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Leptin as a Modulator of Neuroendocrine Function in Humans

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Leptin, a peptide hormone secreted by adipocytes in proportion of the amount of energy stored in fat, plays a central role in regulating human energy homeostasis. In addition, leptin plays a significant permissive role in the physiological regulation of several neuroendocrine axes, including the hypothalamic-pituitary-gonadal, -thyroid, -growth hormone, and -adrenal axes. Decreased levels of leptin, also known as hypoleptinemia, signal to the brain a state of energy deprivation. Hypoleptinemia can be a congenital or acquired condition, and is associated with alterations of the aforementioned axes aimed at promoting survival. More specifically, gonadotropin levels decrease and become less pulsatile under conditions of energy deprivation, and these changes can be at least partially reversed through leptin administration in physiological replacement doses. Similarly, leptin deficiency is associated with thyroid axis abnormalities including abnormal levels of thyrotropin-releasing hormone, and leptin administration may at least partially attenuate this effect. Leptin deficiency results in decreased insulin-like growth factor 1 levels which can be partially ameliorated through leptin administration, and leptin appears to have a much more pronounced effect on the growth of rodents than that of humans. Similarly, adrenal axis function is regulated more tightly by low leptin in rodents than in humans. In addition to congenital leptin deficiency, conditions that may be associated with decreased leptin levels include hypothalamic amenorrhea, anorexia nervosa, and congenital or acquired lipodystrophy syndromes. Accumulating evidence from proof of concept studies suggests that leptin administration, in replacement doses, may ameliorate neuroendocrine abnormalities in individuals who suffer from these conditions.

Key Words: Leptin, leptin deficiency, amenorrhea

INTRODUCTION

Leptin, an adipocyte-secreted hormone, was first discovered in 1994 through positional cloning of the *ob/ob* mouse model of obesity.[1] Since then, much has been discovered about leptin's role in regulating energy homeostasis, neuroendocrine, and immune functions. Leptin is secreted in direct proportion to the amount of energy stored in adipose tissue, and circulating leptin levels serve as a peripheral sig-

nal to the central nervous system about the amount of energy reserves available for reproductive and other essential functions.[2] Thus, circulating leptin levels dictate the body's energy homeostasis and neuroendocrine, immune as well as metabolic function.[3-5] More recently, human and rodent studies have demonstrated that leptin plays an important role in regulating neuroendocrine axes such as the hypothalamic-pituitary-gonadal axis,[6-8] the thyroid axis,[6,7] the growth hormone axis,[7,9] and the adrenal axis.[10,11] Leptin levels are also influenced by several other factors including acute change in energy intake; leptin levels decrease in response to acute and chronic energy deprivation.[7,8,12] It appears that leptin may be especially important in mediating neuroendocrine adaptations that act in a concerted manner to conserve energy.[6-8] We review herein the current state of knowledge about the effects of leptin, leptin deprivation, and leptin replacement on these neuroendocrine axes.

Biology of leptin; an overview

Leptin, the product of the *ob* gene in rodents[1] and the *LEP* gene in humans, is a 167-amino acid, 16 kDalton, adipocyte-secreted hormone. It structurally resembles a cytokine and as it was one of the first adipocyte-secreted hormones to be discovered with this cytokine-like structure, it is the prototype of the adipocytokine proteins. Orthologs of leptin with slightly varied amino acid sequences but conserved functional properties and tertiary structure have been found in several species including fish and reptiles.[13] Leptin is produced primarily by the white adipose tissue, but is also expressed in other tissues such as the brown adipose tissue,[14] the primary and secondary lymphoid organs,[15] the bone marrow, the mammary epithelium, the ovaries, the skeletal muscle, and the placenta to mention a few.[16] Leptin levels in humans are secreted in proportion to energy stored in fat (total body fat mass).[17,18] Acute changes in caloric intake, mainly acute decreases in food intake, have been shown to have a dramatic effect on circulating leptin concentrations. For example, fasting for one or three days results in a marked decrease of leptin levels (by 50% and 80% respectively).[7,8,12] Leptin levels in humans are also influenced by sex steroid levels,[3,19-21] thyroid hormones, cytokines, and other factors to a lesser degree than the factors mentioned above.[22-24]

