Leptin and Reproduction: Is it a Critical Link Between Adipose Tissue, Nutrition, and Reproduction?

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Catalogue Data

Key words: female athletes, ob gene, exercise, energy balance, amenorrhea
Mots-clés: femmes athlètes, gène ob, exercice physique, équilibre énergétique, aménorrhée

Abstract/Résumé

Exercise-associated reproductive disorders are frequently reported among recreationally active and elite female athletes. Although an association between exercise and menstrual disorders has been established, the mechanism by which exercise disrupts reproductive function remains unknown. Recent findings suggest that low energy availability rather than inadequate body fatness or exercise stress is likely the mechanism by which exercise impinges negatively on the hypothalamic-pituitary-ovarian axis in female athletes. The peripheral signal that informs the neural network of energy availability remains unknown. The identification of the adipocyte-derived ob gene product, leptin, and subsequent findings of its association with reproduction in both rodents and humans, led to speculations that it may be involved in the interactions between nutrition and reproduction. This review article focuses on leptin's role in modulating reproduction, and in particular, as a peripheral signal of nutritional status that integrates adipose tissue, nutrition, and reproduction in female athletes.

Les troubles de reproduction associés à l’effort physique sont fréquents chez les femmes qui s’y adonnent de façon compétitive ou récréative. Bien que l’association entre l’exercice physique et les troubles menstruels soit documentée, le mécanisme par lequel l’exercice physique affecte la reproduction n’est pas encore établi. D’après des observations récentes, le dérangement de l’axe hypothalamo-hypophyso-ovarien chez les femmes athlètes serait dû à une faible disponibilité d’énergie plutôt qu’à une sous-adiposité corporelle. On ne connaît pas encore le signal périphérique renseignant le système nerveux sur la disponibilité...

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d’énergie. La caractérisation de la leptine, produite par le gène ob dans l’adipocyte et des observations subséquentes de son association à la reproduction chez les rongeurs et les humains laissent croire que la leptine jouerait un rôle dans la nutrition et la reproduction. Cet article met l’emphase sur le rôle modulateur de la leptine dans la reproduction et en particulier, sur l’indication de l’état nutritionnel intégrant le tissu adipeux, la nutrition et la reproduction chez les femmes athlètes.

Introduction

The hypothalamic-pituitary-ovarian (HPO) axis which governs reproductive function is sensitive to changes in its environment, and factors including exercise and nutrition can adversely affect its function. This is exemplified by a high incidence of reproductive disorders observed in both recreationally active and elite female athletes (Broocks et al., 1990; Loucks et al., 1989). The etiology of exercise-associated reproductive disorders remains unknown, although a myriad of causative factors have been proposed, including negative energy balance, the stress of exercise, and extreme leanness (critical-fat hypothesis). The critical-fat hypothesis has since been refuted but it continues to receive widespread support, especially by the general public. Although an absolute level of body fatness capable of governing reproduction is simplistic and somewhat preposterous, body fat is indirectly involved in modulating reproduction. Adipose tissue stores represent a vital component of whole-body energy balance, and reproductive function is dependent on energy availability (Bronson and Manning, 1991). Interest in the relationship between fat and fertility was restored with the recent identification of the adipocyte-derived ob gene product, leptin, and subsequent findings of its association with reproduction in both rodents and humans.

Although the area of leptin research is new, it is rapidly advancing and evolving; more than 1,000 papers have been published since 1994. However, research has focused predominantly on leptin’s role in obesity. This review article will not address leptin and obesity. Excellent reviews on that topic include those by Friedman and Halaas (1998) and Stephens and Caro (1998). Instead, this review article will focus specifically on leptin’s proposed role in regulating reproduction, and its potential link to exercise-associated reproductive disorders in female athletes.

Exercise-Induced Menstrual Cycle Changes

A high incidence of reproductive disorders is frequently reported among female athletes who participate in strenuous exercise training (Broocks et al., 1990; Loucks et al., 1989). The most commonly reported exercise-associated reproductive disorder is secondary amenorrhea, or cessation of menstrual function. The prevalence of secondary amenorrhea is estimated to be as high as 44% among female athletes, compared to 1 to 5% in the general population (De Souza and Metzger, 1991). However, prospective studies have demonstrated induction of subtle ovulatory disturbances, such as luteal phase defect or suppression by prolonged, moderate exercise training in recreationally active women (Beitins et al., 1991; Broocks et al., 1990). The menstrual cycles of these athletes are like those of eumenorrheic sedentary women, for example, a lengthened follicular phase compensates for the
shortened luteal phase, resulting in a cycle compatible with that of normal duration (Loucks et al., 1989). Since the presence of menstrual bleeding is often used retrospectively as an indicator of the integral function of the HPO axis, these less conspicuous reproductive anomalies are often undiagnosed (Loucks et al., 1989). Thus it cannot be assumed that all physically active "regularly menstruating" women have normal reproductive function.

