REVIEW ARTICLE

Leptin: A new aspect of a multifunctional protein

El-Yassin D. Hedef *

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ABSTRACT

The Maintenance of an appropriate body weight is very important for the survival of higher organisms. In order to have a constant weight, there must be an energy balance. Despite short-term mismatches in energy balance, energy intake can generally be matched to energy expenditure with great precision due to the existence of several types of signaling biomolecules such as leptin.

Leptin is an adiposity-derived hormone that decreases food intake and body weight via its receptor in the hypothalamus. In rodents, it also modulate glucose metabolism by increasing insulin sensitivity.

Leptin interacts with pathways in central nervous system and through direct peripheral mechanisms.

Leptin appears to have a range of roles as a growth factor in a range of cell types. Surely, more interactions are yet to be discovered.

Although the complexity of leptin axis indicates that it is difficult to derive effective treatments for obesity, leptin was the first of a group of adiposite-secreted hormones to be used clinically to treat hypoleptinemic status.

This review sheds a small ray of light on the wide landscape of function, roles, actions and interactions of leptin to improve knowledge and point the way to the underlying physiology which predisposes some individuals to apparently unregulated weight gain

INTRODUCTION

Obesity is a serious medical condition with a rising prevalence[1,2,3] and which is in children is of particular concern[4].

Obesity is associated with significant morbidity and mortality[5,6] and poses an immense and increasing public health burden. It can be attributed to increase risk of a number of medical conditions including type 2 diabetes mellitus, hypertension coronary heart disease, which are most common cause of premature mortality in the obese population[5].

Despite the increase in population obesity described above, it should not be forgotten, in individual people, energy balance is usually very precise. Daily intake of food is highly variable and correlates poorly with energy expenditure, whereas over longer periods body weight is stable in most adults[7]. Appetite and food intake are regulated peripherally by adipose tissue and gastrointestinal tract, and these signals are relayed in the hypothalamus (figure 1)[8] .As shown in the figure, leptin is one of the most important peripheral hormones regulating appetite.

* Department of Physiological Chemistry , College of Medicine , University of Baghdad , Baghdad-Iraq