Leptin exerts its effects through binding and activating specific leptin receptors, which are coded for by the *db* gene in mice[25] and the *LEPR* gene in humans.[26] These receptors exist both in the central nervous system, especially the hypothalamus,[27,28] kidneys, lungs,[29] lymphocytes,[28,30-32] adipose tissue,[21,33,34] prostate, ovaries, liver, small intestines, and heart.[16,35] There are at least six isoforms of the murine leptin receptor,[36] whereas in humans, only four alternatively spliced isoforms have been described.[37,38] The ObRb receptor, the long form of the leptin receptor, is considered the active leptin receptor and is highly expressed in the hypothalamus, including in nuclei associated with body weight control.[29]

Circulating leptin levels in humans

Leptin levels in the blood stream display a circadian pattern such that leptin concentration is at the lowest point between early afternoon and mid-afternoon and is at its highest point between midnight and early morning.[39,40] Furthermore, leptin secretion appears to be pulsatile.[11] Though the pulsatility of leptin secretion is similar in obese and in lean individuals, the amplitude of leptin pulses is greater among obese individuals.[11]

Several factors contribute to the inter-individual variability of leptin levels in humans, including gender and total body fat mass.[41] Leptin levels display sexual dimorphism, with women having higher levels than men even after controlling for adiposity.[42] Sex hormones such as testosterone[43] and estrogen[44,45] explain some of the gender variation in leptin levels. Among women, leptin levels appear to be at their highest level during the luteal phase of the menstrual cycle.[46] Distribution of fat also plays a role in variability of leptin levels; subcutaneous fat appears to produce more leptin than omental fat.[47]

NEUROENDOCRINE FUNCTIONS OF LEPTIN IN HUMANS

Data on the neuroendocrine functions of leptin in humans emanate mainly from case reports of congenital leptin deficiency and leptin administration to these individuals,[48-50] as well as from observational and interventional physiology studies involving fasting and/or weight loss in normal subjects followed by leptin administration.[7,8,51,52] In a direct extension of these studies, leptin physiology and pathophysiology has also been studied using various disease states as experimental models. These include conditions associated with relative leptin deficiency, such as hypothalamic amenorrhea and lipodystrophies.[48,53,54] We summarize findings from these studies in the following paragraphs.

GONADAL AXIS

Humans with genetic mutations that lead to congenital leptin deficiency experience hypogonadotropic hypogonadism and failure to undergo puberty;[55] the latter is restored by leptin administration in replacement doses.[48,49,56] These data in the extremely rare subjects with congenital leptin deficiency are consistent with our finding that a rise in leptin levels precedes the onset of puberty in normal boys.[57] Even in rodents, exogenous leptin administration results in earlier onset of markers of puberty including vaginal opening,[58] and leptin antibodies appear to inhibit pubertal onset in female rats.[59] Although *ob/ob* mice have complete leptin deficiency and are infertile, female *ob/ob* mice can ovulate and give birth if they are treated with leptin in replacement doses,[60] which stimulates the secretion of luteinizing hormone (LH) *in vivo*.[61] In mice, fasting-induced hypoleptinemia also diminishes the levels of gonadotropins and impairs the reproductive function and sex hormone levels of these mice; however, leptin administration restores testosterone levels in male mice, estrous cycles in female mice, and LH levels in both.[6]

