support the idea that NPY and leptin control orexin-containing neurons and regulate food intake and appetite.

CRH is a peptide widely expressed in the brain, particularly in the paraventricular hypothalamic area (Sawchenko *et al.* 1996). Similarly to leptin, CRH is a potent anorectic substance when injected into the brain of animals (Richard 1993). Furthermore, treatment with CRH augments energy expenditure in laboratory animals by increasing the sympathetic nervous activity (Richard 1993). Leptin exposure enhances spontaneous (Uehara *et al.* 1998) but inhibits stimulated CRH synthesis and release (Heiman *et al.* 1997). Recent studies have shown that leptin can attenuate the synthesis of CRH within the paraventricular hypothalamus. For instance, leptin has been reported to block both CRH synthesis induced by food deprivation in obese *ob/ob* mice (Huang *et al.* 1998) and CRH secretion from isolated rat hypothalami stimulated by glucose deprivation (Uehara *et al.* 1998).

The proopiomelanocortin neurons in the arcuate nucleus have been established as leptin targets. Circulating leptin crosses the blood-brain barrier and binds to its receptor in the hypothalamus where it activates the JAK (Janus-activated kinase)-STAT3 (signal transducers and activators of transcription) pathway (Tartaglia 1997). The STAT3 protein is expressed in NPY and POMC neurons consistent with the concept that leptin regulates the transcription of these genes (Hakansson and Meister 1998).

Recent findings support the hypothesis that leptin plays a key role in immune responses. Leptin belongs structurally to the long-chain helical cytokine family such as interleukin-2, interleukin-12 and the growth hormone. *Ob/ob* mice display immune defects with lymphoid organ atrophy, mainly affecting thymic size and cellularity. Leptin replacement reverses the immunosuppressive effects of acute starvation in mice. Leptin increases interleukin-2 secretion and proliferation of naive T cells. According to this view, leptin might

represent an important target for immune intervention in a variety of immunopathophysiological conditions (Matarese 2000).

Leptin and sexual dimorphism in humans

Leptin concentrations are higher in females when compared with males (Caro *et al.* 1996). There is as yet no explanation for these gender-based differences in leptin release, which have been observed *in vivo* from the earliest infancy (Garcia-Mayor *et al.* 1997). The observed differences may be explained by a different sex-based codification of the secretory pattern for male or female derived adipocytes or, alternatively, by differences in the hormonal milieu of the fetus that determine a different "adipocyte sex" in terms of leptin regulation (Casabiell *et al*. 1998).

Another reason for these sex differences in leptin concentrations may be explained by differences in the body composition. It is known that, at any given BMI, women are likely to have a greater percentage of body fat than men. Leptin mRNA expression is higher in subcutaneous than in visceral fat depots (Hube *et al.* 1996). Subcutaneous fat mass is the major determinant of plasma leptin (Tai *et al.* 2000).

However, the gender differences persisted after leptin correction for fat mass (Hassink *et al.* 1996). Gender differences in leptin concentrations also suggest that sex steroids could be involved in the control of leptin production. Rosenbaum *et al.* (1996) proposed that circulating androgens, but not estrogens, could have a suppressive effect on leptin secretion (Nedvídková *et al.* 1997). Leptin levels after estrogen replacement therapy were, however, unaffected in postmenopausal women (Kohrt *et al.* 1996).

Regulation of leptin production

Both *in vivo* and *in vitro* studies in rodents and man have shown that *glucocorticoids* enhance leptin gene transcription and leptin levels (De Vos *et al.* 1995) indicating the possibility of a closed feedback loop (Slieker *et al*. 1996). On the other hand, elevated leptin levels have not been found in Cushingoid subjects (Koistinen *et al.* 1996). Leptin levels were elevated in rats given dexamethasone (De Vos *et al.* 1995). *17β-estradiol* also increased leptin secretion into the culture medium of adipose tissue from female rats. Furthermore, the effect of 17β-estradiol was additive to that of dexamethasone effect (Casabiell *et al.* 1998). The effects of 17β-estradiol and dexamethasone are increased in the adipose tissue from female rats compared to that from males, although no gender differences were found in the number of estrogen receptors (Papaspyrou-Rao *et al.* 1997). The higher 17β-estradiol levels and the increased response to estrogens in female rats may, together with an increased response to dexamethasone, contribute to the sex differences in leptin levels (Hamilton *et al.* 1995).

