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Role of Leptin in Reproduction

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ABSTRACT

Leptin, an adipocyte hormone plays an important role in regulating energy homeostasis by inhibiting hunger, interacts with the reproductive axis of mammals at multiple sites with stimulatory effect at the hypothalamus and pituitary and its high dose has inhibitory action on steroidogenesis. As a marker whether nutrition stores are adequate, leptin may act in concert with gonadotrophins and growth hormone axis for the initiation of complex phenomenon of puberty. The expression and secretion of leptin are correlated with body fat mass and are acutely affected by feed intake. Moreover, circulating leptin increases during pubertal development in rodents and domestic animals. Effects of leptin are mediated mainly via receptor activation of the JAK-STAT pathway; however, activation of alternative pathways, such as MAP kinase, has also been reported. Leptin helps in oocyte development and maturation .It also has angiogenic, immuno-modulatory and anti inflammatory activities helps in implantation and prevents embryo rejection by the maternal immune system, invasion of trophoblast and cause mammary growth development.

Keywords: Leptin, hypothalamus, pituitary, gonadotrophins, steroidogenesis

Leptin is a 16-kDa protein that is produced mainly by adipose tissue, but also by placenta, stomach and skeletal muscle. Leptin, whose tertiary structure resembles that of cytokines and lactogenic hormones, is encoded by the *ob* gene which comprises three exons. The 167 amino acid (aa) protein circulates in the blood (at concentrations paralleling the amount of fat reserves), and acts at the hypothalamic level as a satiety factor. Leptin circulates in biological fluids both as a free protein and in a form that is bound to the soluble isoform of its receptor (Ob-Re). Leptin secretion is pulsatile and shows a circadian rhythm, with a nocturnal rise. This pulsatile pattern is synchronized with that of luteinizing hormone (LH). The identification and cloning of the leptin receptor (Ob-R) in mice and humans have given new insights into the complexity of the actions and targets of leptin. Ob-R belongs to the gp130 family of cytokine receptors; it has a single membrane-spanning domain and exists in different isoforms (Ob-Ra, Ob-Rb, Ob-Rc, Ob-Rd and Ob-Re) that derive from alternative splicing of mRNA. All isoforms have an identical ligand-binding domain but differ at the C-terminus. Only Ob-Rb contains a long intracellular domain and carries both of the protein motifs that are necessary for the activation of the Janus kinase signal transducers and activators of transcription (JAK–STAT) pathway. Ob-Rb is mainly expressed in the hypothalamus, and its expression is much

lower in peripheral tissues, where the prevalent isoform is Ob-Ra.

In vivo models of leptin deficiency, resistance and excess

The existence of an animal model with a homozygous mutation of the *ob* gene is a valuable tool for studying the biochemical messages between fat stores and the reproductive axis. The *ob*/*ob* mouse is infertile and has severely impaired spermatogenesis, probably because of an insufficient hypothalamic–pituitary drive and consequent low circulating gonadal steroids. In *ob*/*ob* mice, the administration of leptin leads to weight gain of the uterus and ovaries in the female and of the seminal vesicles and testes in the male, and restores fertility in both sexes. Conversely, caloric restriction does not restore fertility in the *ob*/*ob* mouse, which suggests that obesity *per se* is not the cause of infertility in leptin deficiency, and that leptin is directly related to the modifications of reproductive capacity.A mutation in the *db* gene, the gene encoding Ob-R, results in the synthesis of a truncated leptin receptor that is devoid of the intracellular domain. The *db*/*db* mouse shows alterations of reproductive function similar to those of the *ob*/*ob* mouse, but leptin treatment is unable to either modify the appetite of the animals or to restore fertility because the defect is at the receptor level. Mutations of the genes encoding leptin and its receptor have also been found in humans, and these have phenotypic characteristics similar to those shown in mice The availability of a murine model with chronically raised leptin levels has also provided new insights into the role played by leptin in reproduction. With no apparent adipose tissue and high leptin concentrations, the female transgenic skinny mice exhibit accelerated puberty and intact fertility at younger ages, followed by late-onset hypothalamic hypogonadism that is characterized by prolonged estrus, atrophic ovaries and reduced gonadotropin-releasing hormone (GnRH) and LH secretion. Hyperleptinemia *in vivo* seems to facilitate the onset of puberty but, if chronically persistent, it can later downregulate the central leptin signals that stimulate reproductive function, or interfere with gonadotropin stimulation of peripheral targets. Thus, it is possible that there are multiple sites of leptin action and different concentration thresholds for the several effects of leptin on reproduction.