In a follow-up to our rodent experiments and observational studies in humans, we have also demonstrated that caloric deprivation of normal-weight men decreases testosterone levels as well as LH pulsatility, effects that can be fully normalized by administering leptin in physiological replacement doses.[7,62] Similarly, caloric deprivation that leads to partial leptin deficiency in normal-weight women decreases their LH peak frequency, and this effect can be reversed through leptin administration.[8] Leptin's effect on luteinizing hormone secretion appears to proceed via an indirect mechanism, as neurons that secrete gonadotropin-releasing hormone (GnRH) do not have leptin receptors.[63] There is evidence that leptin may act on groups of neurons that in turn provide input to populations of GnRH secreting neurons in regions of the brain such the preoptic area.[21,64,65] These groups of neurons may include agouti-related peptide/neuropeptide Y and proopiomelanocortin neurons[64] as well as neurons in the arcuate nucleus that express kisspeptin, bradykinin, and dynorphin, that regulate GnRH secreting neurons[66] either directly or through KiSS-1 neurons.[67]

A well-studied mediator of the relationship between leptin and reproduction is kisspeptin. This protein is a product of the *Kiss1* gene.[68] Kiss1 mRNA expression is reduced in the caudal hypothalamus of fasting female rats[69,70] and *ob/ob* mice express Kiss1 mRNA to a lesser degree than do their wild-type counterparts.[67] The expression of Kiss1 mRNA is partially restored with the administration of exogenously administered leptin[67] whereas exogenously administered kisspeptin to rodents that are relatively leptin deficient stimulates the secretion of GnRH[71] and increases levels of LH, follicle-stimulating hormone,[72,73] and testosterone.[73] Populations of neurons with leptin receptors in brain regions such as the hypothalamic ventral premammillary nucleus and the preoptic region have been found in close proximity to both Kiss1 and GnRH neurons.[74] Although leptin's initiation of puberty in mice requires a functional ventral premammillary nucleus, this does not appear to require the action of Kiss1 mRNA expressing neurons.[75]

THYROID AXIS

Similar to leptin, thyroid-stimulating hormone (TSH) has a pulsatile and circadian pattern of secretion, and we have found that both hormones have similar circadian patterns.[76] In congenitally leptin-deficient individuals, we have observed that TSH secretion is disorganized.[76] It has also been reported that leptin-deficient children exhibit an increase in triiodothyronine (T3) and thyroxine (T4), though not in TSH, after leptin therapy in an uncontrolled interventional study;[49] however, one boy, one man, and two women, all of whom had congenital leptin-deficiency, demonstrated normal thyroid function whether or not they were undergoing leptin replacement.[50]

In rodents, leptin deficient *ob/ob* mice demonstrate much more pronounced thyroid disorders, including hypothalamic hypothyroidism since birth.[77] Similarly, fasting decreases serum levels of T4 in mice, though this effect can be ameliorated through leptin administration.[6] Leptin regulates thyrotropin-releasing hormone levels by increasing pro-TRH gene expression in neurons in the paraventricular nucleus of the hypothalamus,[78] probably through the central melanocortin system.[79] Leptin also stimulates the expression of prohormone convertases 1 and 2, which cleave pro-TRH in order to produce TRH in the paraventricular nucleus.[80]

To directly test the role of leptin in regulating thyroid function in humans, we administered leptin in the context of a randomized, placebo controlled interventional study involving fasting of normal-weight men for 72 hours. Fasting-induced hypoleptinemia resulted in altered TSH levels and secretion patterns and leptin administration ameliorated the degree to

which caloric deprivation altered TSH secretion patterns and levels.[7] In contrast to men, in whom significant hypoleptinemia was induced (levels less than 2-3 ng/mL), similarly pronounced effects of leptin administration were not seen in a similar study on normal-weight women[8] in whom leptin levels were decreased but remained within normal limits. We proposed that the reason for the discrepant results between these two otherwise similar studies could be the fact that men experienced a decrease in leptin levels to an average of 0.27 ng/mL (much lower than the lower normal level in our laboratory, 3 ng/mL).[7] In contrast, leptin levels dropped to an average level of approximately 3 ng/mL in women[8] and thus we suggested that leptin appears to have a threshold level for regulation of TSH.[81] In contrast to TSH, T3 and T4 were much less regulated in humans than in rodents.

