INTERNATIONAL CORNER

LEPTIN AND REPRODUCTION

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INTRODUCTION

Leptin, an adipocyte secreted hormone encoded by the obesity (ob) gene (1), was originally thought to be an antiobesity hormone. Extensive research on leptin over the last years has shown that leptin is not only a messenger of the amount of energy stores to the brain (2), but also a hormone/cytokine for diverse physiological processes, such as inflammation, angiogenesis, hematopoiesis, immune function, and reproduction (3).
Although leptin was originally thought to be exclusively expressed in white adipose tissue, subsequent reports showed that leptin is expressed in several other areas, such as the hypothalamus (4), pituitary (5), fundic gastric epithelium (6), skeletal muscle (7), syncytiotrophoblast (8), and mammary epithelium (9).
Leptin receptors (Ob-Rs) have been identified in the hypothalamus, gonadotrope cells of the anterior pituitary, granulosa, theca, and interstitial cells of the ovary, endometrium (10), and Leydig cells (11). This multifocal expression of leptin, as well as the presence of Ob-Rs at all levels of the hypothalamus-pituitary-gonadal (HPG) axis, implies that the nutritional/leptin regulation of reproduction involves a complex network of interactions to regulate the HPG axis in a paracrine and/or endocrine way.
Leptin gene expression is regulated by many hormones, growth factors, and cytokines Estrogens induce (12) whereas androgens suppress leptin production (13), providing an explanation for the sexual dimorphism in serum leptin levels. Insulin increases leptin production (14), and this may contribute to the decrease of plasma leptin levels that occurs during fasting and the hyperleptinemia that accompanies insulin resistance states (15). Glucocorticoids increase leptin gene expression independently of their effect on insulin resistance, but may also induce a relative leptin resistance by inhibiting leptin action (16).

ROLE OF LEPTIN IN REPRODUCTION SIGNALLING

In addition to its role as a signal to the brain sending information on the amount of energy stores to regulate energy homeostasis, leptin has been found to have equally important effects on reproductive organs. The endocrine and/or direct paracrine effects of leptin on the gonads are implied by the expression of functional leptin receptors on the surface of ovarian follicular cells, including granulosa, theca, and interstitial cells (10), as well as Leydig cells (11). Whether injected systemically in vivo or in vitro into isolated perfused ovary, leptin reduces ovulation, even though no changes in steroid secretion or in the number of preovulatory follicles were noted (17).
The indirect effect of leptin on reproduction may be related to its effect on peripheral metabolism by increasing glucose uptake, hepatic gluconeogenesis, and carbohydrate and fatty acid oxidation (18).
Therefore, given the evidence for the effect of leptin on reproductive function at the hypothalamic-pituitary level, it is possible to speculate that leptin has a different action on the HPG axis depending on its serum levels: leptin deficiency results in HPG dysfunction (19), and leptin administration at low doses may have a permissive, threshold effect on the central networks that regulate gonadotropin secretion at the low leptin levels seen in starvation states or eating disorders, whereas leptin may have an inhibitory effect on the gonads at the high serum leptin levels seen in obese people. This may explain the reproductive abnormalities seen in states of both leptin deficiency and excess, as discussed below.

LEPTIN AND PHYSIOLOGY OF REPRODUCTION

Sexual dimorphism of leptin levels

Even after correcting for body weight and fat mass, women have higher serum leptin levels than men (20). This sexual dimorphism in serum leptin concentrations has been associated with various factors: first, the pulse amplitude, but not the pulse frequency, of leptin secretion from adipose tissue is twofold to threefold higher in females than in males (21). Second, fat mass is increased in females, and there is differential fat distribution with a higher subcutaneous/visceral fat ratio in women than men. Leptin mRNA expression is known to be higher in subcutaneous than visceral fat depots (22). Third, women have higher total serum leptin levels but lower leptin-binding protein levels than men, indicating higher free leptin levels (23). Finally, female adipose tissue may be more sensitive to hormones (i.e., insulin and glucocorticoids) or other substances that stimulate leptin production. It is known that sex steroids such as estrogens increase leptin levels (12), whereas androgens decrease leptin levels (13).