The administration of a GnRH agonist to women undergoing *in vitro* fertilization treatment increased leptin levels (Stock *et al.* 1999). The elevation of leptin levels was not coupled with an increase in BMI and thus was not the result of increased body fat mass. Furthermore, the leptin levels correlated with estradiol levels. This indicates that other factors (possibly estradiol) are also important in the regulation of leptin.

Leptin inhibits the secretion of *testosterone* from rat testes *in vitro* independently of the nutritional state (Stock *et al*. 1999). Hypogonadal men with suppressed concentrations of testosterone exhibit elevated leptin levels (Jockenhovel *et al.* 1997). Testosterone substitution in these patients results in normalized levels of leptin.

Insulin can increase leptin production by rat and human adipocytes *in vitro* (Rentsch and Chiesi 1996). Leptin also counteracts insulin-mediated activation of glucose transport, glycogen synthesis, and lipogenesis in isolated rat adipocytes (Muller *et al*. 1997). Relationships between serum leptin and insulin concentrations were observed (Considine *et al.* 1996), but this association does not persist in subjects with impaired glucose tolerance (Turpeinen *et al.* 1997). Leptin may act independently of or synergistically with insulin in the hypothalamus to increase glucose uptake and inhibit NPY release and thereby diminish appetite (Stephens and Caro 1998). In human experiments, hyperinsulinemia induced by clamp techniques leads to a rise in leptin concentrations, but only during longer time (Kolaczynski *et al.* 1996). It is possible that this stimulatory effect of insulin on leptin secretion is due to the trophic effect of insulin on adipocytes (Van Gaal *et al.* 1999). It was shown by Kieffer *et al.* (1997) that leptin receptors are present on insulin secreting β-cells. Normal functional leptin would be able to suppress the secretion of insulin by activating ATP-sensitive K^+ channels in the β-cells. Mutant, leptin-deficient, *ob/ob* mice, which do not exhibit this insulin suppressive effect, develop hyperinsulinemia and insulin resistance. In humans, leptin resistance may follow a similar course due to the failure of leptin to

inhibit insulin secretion by the β-cells (Van Gaal *et al.* 1999). These data suggest that leptin overproduction may modify insulin secretion directly and could be involved in the development of the diabetic syndrome in obese subjects with insulin resistance.

Leptin levels are increased in patients with end-stage *renal disease* (Merabet *et al*. 1997). Patients treated by peritoneal dialysis seem to have higher leptin levels compared to patients treated by hemodialysis. This could be the effect of a marked increase in body fat mass as a consequence of the continuous carbohydrate load (Stenvinkel 2000). It has been speculated that hyperleptinemia may contribute to uremic anorexia and malnutrition (Stenvinkel 2000). The possible influence of chronic inflammation on serum leptin levels in patients with chronic renal failure needs further studies.

Leptin is known to inhibit *water intake* and may act on the hypothalamo-pituitary axis as a diuretic/ natriuretic hormone in long-term observations (Jackson *et al.* 1997). This might be associated with an increase of blood pressure in rodents (Shek *et al*. 1998), but a potential role of leptin in the pathogenesis of *hypertension* in humans remains to be conclusively demonstrated.

Recent data have shown that a relative deficiency in leptin (leptin resistance) may play a role in the obesity related to breathing disorders such as the *obesity hypoventilation* syndrome or *obstructive sleep apnoea*. The profound obesity in *ob/ob* mice is associated with impaired respiratory mechanics and depressed respiratory control, particularly during sleep. Leptin replacement studies in the *ob/ob* mouse indicate that leptin may act both as a growth factor in the lung and as a neurohumoral modulator of central respiratory control mechanisms (O´Donnel *et al.* 2000). Plasma leptin levels were elevated in patients with sleep apnoea, independently of body fat mass. This suggests that obstructive sleep apnoea is associated with resistance to the weight reducing effects of leptin (Phillips *et al.* 2000).

Several *cytokines*, such as tumor necrosis factor-alfa (TNFα) (Zumbach *et al.* 1997), interleukin-1 (Janik *et al.* 1997), and interleukin-6 (Sarraf *et al.* 1997) alter leptin mRNA expression and circulating levels. Further elucidation of the potential interaction between these factors could provide important insights into the feedback system for regulation of body weight and the mechanisms leading to obesity or cachexia in humans. It was recently reported that leptin and TNFα may be responsible for the weight loss in patients with pulmonary tuberculosis (Cakir *et al.* 1999).

