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The Physiological Role of Leptin: A Review

Patani P. V.*, Ghaisas M.M., Molvi K.I., Seth A.K.

Department of Pharmacology, Arihant School of Pharmacy & Bio-Research Institute, Adalaj, Gandhinagar

ABSTRACT:

Obesity is an increasing health problem not only in the industrialized western countries but, also in the developing countries like India. The adipose tissue specific obese (*Ob*) gene and its peptide product leptin were discovered in 1994. Since then, a plethora of studies on its important function in regulating body weight have been undertaken and has opened a whole new field of research. Although the role of leptin on obesity has been reviewed extensively, our knowledge of its physiological role(s) is not fully known and is an ever-growing subject. Leptin binding to specific receptors in the hypothalamus results in altered expression of orexigenic and anorexigenic neuropeptides that regulate neuroendocrine function and energy homeostasis, and recent experimental evidence suggests that leptin plays an important role in the pathogenesis of obesity and eating disorders. This review will attempt to give a summary of the main physiological actions of leptin in the body and to explore the possibility of the leptin feedback system being used as a basis for the development of new drugs to combat obesity.

Key words: Leptin, obesity, neuropeptides

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INTRODUCTION:

Obesity (defined as an excess of body fat, or body mass index [BMI] ≥ 30 kg/m²) remains an ever increasing health problem in late 20th-century and is associated with many common diseases and health problems including non-insulin depended diabetes mellitus (NIDDM), hypertension, coronary artery disease, osteoarthritis, obstructive sleep apnoea and even certain types of cancer. Understandably, there has been much research effort over the years devoted to the study of mechanisms controlling appetite and body weight and to the development of effective therapeutic interventions that could improve the quality of the patient, as well as reduce the risk of cardiovascular diseases and other chronic medical conditions. The discovery of a miracle weight loss drug however, has remained illusive. The recent expansion of knowledge about the hormonal control of metabolism and body composition now offers the hope that such a new agent will become available over the next decade.

Studies utilizing spontaneous monogenic and transgenic mutant models of obesity in rodents have resulted in the identification of a number of proteins, which play a role in regulating energy, balance. Among these proteins, leptin has been found to play a key role in these regulatory processes. It is widely believed that the primary physiologic role of leptin is to prevent obesity by regulating food intake and thermogenesis through actions on hypothalamic centers. Leptin is expressed primarily by white adipocytes in proportion to their size and secreted into blood stream as part of central negative feedback pathway (1). Leptin is also synthesized by the gastric epithelium, placental trophoblast, skeletal muscle and mammary gland (2).

For Correspondence:

Dr. Pragnesh Patani

Department of Pharmacology,

Arihant School of Pharmacy & Bio-Research

Institute, Adalaj, Gandhinagar

E-mail: Pragnesh006@gmail.com

OB gene and Leptin

In 1994, Friedman and his colleagues first reported the cloning of ob gene in mice. Further, characterization of this ob gene identified that it encodes a 4.5 mRNA transcript with a novel 167-aminoacid proteins termed leptin (from the Greek word lepos meaning "thin"). A 21-amino acid signal peptide is cleaved before release of mature leptin into the circulation. Leptin, the protein product of the ob gene, is primarily an adipocyte-secreted hormone, exerts its influence on food intake, energy expenditure, body weight and neuroendocrine function through actions on neuronal targets in the hypothalamus. Leptin levels increase exponentially with increasing fat mass and leptin production is higher in subcutaneous than in visceral fat depots. Leptin levels reflect not only the amount of fat stored but also energy imbalance; prolonged fasting substantially decreases leptin levels, whereas overfeeding greatly increases them.

The tertiary crystalline structure of leptin was originally reported by Zhang et al. (1997) (3). The leptin molecule is thought to have similar structural features to members of the long chain cytokines family that includes growth hormone (GH), interleukin (IL-6), leukemia inhibitory factor and ciliary neurotrophic factor (CNTF) (4). Human leptin is 84% identical to mouse leptin, and 83% identical to rat leptin (5). The 4- α helix bundle structure consist of 146 amino acids having single disulfide bond that are located at C-terminal end between cystein residues 96 and 146 that is responsible for maintaining protein stability and biological activity (3).