Leptin in states of starvation

It is well established that alterations in nutritional status and/or energy reserves can disrupt the HPG axis resulting in reduced fertility. This adaptive response would be a compensatory mechanism to avoid the excess metabolic demands imposed by reproduction at a time when insufficient nutrient intake is present (24). Accumulating evidence suggests that leptin may serve as this critical link between adipose

stores and hypothalamic centers that control the gonadal axis in rodents. In food-deprived mice, leptin treatment reverses the delay in onset of estrus associated with caloric deprivation in female mice and significantly blunts the fall in testosterone and LH in male mice (25). Similarly, leptin administered to female rats subjected to chronic food restriction, which prevents vaginal opening, resulted in sexual maturation despite a decrease in body weight (26). In humans, an analogous state of starvation occurs in eating disorders such as anorexia nervosa and results in hypothalamic amenorrhea.

Leptin and puberty

The early observation that food restriction may either delay the onset of puberty or alter adult reproductive function led to the hypothesis that attainment of a critical body weight or fat mass must be achieved before puberty can occur (27).

In humans, leptin levels exhibit significant changes during progressive pubertal stages, with a distinct dimorphism between boys and girls. Whether studied longitudinally from prepuberty to late puberty or cross-sectionally at different ages, serum leptin levels in boys appear to peak just before puberty or in early Tanner stages, followed by a decrease to baseline levels as testosterone levels rise. Girls, on the other hand, display a steady rise in leptin levels throughout puberty (28, 29). In addition, a significant relationship exists between the onset of menarche and serum leptin levels, with an increase of 1 ng/mL in serum leptin levels associated with an earlier onset of menarche by 1 month (30).

Although leptin levels maintain a significant correlation with fat mass at all pubertal stages, this difference of leptin levels between genders that occurs throughout puberty leads to significantly higher leptin levels normalized to fat mass in females during late puberty (29), which persists into adulthood. A few cases of either leptin deficiency or resistance (due to inactivating mutations of the leptin gene or leptin receptor gene, respectively) in humans have provided instructive insights on the role of leptin in pubertal development; these included a 34-year-old woman and three adolescent girls with primary amenorrhea and no pubertal development (in the girls), and a 22-year-old man with hypothalamic hypogonadism (31). However, evidence that leptin may have a more permissive role in reproductive function, has been demonstrated by female patients with lipoatrophic diabetes who have normal reproductive function despite low leptin levels due to absence of adipose tissue (32). Therefore, from the animal experiments and the observational studies, it appears that leptin may be a necessary but not a sufficient factor for the initiation of puberty in humans.

Leptin variations during the normal menstrual cycle, the use of oral contraceptives, and menopause

The possible variation of serum leptin levels during the different phases of the menstrual cycle, has been intensely studied. Leptin levels show considerable variation throughout the menstrual cycle, with higher levels in the midluteal rather than the follicular phase (33).

However, conflicting information has been provided about the impact of oral contraceptives upon baseline leptin levels in humans. Rechberger et al found no changes in basal (single sample) leptin levels after 3 and 6 months of therapy with two different oral contraceptives (34), while Messinis et al administered estradiol and progesterone to normal weight women and found an increase in leptin levels (35). Cella et al observed that leptin concentrations were significantly higher in the luteal as compared to follicular phase, but that levels in women taking oral contraceptives did not differ from those in the follicular phase (36).

In a study we recently conducted (unpublished data), we observed the leptin response to an acute 72-hour fast in women taking oral contraceptive. Levels did not differ between the eumenorrheic controls and the subjects on oral contraceptives, and leptin levels fell comparably during fasting in both groups of women. During fasting, leptin levels in both groups of women were profoundly diminished and there was loss of diurnal rhythmicity. Thus, sex steroid milieu did not modify the leptin response to fasting in women. The decline of leptin during fast was marked, rapid, and proportional to the loss of body weight, but not associated with demonstrable reproductive compromise.