Prolonged and strenuous *exercise*, such as the marathon, may decrease leptin concentrations (Landt *et al.* 1997). The relation between exercise or energy expenditure and leptin appears to be modified by the gender and intensity of exercise.

Cholecystokinin (CCK) is one of the satiety factors and has been extensively studied for its role in ingestive behavior (Smith and Gibbs 1994). CCK inhibits food intake in human subjects (Gibbs and Smith 1986). The coadministration of CCK with leptin potentiates its ability to reduce food intake (Matson *et al.* 1997). These findings support a concept in which leptin is involved in long-term energy homeostasis by influencing food intake *via* an interaction with short-term CCK signals that controls the size of individual meals (McMinn *et al.* 2000). The observation that *ob/ob* mice with genetic obesity caused by mutation of the leptin gene are relatively insensitive to CCK-induced satiety provides additional support for this concept (McLaughlin *et al*. 1981). Matson *et al.* (2000) reported that repeated daily combination of intracerebroventricular leptin and intraperitoneal CCK resulted in a significantly greater loss of body weight than does leptin alone. These data suggest a role of CCK in body weight regulation that may not depend entirely on reduction of feeding behavior and suggest a strategy for enhancing the effects of leptin in leptin-resistant obese individuals.

Leptin and thyroid axis

Patients with thyroid disease usually exhibit disturbances of body weight, food intake and thermogenesis. Increased body weight, decreased appetite and decreased thermogenesis are characteristic for hypothyroidism, while the reverse is true for hyperthyroidism. Both thyroid hormones and leptin influence similar aspects of body homeostasis. Leptin decreases appetite and enhances thermogenesis (Korbonits 1998).

Thyroid hormones regulate the expression of leptin mRNA and secretion of leptin by adipocytes *in vitro* (Yoshida *et al.* 1997). Patients with a leptin receptor mutation (Clement *et al.* 1998) are hypothyroid with delayed TSH response to TRH stimulation, indicating hypothalamic hypothyroidism. Recent data suggest that leptin promotes *in vitro* TRH biosynthesis through the action on TRH neurons (Nillni *et al.* 2000). Flier *et al.* (2000) propose that the dominant signal to the brain, which suppresses TRH expression in the paraventricular nucleus, is a drop in the level of leptin. Recent data

support the contention that the effects of leptin on TRH are mediated by other neuropeptides present in the arcuate nucleus. Legradi *et al.* (1998) chemically ablated the arcuate nucleus in rats and observed that starvation failed to suppress thyroid levels.

A number of central effects of leptin are mediated by melanocortins (Seeley *et al.* 1997, Watanobe *et al.* 1999). A novel endogenous melanocortin antagonist, agouti-related peptide (AgRP), has been identified in the arcuate nucleus of the hypothalamus near NPY neurons. AgRP is suppressed by leptin (Fan *et al.* 1997). Transgenic overexpression of AgRP results in obesity. The product of proopiomelanocortin (POMC), such as α-MSH, is an agonist at melanocortin receptors (Fan *et al*. 1997) and is induced by leptin (Kim *et al.* 2000). Some of the leptin effects are due to the effects on α-MSH and AgRP neurons acting on subpopulations of neurons containing melanocortin receptors. Kim *et al.* (2000) demonstrate that α-MSH increases TSH levels when it is administered centrally to rats. Furthermore, AgRP blocks release of TRH by antagonizing α-MSH and thereby opposes the action of leptin (Nillni *et al.* 2000). These data suggest that the melanocortin pathway also plays an important role in the regulation of the thyroid axis by leptin, perhaps by promoting contacts between functionally antagonistic leptin-regulated neurons in the arcuate nucleus and TRH neurons in the PVN.

The biochemical regulation of energy expenditure involves a group of mitochondrial transport proteins (uncoupling proteins, UCPs). One of these proteins, UCP3, is expressed in a variety of tissues including the muscle and brown adipose tissue and is influenced by both leptin and thyroid hormones (Gong *et al.* 1997). UCP3 is upregulated in *ob/ob* rats during leptin treatment (Liu *et al.* 1998). Hypothyroidism decreases UCP3 levels while hyperthyroidism increases them (Gong *et al.* 1997). It is likely that the lower thyroid hormone levels together with the suppression of uncoupling protein activity limit energy expenditure and prevent protein catabolism during food deprivation or illness (Ahima *et al.* 2000). These observations suggest that leptin is involved in the regulation of thyroid hormones and *vice versa*.