Cross-sectional studies have reported abnormal follicular development (suppressed luteinizing hormone [LH] pulse frequency) and suppressed ovarian function (decreased estrogen and/or progesterone levels) in both amenorrheic and eumenorrheic athletes (Laughlin and Yen, 1996; Loucks et al., 1989). Progesterone levels in the luteal phase are decreased in recreationally active athletes (Broocks et al., 1990; Loucks et al., 1989) and in sedentary eumenorrheic women who undertake a strenuous exercise training program, indicative of luteal phase defects (Beitins et al., 1991). The proximal cause has been identified at the level of the hypothalamus, and not to decreased pituitary responsiveness to gonadotropin releasing hormone (GnRH) (Laughlin and Yen, 1996; Loucks et al., 1989). The mechanism that disrupts the GnRH pulse generator remains unknown.

Proposed Etiology of Reproductive Disorders Associated With Exercise

Although the etiology of exercise-associated reproductive disorders remains unknown, a multitude of factors have been postulated as probable causes. Most of these "causative factors" were derived from descriptive characteristics of female athletes with exercise-induced menstrual cycle changes, including inadequate body fat, intensity and duration of exercise training, type of exercise, nutritional deficiencies, predisposition to menstrual irregularities (genetic factor), delayed menarche, and weight loss (Bonen, 1994).

Frisch and McArthur (1974) suggested that delayed menarche and menstrual cycle changes in female athletes are the result of their body fatness falling below the critical levels of 17% and 22%, respectively. The validity of this hypothesis and the proposed mechanisms have been refuted. Subsequent studies do not support the notion that low body weight or extreme leanness disrupts the GnRH pulse generator and LH pulsatility (Laughlin and Yen, 1996; Loucks and Heath, 1994a; Sanborn et al., 1987). Although the concept that reproductive function is regulated by a set level of body fatness universal to all women is simplistic and inconceivable, reproductive function is invariably related to whole-body energy balance, and adipose tissue stores are a vital component of the body's energy reserves (Bronson and Manning, 1991).

It is well documented that when energy is limited, female mammals allocate available energy to processes needed to survive while temporarily sacrificing less important functions such as reproduction and accumulation of body fat. These processes can resume when energy becomes more available (see reviews by Bronson and Manning, 1991; Wade et al., 1996). The dependence of reproduction on energy availability in mammals, including humans, led to the proposal that reproductive disturbances in female athletes may not be related to exercise per se, but are instead the consequence of inadequate energy intake to meet the increased demands of exercise (Loucks et al., 1998). The low-energy-availability hypothesis
Table 1  Caloric Intakes (kcal/day) of Amenorrheic and Eumenorrheic Athletes vs. Controls

<table>
<thead>
<tr>
<th>Study</th>
<th>Eumenorrheic sedentary controls</th>
<th>Eumenorrheic athletes</th>
<th>Amenorrheic or oligomenorrheic athletes</th>
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<tr>
<td>Kaiserauer et al., 1989</td>
<td>1688</td>
<td>2490</td>
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<td>Loucks et al., 1989</td>
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<td>Myerson et al., 1991</td>
<td>1776</td>
<td>1934</td>
<td>1730</td>
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<tr>
<td>Laughlin and Yen, 1996</td>
<td>1597</td>
<td>1739</td>
<td>2106</td>
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<td>Harber et al., 1998</td>
<td>2072</td>
<td>2079</td>
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<td>Thong, 1998</td>
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is supported by the observation that female athletes frequently report similar or lower dietary energy intakes compared to their sedentary counterparts, despite their higher energy expenditure (see Table 1). The energy intake of these athletes is insufficient to meet the increased energy demands from their activity level.