Leptin and gonadotropin secretion

Circulating levels of leptin are closely correlated with the degree of adiposity in the fed state, and fall rapidly on caloric restriction. The long signaling isoform of Ob-R has been localized mainly within the hypothalamus, in the arcuate and ventromedial nuclei, areas controlling both food intake and sexual behavior. In these hypothalamic nuclei, leptin mediates the peripheral messages of energy balance and energy stores, linking together metabolic status and brain function. Much evidence indicates that leptin, as a signal for starvation, mediates the under nutrition induced alterations of the reproductive axis. In a pioneering study performed in mice, it has been found that preventing the starvation induced fall in leptin with leptin administration substantially blunts the changes seen in the gonadal axis in males and prevents the starvation-induced delay in ovulation in females. In subsequent studies, in primates, it has been shown that intravenous leptin infusion maintains LH pulsatility in fasted male rhesus macaques, thereby demonstrating the ability of leptin to counteract the inhibitory effect of fasting on gonadotropin secretion. Furthermore, in experiments designed to mimic the fall of leptin during fasting (leptin antiserum was administered into the lateral ventricles of normally fed rats), LH pulsatility decreased and estrous cyclicity ceased. These data strongly suggest that leptin exerts a tonic facilitatory effect on the central networks that regulate pituitary gonadotropin secretion. However, leptin does not overcome the suppression of sexual behavior in food-deprived animals.

Leptin and GnRH neurons

In vitro observations have shown that subnanomolar concentrations of leptin stimulate GnRH release from rat median eminence–arcuate nuclear explants after acute incubation. These data were confirmed by later experiments performed in an immortalized GnRH-secreting neuronal cell line (GT1-7) that expresses Ob-R. In these cells, GnRH release was stimulated by exposure to low (10^{-12} to10^{-10} M). Taken together, these data strongly suggest that leptin acts centrally to influence reproduction, and that the stimulatory effect is exerted only over a relatively narrow range of leptin concentrations. It is debatable whether leptin actions are exerted directly on GnRH neurons or through an interneural circuit. So far, double-labeling studies conducted on rodents and primates have failed to demonstrate the coexpression of GnRH and leptin receptor in cell bodies, and the hypothesis of neuronal intermediaries between leptin and GnRH neurons seems the most probable. It has been shown that there is abundant coexpression of Ob-R with proopiomelanocortin (POMC), neuropeptide Y (NPY) and Agouti-related peptide (AgRP) neurons in the arcuate nucleus of rat, that *POMC* expression is reduced by 50% in *ob*/*ob* mice, and that leptin administration restores *POMC* mRNAexpression to control levels. It is known that the inhibitory actions of leptin on food intake are mainly mediated by signals transduced by melanocortin receptors, in particular by the predominant neuronal isoforms MC_3 receptor and MC_4 receptor. POMC-deficient mice and humans are hyperphagic, obese and leptin resistant. Central melanocortin antagonism produces a similar phenotype, whether accomplished by overexpression of the MC receptor antagonist peptide AgRP, or by infusion of a synthetic antagonist. In addition, disruption of the genes encoding MC_3 receptor and MC_4 receptor increases fat mass and causes obesity in the mouse. These observations suggest that leptin might modify reproductive function by modulating the availability of the POMC protein in the brain. However, recent data derived from studies in MC_3 receptor-, MC_4 receptor- and NPY-deficient mice did not show major alterations of the reproductive axis or fertility, thereby indicating that it is unlikely that these systems mediate the reproductive effects of leptin.

The cocaine- and amphetamine-related transcript (CART) peptide has been shown recently to be an endogenous inhibitor of food intake regulated by leptin, and to mediate the acceleration of pulsatile GnRH secretion. Interestingly, an anti-CART antiserum partially prevented the leptin-induced reduction of the GnRH interpulse interval from hypothalamic explants of peripubertal rats, suggesting that CART might be an important mediator of leptin action on GnRH-secreting neurons, specifically at the time of the onset of puberty. Further studies are necessary to elucidate the neural circuits responsible for the actions of leptin on GnRH neurons.