There have been conflicting reports on the effect of hypothyroidism and hyperthyroidism on leptin levels and on the interaction between leptin and the pituitary-thyroid axis in humans (Haluzík *et al.* 2000). It has also been documented that plasma leptin levels are elevated in hypothyroid patients and decreased in hyperthyroid patients (Valcavi *et al.* 1997, Pinkney *et al.* 1998, Diekman *et al.* 1998, Ozata *et al.* 1998). On the contrary, other authors have not reported any significant changes in leptin levels in hypo- or hyperthyroidism (Sreenan *et al.* 1997, Mantzoros *et al.* 1997a, Syed *et al.* 1999).

Leptin and obesity

The role of the *ob* gene in pathogenesis of obesity in man has, as yet, only been partly elucidated.

Leptin levels are adapted to changes in energy balance. During fasting (Kolaczynski *et al.* 1996) or weight loss (Considine *et al.* 1996), leptin concentrations decrease, while they increase in overfeeding or weight gain. Patients with morbid obesity were treated surgically and they achieved ideal body weight in 12 months and this persisted for at least 6 months. In these patients, leptin levels were completely normalized. This suggests that hyperleptinemia is not the primary cause. On the contrary, relative hypoleptinemia may be the primary mechanism (Stephens and Caro 1998).

Obese humans show high leptin concentrations (Considine *et al*. 1996). Leptin mRNA expression in fat cells correlates significantly with body fat mass (Considine *et al.* 1996). Hyperleptinemia is thought to be indicative of "leptin resistance", and may play a role in the pathogenesis of obesity (Considine *et al.* 1996, Maffei *et al.* 1995). Potential mechanisms that may mediate leptin resistance include impairment of brain leptin transport and abnormalities of leptin receptors and/or postreceptor signaling.

The upregulation of the *ob* gene in a large group of obese subjects has suggested the possibility of important mutations in the gene or in gene coding for its corresponding receptor. However, extensive screening of different patient categories is presently ongoing in many laboratories, and so far no frequent mutations in the human *ob* gene have been reported. Rare mutations of the human *ob* gene cannot explain the frequently observed elevation of leptin mRNA and plasma leptin in obese subjects.

An alternative and a more likely explanation is that the increased leptin mRNA levels in man are secondary to a decreased sensitivity in the hypothalamic satiety receptor itself, by analogy with the diabetes (*db*) mutant mouse, which has recently been shown to have a defective leptin receptor (Lee *et al.* 1996). Recombinant leptin normalized body weight by decreasing food intake

and increasing energy expenditure in *ob/ob* mice but not in *db/db* mice (Campfield *et al.* 1995). Patients, who are homozygous for leptin receptor mutations, manifest an early onset of morbid obesity, lack of pubertal development and a dysfunction of the growth hormone and thyroid axes (Clement *et al.* 1996).

SOCS-3 (suppressor of cytokine signaling-3) is an intracellular protein, which is induced by activation of cytokine receptors such as the leptin receptor (Tartaglia 1997). Bjorbaek *et al.* (1998) reported that leptin treatment of *ob/ob* mice elevated the levels of mRNA encoding SOCS-3 in the arcuate nucleus and hypothalamic dorsomedial nucleus. It was postulated that the resistance of obese people to leptin is the result of SOCS-3 overactivity (Bjorbaek *et al.* 1998).

The corticotropin-releasing hormone exhibits anorectic properties (Morely 1987). It has been proposed that the actions of leptin might be mediated *via* hypothalamic CRH. Recent studies have shown that leptin-induced reductions in food intake are mediated through the opioidergic pathway. For example, agonists of the melanocortin (MC4) receptor reduce food intake, and targeted mutation of the MC4 receptor causes obesity (Seeley *et al.* 1997). There are high levels of leptin receptor expression on proopiomelanocortin neurons in the arcuate nucleus. Recent findings have shown that human obesity and hyperleptinemia are linked to a segment of chromosome 2 near the POMC gene locus (Krude *et al.* 1998).

Another cause of leptin resistance could be due to a transport problem at the level of the blood-brain barrier (Caro *et al.* 1996). Abnormal leptin catabolism does not seem to be the underlying mechanism for the development of human obesity because the estimated half-life as well as the biological activity of circulating leptin is similar in both lean and obese humans (McGregor *et al.* 1996). Antileptin antibodies and leptin-binding proteins do not inactivate leptin in obese persons (Auwerx and Staels 1998). Understanding the mechanisms underlying leptin resistance is expected to elucidate the pathogenesis of obesity and contribute to the development of specific and effective treatment.