In both humans and experimental animals, the GnRH pulse generator is disrupted by acute (short-term fasting or increased exercise expenditure) and chronic (anorexia nervosa) negative energy balance. Subsequently, gonadotropin secretion from the pituitary is altered and ovarian function is suppressed (Loucks et al., 1998; Loucks and Heath, 1994a; Williams et al., 1995). Alterations in LH pulse parameters can occur and resume rapidly, prior to any noticeable changes in body fat, implying that subtle and complex neuroendocrine changes can disrupt the GnRH pulse generator with body fat remaining relatively immutable (Bonen, 1994; Cameron et al, 1993).

The low-energy-availability hypothesis is further supported by observations that amenorrheic athletes display signs of energy insufficiency, including low triiodothyronine (T3) levels, which are likely responsible for the decrease in resting metabolic rate, mildly elevated cortisol, and decreased insulin and glucose levels (Loucks and Heath, 1994b; Loucks et al., 1992; Myerson et al., 1991). Paradoxically, despite the energy imbalance, body weight in amenorrheic athletes remains relatively stable (Laughlin and Yen, 1996; Myerson et al., 1991). These physiological and metabolic changes along with suppressed reproductive function are likely elicited as adaptive responses to conserve metabolic fuels (Laughlin and Yen, 1996, 1997). As a corollary, the teleological relationship between fat and fertility may have evolved to prevent attempts at reproduction when a woman’s energy stores are insufficient to support the ensuing energetically costly pregnancy and lactation (Wade et al., 1996).

The mild hypercortisolism observed in amenorrheic athletes led to suggestions that exercise training may act as a chronic stressor which activates the hypothalamic-pituitary-adrenal (HPA) axis. In turn, mediators of the HPA axis, including corticotropin-releasing hormone (CRH), adrenocorticotropic hormone (ACTH), and cortisol, can disrupt the HPO axis. Support for this theory has arisen primarily from reports that HPA mediators suppressed GnRH and LH release in nonhuman
studies (Loucks et al., 1998). Several studies have found that fasting-induced changes in LH pulsatility were unaffected by cortisol, but were reversed upon refeeding, in rhesus monkeys (Cameron et al., 1993). Four days of extreme exercise training which resulted in a 10% increase in cortisol levels did not affect LH pulsatility when energy expended via exercise was matched by a concomitant increase in energy intake, suggesting that activation of the HPA axis does not have a negative impact on the HPO axis when the subjects are in a state of energy balance (Loucks et al., 1998). However, the effects on the HPO axis directly by chronic exercise training (i.e., independent of its effects on energy balance) or secondary to the effects of prolonged undernutrition on the HPA axis remain to be determined.

Although the efferent aspects of nutritional infertility associated with energy imbalance are well documented, very little is known about the afferent signals (Wade et al., 1996). The identity of the peripheral signal that reports nutritional status to the hypothalamic regulators of reproduction and how this factor modulates the GnRH pulse generator remains to be determined (Wade et al., 1996). The availability of metabolic fuels, such as glucose and circulating insulin levels, has been suggested as possible signals (Bronson and Manning, 1991; Wade et al., 1996). The recent cloning of the ob gene and subsequent identification of the adipocyte-derived protein, leptin, has led to an interest in its potential involvement in the interaction between nutrition, adipose tissue, and reproduction.

The **OB Gene and Leptin**

**DESCRIPTION CHARACTERISTICS**

The cloning of the obese (ob) gene in mice and its human homologue and the identification of leptin have provided new insights into the mechanism of regulating energy balance and body weight homeostasis (Zhang et al., 1994). The ob gene is most highly expressed in the adipose tissue (Zhang et al., 1994), although pre-ovulatory follicles may be a potential source of leptin (Cioffi et al., 1997). Leptin receptors have been identified in the hypothalamus, other brain areas involved in regulation of energy balance (Håkansson et al., 1997; Schwartz et al., 1996b; Tartaglia et al., 1995), as well as in peripheral tissues (Cao et al., 1997; Karlsson et al., 1997), suggesting that leptin’s actions can be mediated both centrally and at the level of the target tissues.