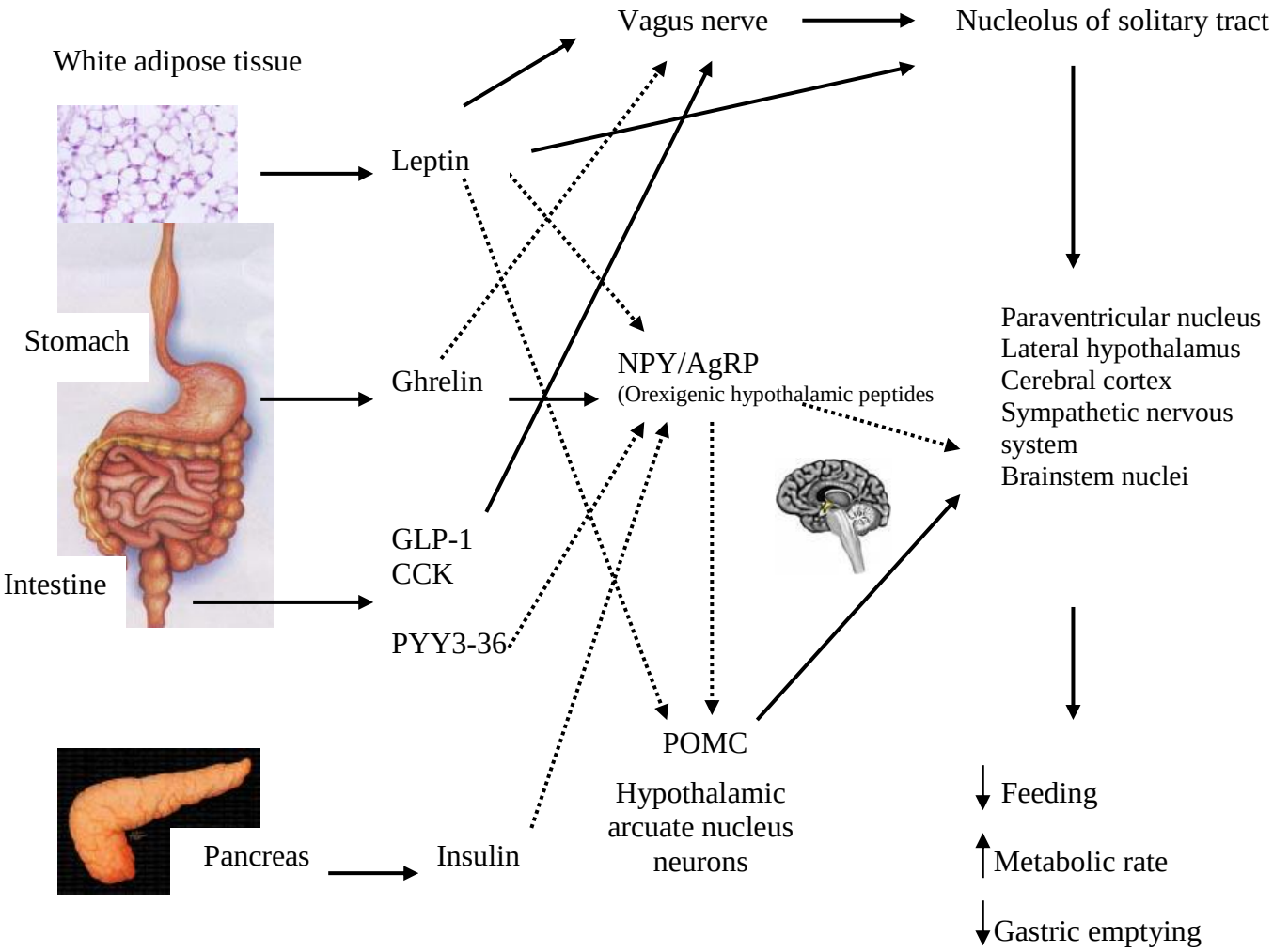

Figure 1 . Circulating gastrointestinal and adipocyte hormones and neural circuits involved in energy homeostasis. Solid lines represent net stimulatory effect, dashed lines represent net inhibitory effect.(Glucagon-like-peptide(GLP-1), Cholecystokinin (CCK), Peptide YY(PYY).

Adipose tissue is the main source of circulating leptin, but leptin is also expressed in non-adipose tissue sites such as the placenta[9], mammary epithelium[10], skeletal muscle[11] and in particular, the stomach[12,13]. The initial view of leptin has been extended to a wider neuroendocrine perspective, being involved in the regulation of a variety of functions including metabolism, neuroendocrine and immune function, and development, all of which are related to energy balance, and acting both through central and peripheral mechanisms[14].

Historical Perspective:

In 1994 there was a major breakthrough when the obese (ob) gene was identified from the examination of naturally occurring mutant ob/ob mice[15]. The protein encoded by the ob gene was named leptin (from the Greek leptos meaning thin), synthesized predominantly in adipose tissues. Circulating leptin levels are directly proportional to adiposity in animals and humans and correlate better with total fat mass than with body weight[16,17,18] . Central and peripheral administration of leptin in rodents causes a profound decrease in food intake and weight loss[19].

1998 sighted the discovery of the production of leptin by the stomach, in addition to its production by adipose tissue[12]. This discovery initiated new investigations on the possible role of this protein in the digestive physiology, in particular in the short-term control of energy balance[20,21]. Leptin has been identified in the lower half of the stomach glands both in the pepsinogen granules of chief cells and in the granules of a specific endocrine cell type, suggesting that leptin action exerted by both exocrine and endocrine pathways[14].

When it was discovered in 1994, leptin thrilled scientists because it seemed so basic to obesity and appetite. Overweight rodents fed leptin lost weight and studies quickly showed that some overweight people had unusually low levels of the hormone[22].

But leptin's effect was not so straightforward in humans, and it became clear that simply injecting obese people with it was not going to make them lose weight[19] . In fact it was found that exposure to leptin early in life affected brain structure involved in weight regulation and so leptin plays an important role in brain development by acting specifically on the clusters of brain cells that regulate food intake[23,24].