The importance of leptin as an adiposity signal to the brain is supported further by the phenotype of animals that either do not synthesize it (*ob/ob* mice that have a mutation in the leptin gene) (Zhang *et al.*, 1994) or that have genetic mutations that compromise functioning of the leptin receptor (*db/db* mice and fatty Zucker *fa/fa* rats). These animals are characterized by hyperphagia and extreme obesity. Administering small amounts of leptin into the brains of *ob/ob* mice reverses this syndrome.

Mutation of the mouse ob gene results in a syndrome that includes obesity, increased body fat deposition, hyperglycemia, hyperinsulinemia, hypothermia and impaired thyroid and reproductive function in both male and female homozygous *ob/ob* obese mice (6). Two distinct mutations of the ob gene have been identified. One mutant, SM/Ckc + Dacob2J/ob2J, expresses no leptin mRNA (7). The other, C57BL/6J, over expresses by 20-fold an mRNA species resulting from a single base mutation at codon 105 (8). This mutation converts the coding sequence for arginine (Arg105) in leptin to a premature stop codon, resulting in the production of a truncated mRNA for leptin, which is translated into a protein that appears to be degraded in the adipocyte.

Leptin receptor and signal transduction

The leptin receptor (LR) gene was first cloned from mouse choroid plexus cDNA. LR mRNA was also found in a wide range

of peripheral tissues e.g. heart, liver, skeletal muscle, pancreas ovaries, testes, spleen, adipose tissue as well as the hypothalamus. The closest relatives of LR encoded as gp130 (9), the G-CSF receptor (10) and the leukemia inhibitory factor receptor (11). Multiple transcripts of the leptin receptor, resulting from alternative splicing of Ob-R mRNA, encode at least six Ob-R isoforms (12, 13). All isoforms of the receptor share an identical extracellular domain at the amino terminus, but have cytoplasmic domains of different lengths arising from alternative RNA splicing at the most C-terminal coding exon. Five of the known receptor isoforms, LRA, LRB, LRC, LRd and LRF, contain transmembrane domains. LRE, lacking both transmembrane and cytoplasmic domains, circulates as a soluble receptor (12, 14).

The roles of the short intracellular domain forms of Ob-R remain to be defined. It is tempting to speculate that the high levels of the short intracellular domain form in the choroid plexus play a role in transporting leptin from the blood into the CSF by a specific and saturable transport mechanism (15), where it can then move by diffusion to the brain centers that regulate body weight. The short isoform (LRA) can transduce signals through insulin receptor substrates and JAK-dependent signaling to mitogen-activated protein kinase pathways. Short LR forms may play a role not only in transport but also in clearance or as a source of soluble receptor; it is assumed that proteolytic mechanisms exist for releasing the extracellular domain from the cell surface (16).

The long receptor isoform LRB contains motifs within its intracellular domain that are required for signal transduction. LRB is a single membrane-spanning receptor that belongs to the class I family of cytokine receptors (16). The homology of Ob-R to class I cytokine receptors immediately provided important clues as to possible intracellular mediators of leptin receptor activation. Leptin binding activates the Janus kinase (JAK)-signal transduction and activator of transcription (STAT) signaling cascade (17). Typically, JAK proteins are associated constitutively with membrane-proximal sequences of the receptor intracellular domain (ICD) and phosphorylate the receptor ICD upon ligand binding. The phosphorylated ICD then provides a binding site for a STAT protein, which is activated upon binding the phosphorylated receptor ICD. The activated STAT proteins then translocate to the nucleus and stimulate transcription. Activation of LRB also promotes JAK-dependent signaling to mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase pathways, and it is possible that Ob-R's ability to control body weight may depend upon these signals as well (17).