Recent studies have proposed that leptin affects the central nervous system by increasing sympathetic outflow independent of feeding. The effects of leptin on glucose and fat metabolism could in part be mediated by the sympathetic nervous system. Disturbances of leptin signaling could cause obesity and impaired glucose tolerance in rodents and humans (Nonogaki 2000).

Leptin and anorexia nervosa

Anorexia nervosa is a syndrome of unknown etiology. It is associated with multiple endocrine abnormalities such as hypothyroidism, low levels of LH and FSH, absence of normal pulsatility in LH secretion and hypercortisolism. Leptin levels are reduced significantly and correlate with body weight, percentage body fat and IGF-I in subjects with anorexia nervosa (Haluzík *et al.* 1999a). These patients have reduced plasma and cerebrospinal fluid leptin concentrations compared to the controls and they normalize their cerebrospinal fluid and plasma leptin levels during the recovery of weight gain (Mantzoros *et al.* 1997b). No significant differences in serum lipids, total protein, albumin and prealbumin concentrations were found in patients with anorexia nervosa before and after refeeding (Haluzík *et al.* 1999b, c). Haluzík *et al.* (1999d) proposed that serum leptin levels may serve as a sensitive parameter of nutritional status in patients with anorexia nervosa which reflects changes of the body fat content before and after realimentation earlier than other biochemical parameters (cholesterol, prealbumin, albumin). Osteopenia is a frequent and often persistent complication of anorexia nervosa (Bachrach *et al.* 1990). IGF-I functions as a bone trophic hormone, which positively affects bone growth and bone turnover. Soyka *et al.* (1999) found a high correlation between leptin and IGF-I levels, indicating that undernutrition is a major cause of reduced IGF-I levels in adolescents with anorexia nervosa. A recent study has shown that leptin has receptors in bones (Ducy *et al.* 2000). Warren *et al.* (1999) speculate that leptin may be a physiological regulator of bone mass, and thus may be the link between amenorrhea and osteopenia.

Leptin and puberty

The influence of adequate nutrition on the timing of puberty has been discussed for many years. Inadequate nutrition retards growth and delays sexual maturation, high nutrition and rapid growth advance maturation.

Frisch and McArthur (1974) proposed the concept of critical body weight according to which menarche is triggered when a critical percentage of fat is attained, and the maintenance of menstrual cycles requires the persistence of a minimal level of body fat. This association was found to be useful clinically, but these authors were unable to prove any causal relationship between fatness and reproduction. An acceptable neuroendocrine mechanism is still lacking as to how the brain can detect the status of body fat in order to increase GnRH secretion. Leptin seems to be a signal for the brain about the adequacy of fat stores for sexual maturation and reproduction.

The *ob/ob* mouse, which is morbidly obese and infertile, can attain sexual development and become fertile following weight loss induced by exogenous leptin (Chehab *et al.* 1996). In particular, the administration of leptin to normal young female mice has been shown to accelerate the onset of puberty (Ahima *et al.* 1997). Barash *et al.* (1996) found that leptin administration increases basal LH levels in *ob/ob* mice. While many investigators have assumed that leptin acts within the brain to affect secretion of GnRH, the actual mechanism is still not clear. The simplest possibility is that the fat-derived hormone regulates GnRH activity directly. This is problematic because it is difficult to alter the levels of leptin experimentally without affecting many other nutritional factors, which could also be involved in the modulation of reproduction. Yu *et al.* (1997) demonstrated that leptin stimulates gonadotropin release from rat pituitaries *in vitro* indicating that leptin may have a direct effect on the pituitary tissue. Little attention has yet been paid to the possibility that leptin may act indirectly or at least in concert with other metabolic signals. For example, it has been reported that leptin may regulate insulin-stimulated glucose uptake, one of the factors regulating glucose availability. Glucose availability variations may be a metabolic signal that can provide information for the control of GnRH secretion (Foster and Nagatani 1999). It seems, according to some reports in the literature (Hassink *et al.* 1996, Ambrosius *et al.* 1998, Ahmed *et al.* 1999), that leptin acts rather as a permissive factor for the onset of puberty and that leptin itself is not capable of initiating puberty, although a minimal threshold level of leptin is necessary for pubertal development.

Leptin in the neonate

The placenta also produces leptin (Schubring *et al.* 1997). Placental leptin may exert angiogenic and immunomodulatory activities, which affect the placenta in an autocrine or paracrine manner (Ashworth *et al.* 2000).