The effect of leptin does not seem to be confined to the regulation of body fat or insulin. Gainsford et al.[25] found that both the mRNAs encoding long and short forms of the human leptin receptor were expressed in a diverse group of cells in the hemopoietic organs in both human and mouse. A subset of these cells was able to bind leptin. Leptin enhanced phagocytosis and cytokine production in these cells. These findings show that leptin is a cytokine, which targets various cells in the body.

Synthesis of Leptin :

Leptin is the name given to the protein product of the detective gene discovered in the ob/ob mouse[15].

Transcription of the leptin gene in mice yields a mRNA of ~3.5kb that is expressed primarily in adipose tissues, but recent studies have confirmed that some other tissues also express leptin, including placenta, ovaries, skeletal muscle and stomach[11,12,26,27,28]. In humans, leptin is encoded by a gene located in human chromosome 7q31.3, and is similar to that in rodents[29].

Leptin is translated as a 167 amino acid protein with an amino-terminal secretary signal sequence of 21 amino acids. The signal sequence is functional, and results in the translocation of leptin into microsomes with the subsequent removal of the signal peptide[15] . Therefore, leptin circulates in the blood as protein of 146 amino acid residues.

Leptin Receptors :

The leptin receptor is encoded by a single gene with multiple isoforms being produced by alternative splicing[30,31]. The receptor is a member of cytokine receptor superfamily and has a large extracellular domain, a single transmembrane domain and a cytoplasmic tail, which varies in length between isoforms[32,33,34].The long form of the receptor, which contains at least two suites capable of interacting with JAK proteins, is highly expressed in brain, and particularly in hypothalamic nuclei such as the arcuate which are known to have a role in appetite control[35,36] (figure 2)[8].

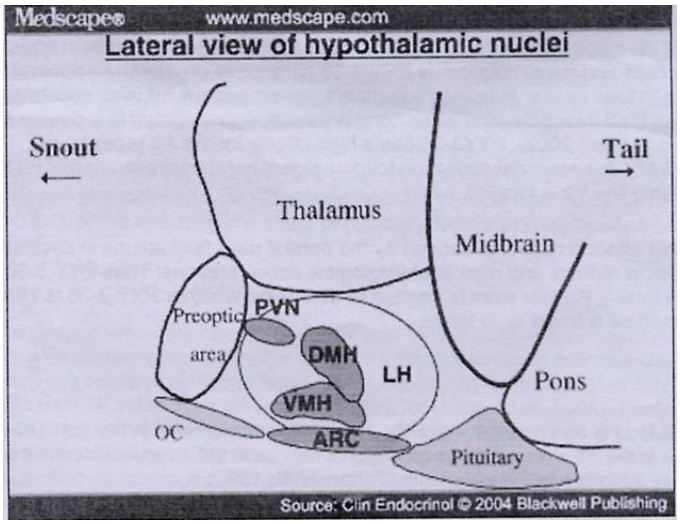

Figure 2 . Hypothalamic nuclei involved in energy homeostasis (lateral view) ARC, arcuate nucleus; PVN, paraventricular nucleus; VMH, ventromedial hypothalamus; DMH, dorsomedial hypothalamus; LH, lateral hypothalamic area; OC, optic chiasm.

The other receptors, whose role in intracellular signaling is much less clear, are widely expressed throughout the body, in which there are numerous reports of the expression of leptin receptors in peripheral tissues, including liver, heart, kidneys, lungs, small intestine, pituitary cells, testes, ovaries, spleen, pancreas, adrenal gland and adipose tissue[26,27,31,37,38,39,40,41,42,43,44] . A mutation in the leptin receptor gene which selectively affects the production of the long form of the leptin receptor, is found in severely obese db/db mice[45].

Sites of Action of Leptin :

Leptin , produced by gastric cells and by adipocytes , could act on both acute and chronic regulation of feeding behaviors respectively, giving information to the brain on the availability of external (food) and internal (fat depots) energy resources, thus participating in short- and long-term satiation[46] Leptin acts on receptors in the hypothalamus of the brain where it:

Counteracts the effect of neuropeptide Y

Counteracts the effect of anadamide .