Leptin action in the hypothalamus and clearance

LRB is expressed at many sites in body, the highest levels of LRB expression in the body is found in neurons of the nuclei of the basomedial hypothalamus-including the arcuate (ARC), dorsomedial hypothalamic (DMH) and ventromedial hypothalamic (VMH) nuclei. Chemical or physical ablation of these nuclei results in increased feeding and neuroendocrine

abnormalities that are similar to the phenotypes of *db/db* or *ob/ob* mice, suggesting that these hypothalamic nuclei (which is so-called "satiety center") are critical sites of leptin action (18, 19) Within the nuclei of the basomedial hypothalamus, LRB is expressed at its highest levels in the ARC. Within the ARC, LRB is found in at least two distinct populations of neurons :

- 1) Neurons that coexpress neuropeptide Y (NPY) and agouti-related peptide (AgRP) and
- 2) Neurons that express pro-opiomelanocortin (POMC) (18, 19)

POMC is processed to alpha melanocyte-stimulating hormone (α -MSH) in the LRB/ POMC neuron. α -MSH mediates a powerful anorectic (appetite-suppressing) signal; LRB stimulates the expression of POMC and activates the LRB/POMC neuron (19, 20). AgRP is an antagonist of α -MSH signaling and NPY is itself an orexigenic (appetite-stimulating) hormone that also acts to suppress the central LRB growth and reproductive axes (21-24). Leptin acts via LRB to inhibit the NPY/AgRP neurons and to suppress expression of these neuropeptides. Thus, LRB signaling stimulates the production of anorectic neuropeptides and suppresses levels of orexigenic peptides. Conversely, when leptin action is decreased or deficient (e.g., starvation, *ob/ob* or *db/db* mice), appetite is stimulated via the suppression of anorectic neuropeptides e.g., POMC) and by increased expression of orexigenic peptides (e.g., NPY, AgRP) (25) LRB-expressing ARC NPY/AgRP and/or POMC neurons also regulate energy expenditure and other elements of neuroendocrine function (26). Other distinct neurochemical properties of LRB-expressing neurons in the DMH, VHM and populations of LRB-expressing neurons may be found in the ARC (27). The elsewhere (including the brainstem) are poorly defined. The relation of leptin to other hypothalamic neuropeptides, such as orexin, the tubby transcript (28, 29), melanin-concentrating hormone, neurotensin, and cholecystikinin, has only recently begun to be deciphered (30-32).

Leptin receptors are also expressed in peripheral tissues, including lung, kidney, liver, pancreas, adrenals, ovaries, hematopoietic stem cells and skeletal muscle, whereas the soluble leptin receptor isoform that circulates in the serum functions as a leptin-binding protein (33-42). Although this wide expression may imply that the role of leptin is much broader than that of a circulating satiety factor, the full array of leptin's actions through activation of these receptors has not been fully clarified. However, it seems that short receptor isoforms present in the kidney may mediate leptin clearance (33, 34), whereas those in the brain capillary endothelium (35) and the choroid plexus (36) transport leptin from blood into the brain interstitium and the cerebrospinal fluid by way of a saturable system (38, 39). There is a threshold level of serum leptin (about 25 to 30 ng/mL) above which increases in serum levels are not translated into proportional increases in cerebrospinal or brain leptin levels (38); this, in turn, may result in an apparent leptin resistance and obesity.

Physiological role of leptin

Leptin has a role in the regulation of several hypothalamic-pituitary-endocrine axes, i.e., gonadal, adrenal, thyroid, pancreatic islets, and growth hormone (Figure 1). Leptin has also been implicated as having a role in reproduction system, hematopoiesis, angiogenesis, immune function, osteogenesis and wound healing.