The level of leptin in cord blood is positively correlated with body weight and fat mass of the neonate (Harigaya *et al.* 1997). It decreases in response to maternal smoking (Mantzoros *et al.* 1997d). Leptin concentrations in breast milk – 73.2 ng/ml (Smith-Kirwin *et al.* 1998) are higher than serum leptin levels in normal – 7.5 ng/ml, obese – 31.3 ng/ml (Considine *et al.* 1996) and pregnant – 29.8 ng/ml (Butte *et al.* 1997) individuals. Human mammary epithelial cells produce and secrete leptin (Smith-Kirwin *et al.* 1998). Leptin is secreted into the milk and passes from the gastrointestinal tract into the blood (Casabiell *et al.* 1997), suggesting that an important role in regulating neonatal food intake and/or growth. Leptin may play a role in fetal bone metabolism as a part of its effect on fetal growth and development. Ogueh *et al.* (2000) suggested that leptin may decrease fetal bone resorption thus increasing bone mass.

Leptin and polycystic ovary syndrome

The polycystic ovary syndrome (PCOS) is the most common endocrine disorder causing anovulatory infertility (Franks 1995). Preliminary data obtained in rodents indicate that leptin may also be involved in the regulation of gonadal function and fertility (Chebab *et al.* 1996). *Ob/ob* mice are characterized by severe obesity, insulin resistance and infertility. The injection of leptin increases the levels of gonadotropins, promotes ovarian follicular development and restores fertility. On the contrary, leptin administration in the rat resulted in fewer ovulations, both *in vivo* and *in vitro* (Duggal *et al.* 2000).

Expression of both leptin (Cioffi *et al.* 1997) and its specific receptors (Cioffi *et al.* 1996) has been found in the human ovary. Karlsson *et al.* (1997) found that leptin inhibited LH-stimulated estradiol production by granulosa cells. Perhaps the effect of high circulating concentrations of leptin on the ovary of obese patients with PCOS explains their otherwise impaired response to gonadotropin stimulation (White *et al.* 1996). It seems likely that the response of the ovaries of such patients represents a balance between the stimulatory effects of insulin and the inhibitory effects of leptin (Jacobs and Conway 1999).

The present study suggests that the human endometrium is a novel target for leptin. Alfer *et al.* (2000) investigated subfertile women. These patients were deficient in the expression of the functional leptin receptor. These analyses provide evidence that the lack of the leptin receptor in an anovulatory cycle may contribute to subfertility by a hitherto undefined endometrial factor.

Women with PCOS are frequently obese, insulin-resistant and have hyperinsulinemia. Insulin stimulates the expression of the *ob* gene and secretion of leptin in rats (Cusin *et al.* 1995). Human studies of leptin regulation by insulin have yielded conflicting results. Prolonged exposure of cultured human adipocytes to insulin increased the levels of leptin mRNA and its protein (Kolaczynski *et al*. 1996). Hyperinsulinemia induced by the clamp technique increased leptin concentrations, but only in longer observations (Kolaczynski *et al.* 1996). Insulin and leptin concentrations seem to be associated, but plasma insulin levels do not acutely regulate leptin production. It is possible that this stimulatory effect of insulin on leptin secretion is due to the trophic insulin effect on adipocytes (Van Gaal *et al*. 1999).

In one study, serum leptin concentrations in women with PCOS have been reported to be higher than in regularly cycling controls (Brzechffa *et al.* 1996). In most other studies, serum leptin levels in PCOS did not differ from those of normal women with similar BMI or adiposity (Chapman *et al.* 1997, Mantzoros *et al*. 1997c, Rouru *et al*. 1997). A complex etiopathogenic role of leptin in PCOS can be postulated through its peripheral as well as central effects, but this still remains only speculative. Further studies should focus on the effects of exogenous leptin on the reproductive axis and ovary in women with PCOS. The interaction of leptin with gonadotropins and sex steroids, especially androgens, is still not clear.

Conclusions

Serum leptin levels correlate with the amount of body fat and regulate energy intake and expenditure by interacting with hypothalamic leptin receptors. Leptin promotes hematopoiesis, influences pubertal development and fetal growth, but the mechanism of leptin action in these processes is not clear. The significance of the leptin effect in the pathogenesis of obesity, anorexia nervosa, insulin resistance, hypertension and the polycystic ovary syndrome must be examined in various populations and under various experimental conditions. Despite a certain skepticism to leptin, new insights into a number of physiological and pathophysiological states were obtained.

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