Promotes the synthesis of α-MSH[47]

Leptin interacts with the reproductive axis at multiple sites, with stimulatory effects at the hypothalamus and pituitary and, inhibitory actions of the gonads[48]. It acts on the hypothalamic

neurons responsible for :
Stimulating the secretion of gonadotropin-relesing hormone (GnRH)[49,50]
Suppressing bone formation. The action of leptin on bone seems to be mediated by sympathic nervous system[51].
In addition to its effect on the hypothalamus, leptin induce metabolic regulation by two major mechanisms:
In muscle via 5'AMP, which regulates the ability of leptin to control fatty acid oxidation[52].
In liver via triglyceride synthesis, in which leptin controls its rate determining step[53] .
It also acts directly on T cells where it enhances the production of Th1 cells promoting inflammation[54]. Mice without leptin are protected from autoimmune disease (which may account for the reports that restricting food intake helps humans with rheumatoid arthritis).
Leptin may have more complex roles than initially thought, raising the question of additional physiological functions of leptin in different tissues[55] .

Leptin and Insulin :

Insulin is an important regulator of energy homeostasis. It stimulates glucose, free fatty acid and amino acid uptake by tissues and tissue anabolism. It is not surprising that a link between leptin and insulin should exist in the regulation homeostasis.
Current knowledge[55] suggests that insulin plays a chronic role in the regulation of leptin gene expression and production by white adipose tissue(WAT). Some studies have shown that hyperinsulinaemia increased plasma leptin concentrations and gene expression in WAT in both rodents and human[56-65].

Leptin and Glucocorticoids :

Adipocytes culture studies have shown that glucocorticoids stimulate leptin gene expression[66,67] . Glucocorticoid secretion is linked with meal times in normal humans and rodents[68] and is increased in Cushing's disease or by glucocorticoid administration to normal volunteers[69] . In humans , glucocorticoids stimulate leptin gene expression and secretion independently of effects on food intake, although increases in insulin or lipogenesis associated with food intake may contribute to leptin production[70,71] .
The mechanism(s) of glucocorticoids stimulation of plasma leptin is still unknown. Wabitsch and co-workers[67] have postulated that glucocorticoids may influence leptin gene expression directly and independently of their differentiation-promoting effects on adipocytes, or they can induce changes in plasma insulin sensitivity. De Vos et al. have shown similar effects on leptin expression in rat[72], however , a later study by the same group[73] indicates that the leptin gene promoter region does not contain a binding site for the glucocorticoid receptor , thus the effect does not rely on the classical molecular mechanism of glucocorticoid receptor action. However, another possible mechanism includes modulations via the CNS, mediated by NPY[74].

Leptin and Growth Hormone :

Although most of the interactions of leptin with growth hormone are likely to be centrally mediated, interactions of leptin with major components of the growth hormone axis such as insulin-like growth factor-1 (IGF-1), appear to be more direct.
Several groups have reported that leptin regulates growth hormone secretion in humans[75,76], rodents[77,78,79], sheep[80,81,82], and pigs[83].

Studies of the effects of IGF-1 on serum leptin and leptin expression are contradictory. Isozaki et al.[84] showed that IGF-1 did not change the percentage body fat or leptin mRNA in visceral fat tissue in Zucker rats while Boni-Schnetzler and co-workers[85] found that IGF-1 decreased leptin mRNA in epidydimal fat tissue of hypophysectomized rats.

Leptin and other Cytokines :

The influence of cytokines on leptin mRNA expression and circulating concentrations has been investigated in human subjects[86,87,88,89] Interleukin-1 (IL-1) was found to induce leptin levels directly or indirectly by increasing the activity of the hypothalamic-pituitary axis[86,90] Mantzoros and co-workers[89] reported a positive independent association between tumor necrosis factor-α (TNF-α) levels and circulating leptin concentrations, suggesting that TNF-α may directly induce leptin gene expression in humans, as it does in rodents[90,91]. These studies may implicate a role for leptin in the pathogenesis of cachexia that is accompanied by increased levels of cytokine in advanced stages of AIDS and cancer[92].