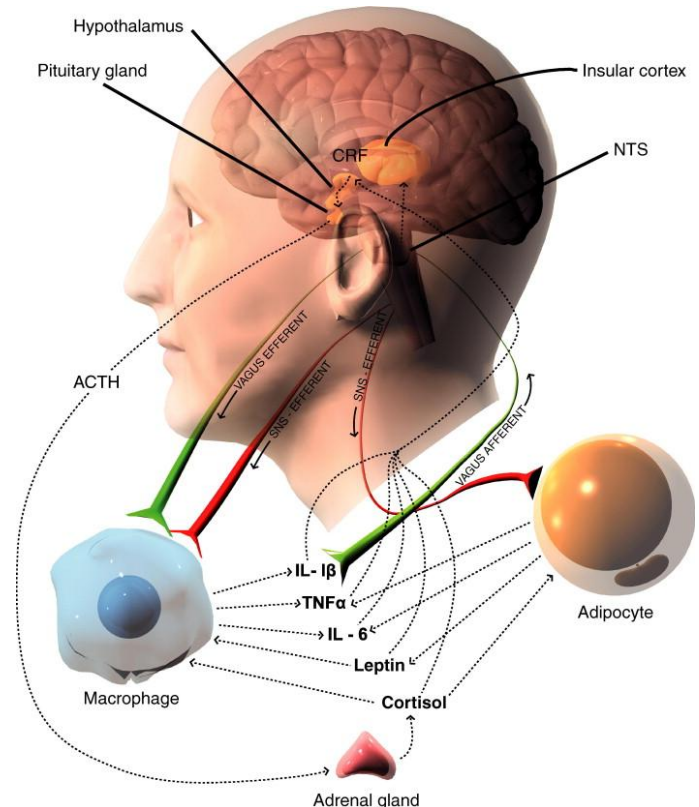


Figure 1 Leptin - in the regulation of several hypothalamic-pituitary-endocrine axes

Leptin in reproductive system

Recombinant leptin therapy initiates puberty and also restores fertility in homozygous *ob/ob* female mice and accelerates the onset of puberty in wild type mice. It is well documented that Leptin play major role in development of human menarche, pregnancy and lactation (43). Early sexual development is normal in *ob/ob* females however ovulation does not occur and the mice remain prepubertal with no occurrence of estrous cycles. Restoration of reproductive functions of *ob/ob* females could be possible by administration of hypothalamic extracts to the third ventricle with pituitary extracts, gonadotropins, progesterone and relaxin (44). These observations indicate that the infertility of *ob/ob* females is related to hypothalamic and pituitary hormone insufficiencies, and mutations of the *ob* and *db* genes have been shown to be associated with hypothalamic hypogonadism in humans. One clinical trial has been published in 1999 on a 9-year-old leptin-deficient girl with severe early-onset obesity. She received daily recombinant methionyl human leptin by s.c. route for

12 months which resulted in sustained reduction in body fat mass (45-46). Leptin gradually increased both basal and stimulated serum gonadotropin levels in this patient during the 12 months of treatment. Pelvic ultrasonography indicated a juvenile uterus and ovaries and also there was no evidence of development of secondary sexual characteristics, however, the nocturnal pattern of gonadotropin release was pulsatile, suggesting an early onset of puberty that progressed to normal luteinizing hormone (LH) and follicle stimulating hormone (FSH) pulsatility with continued replacement therapy (47, 48). In another model, long term physiological leptin replacement for 18 months in three leptin deficient, hypogonadal adults resulted in clinical signs of puberty and increases in LH pulsatility and testosterone levels in a 27 year old man and development of ovulatory menstrual cycles in two adult women with luteal phase defect (49,50). These models of leptin deficiency in animals and human indicate that leptin has an important role in reproduction by influencing hypothalamic - pituitary - gonadal axis.

Leptin and haematopoiesis

Leptin receptor distribution in hematopoietic tissue and stem cells during embryonic development (51). Secretion of leptin by bone marrow adipocytes may provide a local source of leptin to precursor cells. Recent studies indicate that leptin, together with other cytokines, influences the development of specific lineages of cells, particularly T cells and macrophages, very early in haematopoiesis. A strong correlation between plasma leptin levels and white blood cell count has been observed in obese humans (52).

Leptin and immune function

A large body of evidence suggests that leptin-mediated signaling pathways play an active role in innate and adoptive immunity through alteration of transcription of various target genes. Impaired immunity due to reduction in T cell function has been noted in both leptin deficient *ob/ob* and leptin deficient *db/db* mice (53). These impairments have been observed principally in cell mediated immune responses, in resistance to viral and bacterial infections, and in macrophage function. Leptin can induce a shift of T cells in *ob/ob* mice to a predominantly Th1 response by increasing interferon- γ and interleukin-2, and decreasing interleukin-4 cytokine production in vivo (53). These helper cells also express the long isoform of the leptin receptor. These data strongly suggest a leptin receptor-mediated effect on T cell production and function.