Leptin and pituitary

mRNA transcripts encoding the leptin receptor have been found in adult rat pituitary, human fetal pituitary, pituitary adenomas and ewe anterior pituitary. Immunohistochemical studies demonstrated Ob-R protein expression in 29% and 90% of the gonadotropes of the pars distalis and the pars tuberalis of ovine anterior pituitary, respectively. Other studies confirmed the presence of both the long isoform of the receptor and leptin in normal and neoplastic human pituitary tissue[47], which indicates that leptin has the potential to regulate pituitary function via endocrine and paracrine and/or autocrine mechanisms. In recent experiments, 16 out of 47 pituitary adenomas released leptin into the incubation medium, and leptin release did not correlate with tumor type or with the release of other pituitary hormones. *In vitro* studies showed that incubation of male adult rat anterior hemi-pituitaries and pituitary tumors with leptin led to a dose-dependent

release of gonadotropins. Consequently, leptin might directly and positively influence the function(s) of the gonadotropes by amplifying the stimulatory actions on the hypothalamus–pituitary–gonadal (HPG) axis exerted at the hypothalamic level; in addition, leptin might be involved in the regulation of the growth and differentiation of pituitary cells.

Leptin and puberty

Early indications that leptin might have an impact on pubertal onset came from the observation that *ob*/*ob* and *db*/*db* mice are infertile and fail to undergo normal sexual maturation because of alterations of hypothalamic–pituitary function. There is much debate about the pathophysiological effects of leptin on the onset of puberty, and it is difficult to correlate the complex mechanisms of puberty with modifications of putative single signals such as leptin. It is well established that food restriction delays pubertal onset, whereas refeeding abolishes this delay. In addition, murine and human genetic models of leptin deficiency fail to enter puberty, and treatment with leptin can establish a pulsatile secretory pattern of gonadotropins that is characteristic of early puberty. The female transgenic skinny mouse, which is an *in vivo* model of chronic hyperleptinemia in the absence of adipose tissue, enters puberty precociously Thus, the question arises whether leptin might be a 'permissive factor'(tonic mediator), whose concentration above a certain threshold is required for pubertal onset, or a 'trigger'(phasic mediator) that determines the pubertal spurt through a rise in serum concentration at an appropriate time of development.

The temporal correlation between increases in leptin concentration and the initiation of LH pulsatility over the peripubertal period has been studied in several species. In men it has been shown that leptin levels rise by 50% before the onset of puberty, and decrease to baseline after the initiation of puberty. Other cross-sectional studies showed that age has a significant effect on serum leptin concentrations through prepuberty into early puberty. It has been reported repeatedly that there are no significant changes in leptin levels over the peripubertal period in male rhesus macaques however; more recent studies performed in castrated male monkeys showed that nocturnal levels of leptin increase just before the nocturnal prepubertal increase in pulsatile LH release. A possible explanation for such contrasting reports in monkeys could be the sampling of nocturnal rather than diurnal blood. Indeed, in primates, prepubertal changes in nocturnal LH release occur approximately five months before diurnal variations. Another reason might be the use of different models: agonadal monkeys were treated with intermittent exogenous GnRH to sensitize the pituitary to endogenous GnRH, thus magnifying the LH release independently from gonadal influences. In the same study, the leptin rise was accompanied by a sustained increase in nocturnal GH and IGF-I concentrations before the onset of puberty, which is defined as the increase in nocturnal pulsatile LH secretion.

The sexual dimorphism in leptin concentrations becomes evident after puberty. In males, leptin levels rise throughout childhood, reach a peak in the early stages of puberty and then decline, whereas they increase steadily during pubertal development in females. Consequently, leptin levels are three to four times higher in females than in males. The reason for this postpubertal sexual dimorphism in leptin levels is not clear. After puberty, serum testosterone and testicular volume are inversely related to leptin levels in males, whereas in females, when adjusted for adiposity indexes, estradiol is directly correlated with leptin levels. These observations indicate that androgens and estradiol might account, at least in part, for the gender differences in circulating leptin levels. This is also supported by *in vitro* studies which show that androgens and estrogens inhibit and stimulate leptin expression and release from

human adipocytes in culture, respectively. Thus, puberty represents a turning point in the sexual dimorphic relationships between the HPG axis and leptin by determining the steroid milieu that leads to a different regulation of leptin secretion in the sexes.

Leptin and gonadal steroidogenesis

Leptin and ovary

Not only does leptin participate in the control of gonadotropin secretion via its hypothalamic/pituitary actions, but circulating or locally produced leptin may also provide direct modulation of ovarian function. Leptin protein has been found in follicular fluid, with concentrations corresponding to those reported in serum. Leptin plays a role in both follicular development, where leptin transcript has been detected at early follicular stages, whereas leptin protein appears only in mature follicles, and subsequent luteal function. Moreover, LEPRs have been identified in granulosa, theca and interstitial cells of the human ovary .In these, several in vitro studies have demonstrated that treatment with medium-high physiologic doses (beginning from 10 ng/mL) of leptin-inhibited steroidogenesis in human granulosa and theca cells and lead to a marked decline in the number of ovulated oocytes . Thus, high leptin concentrations in the ovary may suppress estradiol production and interfere with the development of dominant follicles and oocyte maturation, predisposing to anovulation. There-fore, conditions with excess energy stores or metabolic disturbances, such as obesity and polycystic ovarian syndrome, leptin have an inhibitory effect on the gonads.