Role of Leptin in Obesity and Hypertension :

Obesity is usually associated with hypertension, possibly resulting from sympathetic nervous system activity. Obese humans have increased circulating levels of leptin[93] and given the associations with the sympathic nervous system, this might contribute to the pathogenesis of hypertension in obesity. The diminished effect of leptin on satiety, manifested as so-called leptin resistance, with maintained obesity despite elevated leptin concentrations, makes such a mechanism unlikely. However, one can not role out the possibility that the leptin signal, via central leptin receptors is specially inhibited to pathways of satiety but not to other functions, such as to sympathetic nervous system.

Role of Leptin in Diabetes :

In humans, low adiponectin concentrations in plasma are associated with a higher risk of developing type 2 diabetes[94,95]. However, the contribution of low adiponectin levels to metabolic syndromes of leptin-dificient states in humans is not clear.

Role of Leptin in Reproductive System :

Recently it was found that leptin plays an unexpected role in reproduction[49,50,96]. Human ovary and prostate and murine ovary and embryo express mRNA for the leptin receptor[30,43,97] . Although much evidence suggests a central site for the effects of leptin on the reproductive axis, a direct effect on the ovary has also been demonstrated as leptin at physiological concentrations inhibits insulin-induced oestradiol production by granulose cells from both small and larger bovine follicles[28, 98,99].

Role of Leptin in Fertility :

The sterility of male and female ob/ob mice is a recognized feature of this mutation[100]. Hoggard and co-workers[101] found that reproductive hormone levels are reduced in ob/ob females, suggesting a functional defect in the hypothalamic-pituitary axis.
Furthermore, in vitro studies have revealed that oestrogens increase leptin mRNA expression[102, 103].

Testosterone , in contrast, inhibits leptin gene expression in vivo and in vitro[102,104] possibly through a direct suppressive effect. These findings suggest that androgens and oestrogens modulate leptin expression at the mRNA level through sex steroid receptor-dependent transcriptional mechanisms[102].The study of Jockenhovel et al.[105] indicated that testosterone substitution normalized elevated serum leptin levels in hypogonadal men. These investigators have concluded that interaction of testosterone and leptin might be part of a hypothalamic–pituitary-gonadal-adipose tissue axis that is involved in body weight maintenance and reproductive function.

Role of Leptin in Pregnancy :

In females of most mammalian species, high leptin levels may signal the attainment of the sufficient long-term energy stores that are crucial for successful reproduction[49]. Leptin level are known to rise during pregnancy[106, 107].
Hyperleptinemia in early pregnancy appears to be associated with the development of gestational diabetes . But studies investigating maternal leptin concentrations and gestational diabetes have yielded conflicting results[108] .
However, hyperleptinaemia during pregnancy is not associated with decreased food intake or a decline in metabolic efficiency. Explanations for this may be a possible pregnancy-induced state of leptin resistance or a change in leptin bioavailability[109, 110] .
There appear to be number of possible explanations for the increase in leptin levels in pregnancy:
increased production by maternal fat;
increased expression by the placenta;
And increased levels of binding protein(s)[101,111] .

Clinical Uses of Leptin :

Metabolic effects of leptin therapy in obesity
Leptin therapy leads to decreased food intake and weight loss, associated with a marked decrease in body fat percentage, and increases in resting energy expenditure, body temperature and activity levels[22, 112] . See figure 3[113]