Leptin and osteogenesis

Several studies have shown that leptin directly stimulates bone growth in vitro and increase bone density in leptin deficient animals. Clinical studies have shown that obesity is a protective factor for postmenopausal osteoporosis and that bone mass correlates positively with fat mass (54). There is also a positive relationship between serum leptin levels and bone mineral content at several skeletal sites in healthy non-

obese women and in whole body bone mineral density in postmenopausal women (55). The leptin-defective *fa/fa* rat has decreased bone mass, increased bone resorptive activity, and hypercalciuria that cannot be attributed to the diabetic condition of this animal model (56). The *ob/ob* mice respond to leptin treatment with increased osteoblast formation and bone deposition (57). Direct effects on osteoblasts and osteoclasts, which are known to contain leptin receptors, may mediate Leptin's actions on bone, indirect actions of leptin are also suggested. The effects of leptin on the hypothalamic-pituitary axis have been confirmed. Leptin has been shown to regulate the release of growth hormone and somatostatin and to improve the body's response to insulin and insulin like growth factor (58). It is proposed that these may be mechanisms by which leptin indirectly influences homeostatic balance to favor bone formation rather than bone resorption. There is evidence to suggest that leptin-induced enhancement of osteoblast differentiation and suppression of adipocyte differentiation in human bone marrow may be responsible for the negative correlation between bone mineral density and body fat mass (59). Florent Elefteriou et al. (60); have recently proposed that CART (cocaine amphetamine regulated transcript), a neuropeptide whose expression is controlled by leptin and nearly abolished in *ob/ob* mice, inhibits bone resorption by modulating Rankl expression. Their study establishes that leptin-regulated neural pathways control both aspects of bone remodeling, namely resorption of osteoclasts and formation of osteoblasts and demonstrates that integrity of sympathetic signaling is necessary for the increase in bone resorption caused by gonadal failure (60).

Gastric leptin

Adipocyte-specific, the *ob* gene, as well as the leptin receptor, has been found in a variety of other tissues including the stomach. Epithelial cells of stomach synthesize and secrete leptin in rodents and in humans (61). Leptin also detected in the secretory granules of endocrine P cells (62). Leptin secretion in the stomach is regulated by feeding, acetylcholine released by the vagus nerve (63), and intestinal hormones (i.e. cholecystokinin and secretin) (61) Stomach-derived leptin, mainly secreted in the lumen, remains stable in gastric juice even at pH2. It then enters the intestine where leptin receptors have been identified on the brush border. Recent data also suggest that gut leptin may act locally within the gastrointestinal tract to influence intestinal functions, such as nutrient absorption, and thus have pathophysiological implications.

Leptin derived from the stomach can be distinguished from adipocyte leptin through its rapid increased secretion following a meal and through its exocrine secretion (i.e. mainly in the gastric lumen). Gastric leptin act as a neuroendocrine key for satiety. Recent studies shown that Leptin (secreted by the stomach) and CCK can be considered as short-term gastrointestinal signals in the control of feeding. These signals locally activate their receptors on vagal terminals to generate signals that are processed in the NTS

(nucleus of the solitary tract) and the paraventricular nucleus (PVN) of the hypothalamus. The stomach-derived leptin secreted in the lumen enters the intestine in an active form. In the intestine, luminal leptin enhances duodenal CCK release, jejunal absorption of dipeptides transport through PepT-1 transporter, and butyrate uptake by colonocytes via MCT-1. During intestinal inflammation, colon cells express leptin, whereas normal colonic epithelial cells do not, suggesting a role for leptin in inflammation (63). Systemic leptin has also effect on GIT. It can inhibit the active component of galactose absorption mediated by the Na⁺/glucose cotransporter SGLT1 without affecting the passive component of the absorption (62).

Conclusion and feature remarks

The discovery of the *ob* gene and leptin has clearly provided exciting new insights into the mechanisms controlling body weight and composition. Leptin research has also revealed several interexisting new physiological targets which have direct clinical relevance for disease state associated with low leptin concentration and neuroendocrine abnormalities. Since obesity is a chronic disorder, treatment is also likely to be chronic, which raises important issues of tolerability and potential toxicity of any newly developed drug. An enormous amount of progress has been made in understanding leptin physiology since it was first identified 10 years ago (from in-vitro, animal and toxicology studies to human physiology and proof-of-concept treatment studies)- an achievement that tends to be the exception rather than the rule in medical research and that highlights the potential for even greater advances in scientific knowledge and development of treatment in the upcoming years.

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