These data, taken together, suggest that although leptin may have a significant effect in regulating the secretion pattern of TSH in humans, its role in regulating circulating levels of T3 and T4 may not be as important in humans as it is in rodents and may be different in complete leptin deficiency than in relative, acute hypoleptinemia.[21,65,81]

A subsequent interventional but non-randomized study focused on both lean and obese participants who were studied before and after they had lost 10% of their body weight (and thus became relatively hypoleptinemic) over the course of an average of eight weeks.[51] Though all participants experienced decreased levels of leptin, obese subjects still had relatively higher levels, which ranged from 10 ng/mL up to 60 ng/mL.[51] Levels of TSH, T3, and T4 were reportedly all decreased, though leptin replacement only increased T3 and T4 levels in these subjects.[51] The authors of this study suggest that leptin may increase the bioactivity of TSH or stimulate T4 secretion, but these results from this non-randomized study remain to be replicated by future randomized studies involving administration of leptin in physiological replacement doses.[51] We have recently reported that administering leptin in pharmacological doses to subjects undergoing a 6-month-long mild hypocaloric diet does not appear to alter levels of the circulating hormones of the thyroid axis but doses administered were supraphysiological and may have thus suppressed leptin receptors leading to suboptimal results.[82]

GROWTH HORMONE AXIS

Humans who are leptin-deficient due to mutations of the leptin gene demonstrate normal growth velocity in childhood[48,49] although their final height is decreased due to the lack of pubertal growth spurt.[83] We have proposed on the basis of the above and other experimental data from our own physiology studies in humans that leptin may regulate growth hormone's ability to stimulate the secretion of insulin-like growth factor 1 (IGF-1) as well as the corresponding binding proteins in the periphery as opposed to acting directly on pituitary secretion of growth hormone itself.[84]

We have shown by studying a group of normal-weight men who became truly hypoleptinemic through prolonged fasting that leptin administration tends to restore total IGF-1 levels, which are decreased due to caloric deprivation.[7] In normal weight women, though, we found that leptin replacement did not significantly normalize IGF-1 levels.[8] Again, this was interpreted as illustrative of leptin having a threshold for neuroendocrine regulation as the men's leptin levels dropped below 3 ng/mL whereas the women's average leptin level did not.[81] Similarly, administering leptin to euleptinemic subjects undergoing a 6-month-long mild hypocaloric diet did not appear to alter levels of the circulating levels of IGF-1 or other hormones in the IGF axis.[82]

ADRENAL AXIS

In vitro evidence indicates that corticotropin-releasing hormone is released in response to leptin in a dose-dependent manner[10] and suggests that leptin decreases the secretion of corticosterone from cells of rat adrenal cortex.[85] Additionally, leptin decreases the degree to which stress increases ACTH and corticosterone levels.[86]

On the basis of very small and non-randomized studies, it has been suggested that the adrenal axis of individuals with mutated leptin or leptin receptor genes may not be significantly impaired.[26,48,49] Similarly, leptin administration in a group of men who became hypoleptinemic in response to fasting for 72 hours did not appear to have a major effect on cortisol secretion.[7] Likewise, leptin administration did not attenuate significantly the activation of the adrenal axis in a similar study of fasting women.[8] In contrast, in the context of a larger randomized placebo-controlled study, we found that women with hypothalamic amenorrhea who were hypercortisolemic did experience a statistically significant decrease in cortisol levels after they were treated with replacement doses of metreleptin.[87] Along the same lines, we have reported that there appears to be an inverse relationship be-

tween healthy men's fluctuations in circulating levels of leptin and both cortisol and ACTH.[11] Thus, the effect of leptin to regulate the adrenal axis in humans is rather small in magnitude and thus can be detected only in larger, randomized studies.