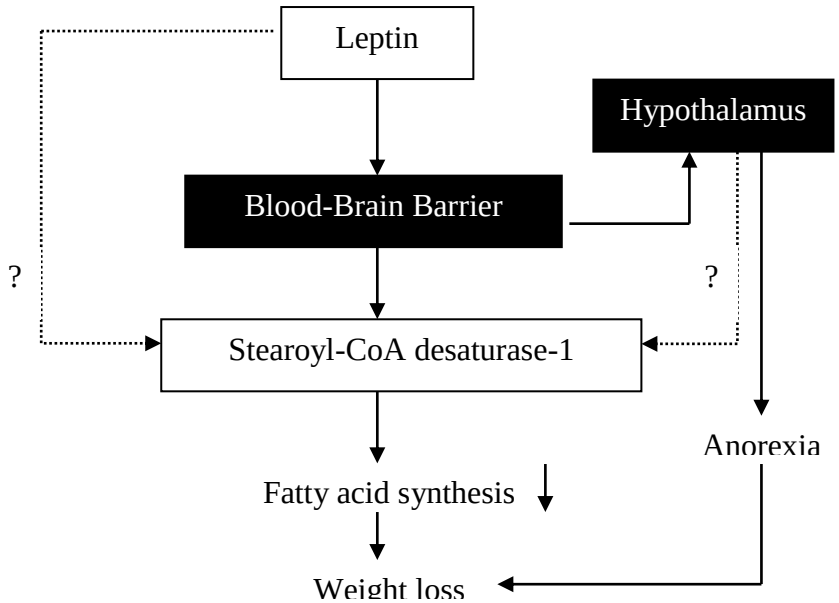

Figure 3 . Effect of leptin on weight regulation by inducing anorexia and by reducing hepatic fatty acid synthesis.

Leptin has been administered to children with ob gene mutation who have essentially no circulating leptin[114, 115] .The major manifestation of the clinical phenotype is extreme obesity and the most immediate effect of leptin therapy both in mice and human is to induce satiety. However the concomitant increase in resting energy expenditure observed in the ob/ob mouse after leptin therapy[116] does not occur in humans. Up to 98%of the weight loss observed in these children caused by a loss of fat mass. Because this is occurring in growing children it is likely that this obscures a loss of lean body mass that is typical of dietary weight loss and leptin therapy in lipodystrophy patients[117] .

Effects of leptin therapy on the hypothalamic-pituitary axis :

Leptin deficiency in the ob/ob or lipoatrophic mouse results in suppression of the gonadal and thyroid endocrine axes and activation of the adrenal axis[118,119,120,121] . In children with leptin mutations, leptin administration is associated with normalization of gonadotrophic possibility[122,123]. In adolescent women with low leptin levels and lipodystrophy, luteinizing hormone (LH) secretion is low , does not respond to LH-releasing hormone[124] . Following leptin treatment, there is a robust response to the releasing factor and most young women acquire regular menses. In humans, leptin therapy does not induce puberty, but appears to be necessary for normal gonadothrophin function once puberty ensues[124].
In normal, 72 hour starvation induces a disruption of both LH and thyroid-stimulating hormone pulsitility, as well as decrease in circulating testosterone[125]. These changes are associated with a fall in serum leptin concentration and are prevented by physiological leptin replacement. There is no significant change in either thyroid or glucocorticoid hormone levels[125,126,127] .
Disruption in hypothalamic-gonadal and other axes due to energy deficit are associated with low levels of leptin and result in hypothalamic amenorrhea. Exogenous recombinant leptin replacement improves reproductive and neuroendocrine function in women with hypothalamic amenorrhea[128].

Role of leptin in hepatic steatosis :

In leptin-deficient rodent (i.e. ob/ob and lipotrophic mice) and humans with congenital leptin deficiency and lipodystrophy, there is a massive accumulation of triglycerides in blood, liver and other tissues. In humans, this is connected with progressive liver disease known as non-alcoholic steatohepatitus. Leptin therapy is associated with a decrease in liver triglycerides, a reduction of liver volume and possibly, prevention of liver fibrosis[126]. This is consistent with the hypothesis that one of the major functions of leptin is to partition lipid away from non-adipose cells where it appears to have a toxic effect[129].

Immunological effects of leptin :

Leptin has the structural characteristics of a cytokine and binds to a cytokine receptor system. In although leptin administration can augment certain lymphocyte subsets and enhance responses to immune stimulators in vitro, there is no clear immunodeficiency phenotype in either congenital leptin deficiency or lipodystrophy[122]. Thus the physiological role of leptin interaction with lymphocytes, monocytes and platelets remains to be determined in patients(clinical uses of leptin).

Leptin may be involved in sympathetic activity of the heart[130] and may also provide a link between obesity and colon cancer [131] . These fields are still to be studied.

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