Finally, several studies (37) but not all (38) have shown that serum leptin levels decline in postmenopausal women, especially obese women, probably due to altered sex steroid levels in the postmenopausal state. However, hormone replacement therapy did not significantly increase serum leptin levels in most (39) but not all (40) studies.

Leptin in pregnancy

Leptin may be important in regulating maternal nutrition and the metabolic adaptation of nutrient partitioning during the energy-consuming processes of pregnancy and lactation (41). More specifically, pregnancy with its associated hormonal changes (especially insulin, glucocorticoids, estrogens, and prolactin) appears to be a state of physiologic hyperleptinemia and leptin resistance, with uncoupling of eating behavior and metabolic activity (42). Thus, leptin has a lipolytic effect and may favor increased fatty acid mobilization from adipose tissue as well as increased peripheral use or oxidation of fatty acids (18).

However, the high leptin levels do not appear to be responsible for the increased appetite and dietary intake associated with pregnancy, which may reflect other hormonal changes (e.g., increase of glucocorticoids and/or prolactin). After childbirth, there is a rapid drop of serum leptin levels (42), which may account for the increased appetite but not for the suppression of reproductive function during lactation. Leptin has also been detected in colostrum and breast milk and results from regional production by mammary epithelial cells and diffusion from the maternal circulation. Further acute decline of leptin levels in lactating rats depends on the degree of metabolic drain from milk production (43).

LEPTIN AND THE PATHOPHYSIOLOGY OF REPRODUCTION

Role of leptin in polycystic ovary syndrome

Polycystic ovary syndrome (PCOS) is a heterogeneous disorder characterized by menstrual dysfunction and hyperandrogenism, often accompanied by metabolic disturbances such as obesity and insulin resistance, as well as by an abnormal secretory pattern of GnRH leading to a high LH/FSH ratio. Despite the evidence that leptin may regulate gonadotropin secretion, a definitive role for leptin in the pathophysiology of PCOS has not been established, as serum leptin levels in PCOS patients do not differ from those in age-matched and weight-matched controls (44, 45). In addition, treatment with antiandrogens, estrogens, and insulin sensitizers have generally not been shown to affect serum leptin levels in humans (44), although one study showed a modest effect of insulin sensitizers after short-term treatment (46).

Despite similar follicular fluid and plasma leptin levels, relatively higher leptin-binding activity in the preovulatory follicle may result in lower follicular fluid free leptin levels (47). Importantly, normal women and women with PCOS who succeeded in becoming pregnant within three cycles of in vitro fertilization had significantly lower follicular fluid leptin levels compared with those who failed to

become pregnant (48). These findings provide supportive evidence for the theory that elevated leptin levels impair reproductive function..

Role of leptin in eating disorders

One of the characteristics of anorexia nervosa (AN) is the amenorrhea that results as patients lose progressively more weight. In AN, the amenorrhea is mainly hypothalamic in origin, and when the weight falls below a threshold of about 70% of body weight, the reproductive disturbance is accompanied by changes in multiple endocrine axes (49). Serum leptin levels in AN patients are significantly lower than in healthy controls of normal weight but correlate with body weight and percentage of body fat (50). Increasing leptin levels in response to dietary treatment correlate significantly with serum LH and FSH, implying that the rise of serum leptin levels secondary to the increase in body weight may activate the HPG axis. Thus, leptin may be a necessary but not a sufficient signal for resumption of menses in AN. For example, weight recovery in patients with AN is not always associated with resumption of menses, and frequently there is no difference in serum leptin levels between amenorrheic and eumenorrheic women with AN, indicating that other endocrine axes, including the GH/IGF-I axis, should be normalized too (51).