However, in suboptimal nutritional status, such as eating disorders, exercise-induced amenorrhea, and functional hypothalamic amenorrhea, leptin deficiency results in HPG dysfunction raising the possibility that relative leptin resistance or deficiency may be at least partly responsible for the reproductive abnormalities that occur in these pathophysiological conditions.

The recent identification of Ob-R expression in several peripheral tissues (e.g. ovary, testis and adrenal gland), suggested that leptin might have a direct effect on downstream endocrine targets of the reproductive axis. Several studies have focused on the ovary, where Ob-R expression is abundant. *In vitro* studies conducted on thecal and granulosa cells showed that leptin has a negative effect on ovarian steroid output, both in rodent and bovine models. In particular, it has been found that: (1) leptin inhibits insulin-induced progesterone and 17β -estradiol production by isolated bovine granulosa cells (2) leptin prevents insulin-induced progesterone and androstenedione secretion in bovine ovarian thecal cell and (3) leptin impairs the hormonally stimulated release of17β -estradiol by rat granulosa cells in culture.

Role of leptin in implantation

Embryo implantation represents the most critical step of the reproductive process, involving a complex sequence of signaling events that are crucial to the establishment of pregnancy. A large number of identified molecular media-tors have been postulated to be involved in this early feto-maternal interaction, including hormones, adhesion molecules, cytokines, growth factors, lipids and others. In this sense, it has been reported that both leptin and LEPR are expressed in the glandular and luminal tissues of the endometrium throughout the menstrual cycle. More specifically, low LEPR levels observed during the early proliferative phase are followed by a gradual increase and peak in the early secretory phase of the menstrual cycle, suggesting that LEPRs may be regulated by ovarian steroids, and that leptin might have a physiological role in the implantation of a fertilized egg. The obligatory nature of leptin signaling in mammalian implantation was illustrated by experiments in the mouse demonstrating that

endometrial LEPR expression was pregnancy-dependent and that intrauterine injection of a leptin peptide antagonist or a leptin antibody impaired implantation, suggesting that secretory endometrium is also a target tissue for leptin action. In fact, the blastocyst becomes intimately connected to the maternal endometrial surface to form the placenta and oocytes and preimplantation embryos also express LEPR mRNA, as mentioned above, indicating that leptin may be necessary for embryonic development. In line with this, a deficiency in functional LEPR expression in the endo-metrium has been found in patients with subfertility who had evidence of an endometrial maturation defect. Further evidence for the importance of leptin in implanta-tion is the fact that in cytotrophoblasts, leptin increases the expression of matrix metalloproteinases (MMP-2 and MMP-9), which have been implicated in trophoblast invasion.

Leptin and testis

The first identification of Ob-R expression in the testis was by Hoggard *et al.* who detected mRNAfor the common extracellular domain in murine spermatic cells and in Leydig cells by *in situ* hybridization. The passage of leptin across the blood–testis barrier has also been investigated, and has shown that leptin enters the testis by a passive, non-saturable process. By using reverse transcription (RT)-PCR, Ob-Rb (the long signaling isoform) was shown to be expressed both in primary cultures of adult rat Leydig cells and in a Leydig tumor cell line (MLTC-1). In these models, it was also shown that leptin exerts a rapid and dose-dependent inhibition of LH-stimulated testosterone production in rat cells in culture. hCG-stimulated testosterone suppression was accompanied by a parallel reduction of androstenedione levels and a concomitant rise in the precursor metabolites 17-OH progesterone, progesterone and pregnenolone, compatible with a leptin-induced lesion of 17-20 lyase activity. In accordance with these findings, other studies have shown that leptin inhibits testosterone secretion from adult rat testicular slices incubated *in vitro*, but not from prepubertal testes. These observations indicate that leptin has the potential to modulate the paracrine network that controls gonadotropin-stimulated testicular steroidogenesis, which is analagous to its actions in the ovary. More recent immunohistochemical studies demonstrated that mouse testis germ cells express the Ob-R in a stage- and age-dependent manner. Furthermore, *in vitro* treatment of isolated seminiferous tubules with leptin led to STAT-3 phosphorylation, which indicated that Ob-R is functional and capable of signal transduction in germ cells. These data suggest that leptin might have additional testicular effects, possibly exerted on the proliferation and differentiation of germ cells, and that the lack of its action(s) might be locally involved in the pathogenesis of infertility observed in leptin-deficient mice.