POTENTIAL APPLICATIONS OF LEPTIN IN HUMAN PATHOPHYSIOLOGY AND THERAPEUTICS

Congenital leptin deficiency

Mutations of the leptin gene result in congenital leptin deficiency, a rare condition in humans seen more commonly in populations where consanguineous marriage is relatively more common.[88] Congenital leptin deficiency leads to obesity, which arises early in life, due to uncontrollable hyperphagia.[89] It is also accompanied by neuroendocrine abnormalities such hypothalamic hypogonadism and pubertal failure[55] which can be treated with leptin administration in replacement doses.[48,49,56] As mentioned above, these individuals may have an impaired pituitary-thyroid axis (though one case series did not find abnormal thyroid function[50]), with increased T3 and T4 levels and unchanged TSH levels after leptin replacement.[49] Leptin is currently available on a compassionate basis for the treatment of morbid obesity and hypothalamic hypogonadism of these subjects.

Hypothalamic amenorrhea

Hypothalamic amenorrhea (HA) caused by an imbalance between energy expenditure and energy intake associated with excessive stress, excessive exercise, or inadequate food intake leads to significant neuroendocrine abnormalities, infertility and osteoporosis/stress fractures.[90] In association with low fat mass, women with HA have abnormally low levels of leptin.[91] We have reported that administration of leptin, in replacement doses, may lead to normalization of neuroendocrine action in women with hypothalamic amenorrhea.[54] Leptin replacement normalized LH levels and pulsatility within weeks and ovulation within months in an open label study with a course of treatment of ten weeks.[54] Moreover, in a subsequent placebo-controlled, double-blind, randomized study with a larger sample size we confirmed these results using a course of treatment of nine months.[92]

The aforementioned ten-week study of leptin replacement in women with hypothalamic amenorrhea yielded increased levels of bone-specific alkaline phosphatase and osteocalcin, two bone formation markers.[54] More definitive results in terms of changes in overall or regional bone mineral content or density were reported in the nine-month long randomized placebo-controlled study.[87] In this study, six women underwent nine months of double-blind leptin administration and then elected to receive an additional year of open-label leptin administration; these women experienced significant gains in bone mineral content and density.[87]

Anorexia nervosa

Anorexia nervosa is associated with hypoleptinemia.[53] Among amenorrheic anorexic women with who have gained weight, circulating leptin levels are higher in those whose menses had resumed than in those who had remained amenorrheic.[93] Unfortunately, there may be mild weight and/or fat loss with leptin replacement in lean women,[54,92] which may be a potential reason not to administer leptin to anorexic women.

Lipodystrophy

Lipodystrophy is a condition characterized by abnormal distribution of adipose tissue. It is either inherited, in a very small number of patients, or can be acquired. The latter is much more frequent and most often occurs in HIV-positive subjects being treated with highly-active antiretroviral therapy for human immunodeficiency virus (HIV) infection.[94] Subjects with significant degrees of lipodystrophy have lower leptin levels than unaffected subjects.[95,96] Leptin administration in replacement doses is associated with normalization of neuroendocrine parameters in subjects who have lipodystrophy.[97] Additionally, and probably even more importantly, leptin administration in replacement doses to subjects with lipodystrophy improves their metabolic abnormalities; including hypertriglyceridemia and impaired glucose control which often are resistant to maximum doses of insulin sensitizers or very high doses of insulin.[98,99] The development of anti-leptin antibodies has been considered by many as a factor that may limit the use of this medication in humans but data remain inconclusive.[100,101]

CONCLUSION

In conclusion, it appears that leptin regulates neuroendocrine function in humans and has a special role in mediating the neuroendocrine response to energy deprivation. Directions for future research include further elucidating the anatomical connections between energy homeostasis and

neuroendocrine functions and the specific molecular mechanisms underlying these connections.

Additionally, it appears that leptin levels are decreased in conditions such as congenital leptin deficiency, hypothalamic amenorrhea, anorexia nervosa, and lipoatrophy, conditions in which a leptin measurement in the blood could provide important novel diagnostic information. Small-scale proof-of-concept studies have shown that several of the neuroendocrine and other abnormalities associated with these conditions can be ameliorated via leptin therapy in replacement doses. Future research should involve larger-scale phase III placebo-controlled leptin administration trials to fully establish leptin's therapeutic role in disease states associated with leptin deficiency.

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