Bulimia nervosa (BN) represents the other end of the spectrum of eating disorders in which the body weight tends to remain normal despite binge eating. BN patients have significantly decreased serum leptin levels compared with age-matched and weight-matched healthy controls; levels remain lower even during remission periods of BN (52), but leptin levels in BN are not as low as in AN. Menstrual irregularities have been less well studied in this group but seem to occur less frequently than in AN and are usually hypothalamic in origin (49). Thus, it has been suggested that leptin may play a role not only in appetite regulation and energy homeostasis but also in the menstrual abnormalities of women with eating disorders.

Role of leptin in exercise-induced amenorrhea and functional hypothalamic amenorrhea

Another group of women in whom hypothalamic amenorrhea occurs commonly are elite women athletes who exercise strenuously. Amenorrhea in these women results from suppression of GnRH pulsatility, leading to a low estrogen state. Elite female gymnasts with significantly lower serum leptin levels compared to controls of the same age and percentage of body fat (5) have delayed menarche and retarded bone maturation. It has been suggested that the reproductive abnormalities in women with exercise-induced amenorrhea may be related to a negative energy balance. Because amenorrheic women athletes also have significantly lower leptin levels and a striking absence of the normal diurnal pattern of serum leptin levels present in eumenorrheic athletes (54), it has been suggested that the low leptin levels may also contribute to the amenorrhea of these women.

Hypothalamic amenorrhea may also occur in normal weight, nonathletic women without apparent eating disorders, in which case it is known as functional hypothalamic amenorrhea (FHA). Interestingly, patients with FHA have lower serum leptin levels compared to age-, weight- and body fat-matched eumenorrheic controls (55). Thus, it has been suggested that the decreased leptin levels may be a sensitive indicator of overall nutritional status and may contribute to the amenorrhea that occurs in these women (55).

In summary, several clinical disorders with hypothalamic amenorrhea are associated with decreased leptin levels. These syndromes likely represent a continuum of abnormalities in reproductive function with increasingly severe menstrual abnormalities associated with increasing degrees of disordered eating and/or loss of fat mass. Thus, normal weight women with BN have milder menstrual irregularities that occur less frequently than in amenorrheic athletes or women with AN who have more severe disturbances in body weight and energy balance and thus more significant reproductive disturbances. Because these data are derived from observational studies, they only provide suggestive evidence for the role of leptin in the development of amenorrhea (24).

Role of leptin in obesity-related reproductive dysfunction

Not only are states of starvation associated with reproductive disturbances but the other end of the spectrum, obesity, is as well. This was first documented in animal models of obesity in which the leptin system is primarily defective, mostly due to complete leptin deficiency (ob/ob mice) (56), leptin resistance in association with high serum leptin levels. Similar to the ob/ob, humans who are obese due to mutations in the leptin gene (31, 57) or the leptin receptor gene have also been demonstrated to have reproductive dysfunction. With the exception of these rare cases, human obesity is because of leptin resistance from either receptor downregulation or postreceptor defects rather than leptin deficiency (58).

It has been observed that obese girls reach menarche at an earlier age compared to normal weight girls (59), a fact consistent with the hypothesis that increasing leptin levels contribute to the initiation of puberty, as reported above. It has also been shown that increasing obesity is associated with an increasing frequency of anovulatory cycles (60) and that obese women have increased numbers of atretic follicles (60). These findings are consistent with the direct inhibitory action of high leptin levels on ovarian steroidogenesis leading to ineffective follicular maturation. Thus, it can be proposed that the high serum leptin levels of obese women may contribute to reproductive function or dysfunction at different levels of the HPG axis, that is, a central effect is of increasing leptin levels leading to early menarche, which is later followed by resistance to the response of their gonadotropes to GnRH, combined with a peripheral inhibitory effect of the higher leptin levels on ovarian function predisposing to anovulation.

CONCLUSIONS

Recent research has demonstrated that leptin plays an integral role in the normal physiology of the reproductive system with complex interactions at all levels of the HPG axis. Observational studies have demonstrated that states of leptin excess, deficiency, or resistance can be associated with abnormal reproductive function. Future interventional studies involving leptin administration are expected to further elucidate these complex relationships and potentially provide new and better options in our therapeutic armamentarium for these clinical syndromes.

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