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Leptin and pregnancy

During pregnancy, especially in the second and third trimesters, serum leptin is raised in both animal models and humans and drops sharply after delivery. The main paracrine role of placental derived leptin in the mother is not clear; however, it is likely to have endocrine activity on maternal appetite, energy metabolism and fat deposition. The marked

increase in leptin, an appetite suppressant, during late pregnancy when maternal nutrient requirements are increased suggests an alternative role for leptin or that the mother has some form of leptin resistance. One possible explanation for this contradiction is through the release of the soluble leptin receptor (ObRe) by placental membrane shedding. The binding of leptin to this receptor may protect leptin from degradation or excretion giving a peak in maternal circulating leptin, while preventing it from binding to the signalling form of the leptin receptor, thereby also giving rise to leptin resistance. However, the large increase in bound circulating leptin observed during late pregnancy in mice does not occur in humans or rats.

Autocrine activity on the placenta

The placenta is a complex organ that enables the mammalian embryo to survive within the intrauterine environment. The diversity of functions performed by the placenta is impressive, ranging from anchoring the embryo and preventing its rejection by the maternal immune system to enabling the transport of nutrients and waste between mother and the embryo Concentrations of leptin in cord blood correlate with placental size, indicating a possible mechanism whereby the placenta can regulate its own growth. The mechanism by which this occurs is unknown; however, it is possible that leptin stimulates placental angiogenesis as reported in primary cultures of mice endothelial cells In this regard, it has been reported that gestational hor-mones, such as b-hCG, estrogen progesterone and human placental lactogen (hPL) as well as hypoxia, insulin, glucocorticoids, several interleukins (IL-1a, IL-1b, IL-6), interferon-γ and cAMP, regulate placental leptin expres-sion.leptin may exhibit an angiogenic role possibly through the stimulation of other angiogenic factors, for example vascular endothelial growth factor (VEGF) or placental induced growth factor (PIGF). Placental leptin may also have a local autocrine immuno-modulatory or anti-inflammatory role. Since successful pregnancy is associated with down regulation of intra-uterine pro-inflammatory cytokines such as TNFα and interleukin 1 (IL-1), leptin may function as a local immunomodulator at the maternal–fetal interface .This may be a protective response to counter the effects of pro-inflammatory cytokines.

Paracrine activity on the fetus

Although no correlation has been found between maternal leptin concentrations and birth weight, several recent studies have reported a positive correlation between leptin concentrations in cord blood and birth weight. As a result, leptin has been implicated as an important new growth factor in intrauterine and neonatal development, studies showing localization of leptin to villous endothelial cells and reports of higher leptin concentrations in venous compared with arterial umbilical blood indicate that placental leptin is important for fetal growth and development, although a possible contribution from leptin from the fetus cannot be excluded. Our studies have shown that concentrations of leptin in placental and cord blood are decreased in pregnancies complicated with fetal growth retardation and increased in those complicated with maternal diabetes. In a 33 week twin pregnancy in which one twin was growth retarded and the other of normal size, leptin concentrations in placental and cord blood associated with the growth retarded fetus were lower. Evidences are reported the role of leptin to regulate mammogenesis during pregnancy and involution. In mammary gland, leptin has been observed to exert also an autocrine and/or paracrine activity which affects the development of duct, formation of gland alveolus and expression of milk protein gene in mammary gland.

CONCLUSION

Leptin an adipocyte hormone plays an

important role in regulating energy homeostasis by inhibiting hunger, interacts with the reproductive axis of mammals at multiple sites with stimulatory effect at the hypothalamus and pituitary and its high dose has inhibitory action on steroidogenesis. As a marker whether nutrition stores are adequate, leptin may act in concert with gonadotrophins and growth hormone axis for the initiation of complex phenomenon of puberty. The expression and secretion of leptin are correlated with body fat mass and are acutely affected by feed intake. Moreover, circulating leptin increases during pubertal development in rodents and domestic animals. Effects of leptin are mediated mainly via receptor activation of the JAK-STAT pathway; however, activation of alternative pathways, such as MAP kinase, has also been reported. Leptin helps in oocyte development and maturation. It also has angiogenic and immuno-modulatory and anti inflammatory activities helps in implantation and prevents embryo rejection by the maternal immune system, invasion of trophoblast and cause mammary growth development.

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