Leptin is secreted in proportion to adipose tissue size, providing a negative feedback signal to the central nervous system, thus forming an integral part of a complex feedback loop in regulating energy homeostasis (Zhang et al., 1994). Modulation of central systems, including neuropeptide Y, has been suggested as the efferent arm of leptin’s effects mediated centrally (Stephens et al., 1995). Administration of leptin to leptin-deficient obese ob/ob mice resulted in weight loss from a suppression of appetite and increase in thermogenesis. Normalization of hyperglycemia and hyperinsulinemia was observed and this occurred prior to any significant weight loss. Another strain of genetically obese mice, the db/db mice are leptin-resistant in that they have elevated ob mRNA and leptin levels and yet are obese. Their inability to respond to both endogenous and exogenous leptin results from a defect in leptin receptor/signaling system. Leptin administration to lean animals resulted in smaller weight loss but had no effect on metabolic parameters (Halaas et al., 1995; Pelleymouther et al., 1995).
Human obesity appears to more closely resemble the leptin-resistant \( db/db \) mouse in that circulating leptin levels reflect adiposity in obese humans. It has been suggested that the inability of leptin to cross the blood-brain barrier into the cerebrospinal fluid from the circulation, due to saturation of leptin transporters and/or a defect in the postreceptor signaling mechanisms, may contribute to leptin resistance in obese humans (Caro et al., 1996; Schwartz et al., 1996a). Leptin deficiency resulting from a mutation in the leptin receptor gene was recently described in three sisters (Clements et al., 1998). Patients homozygous for this mutation present with early-onset morbid obesity, lack of pubertal development, and diminution in growth hormone and thyrotropin secretion (Clements et al., 1998).

Leptin displays a diurnal rhythm, with levels peaking between midnight and early morning and then declining to a nadir by midafternoon (Saad et al., 1998; Sinha et al., 1996a). Leptin is secreted in a pulsatile manner with a pulse duration of approximately 30 minutes (Saad et al., 1998; Sinha et al., 1996c). Adipocytes are widely dispersed throughout the body, with each fat cell secreting leptin in proportion to its size, thus the mechanism that synchronizes the diurnal and pulsatile rhythm of leptin secretion from each fat cell is intriguing. Leptin circulates in both bound and free forms. Most of the circulating leptin is bound to binding proteins in lean individuals but it circulates in free form in obese individuals (Sinha et al., 1996b). The physiological significance of this binding activity remains to be determined. Circulating leptin exhibits diametrical sexual dimorphism in rodents and humans. In humans, females have higher leptin levels than males at any given level of fatness (Dubuc et al., 1998; Hickey et al., 1996b; Saad et al., 1997), whereas the opposite is observed in rodents (Landt et al., 1998).

**REGULATION OF LEPTIN EXPRESSION AND SECRETION**

In humans and rodents, \( ob \) mRNA and leptin levels are positively correlated with the amount of adipose tissue, although there is a wide interindividual variation in leptin levels at any given level of body fat (Considine et al., 1996; Maffei et al., 1995). Regardless of gender, leptin expression and levels are significantly higher in subcutaneous than in visceral adipose tissue, possibly due to differences in adipocyte size (Hube et al., 1996; Masuzaki et al., 1995; Montague et al., 1997). The mechanism linking adipocyte size to rates of leptin production remains unknown. We often think of fat as a percentage of body weight rather than as absolute mass, yet the latter may be more critical for interpreting findings from leptin research. Leptin expression is higher in subcutaneous adipocytes, especially in women who have more of their fat distributed in subcutaneous sites and less in the viscera, suggesting that regional fat distribution may also contribute to this sexual dimorphism of leptin levels (Hube et al., 1996; Montague et al., 1997; Van Harmelen et al., 1998). However, regional distribution alone cannot account for the sexual dimorphism, as leptin expression and secretion in both subcutaneous and visceral adipocytes are higher in females than in males (Lönnqvist et al., 1997a; Montague et al., 1997). The ability of sex hormones to regulate leptin expression and secretion cannot be excluded.

In general, leptin expression and levels decrease in response to weight loss in both humans and rodents (Considine et al., 1996; Thong et al., 1997; Wadden et al., 1998). In humans, reduction in leptin with weight loss appears to be gender
dependent, with a greater decrease observed in women compared to men in response to similar weight loss (Dubuc et al., 1998). Short-term fasting or overfeeding (36 hrs to 4 days) with a 2–10% change in body weight resulted in a disproportionate change in leptin levels (40–50%), suggesting that during periods of energy imbalance, leptin’s lipostatic role is overridden by an energy balance sensor that can increase or decrease its expression and secretion (Kolaczynski et al., 1996b). The exact mechanism by which adiposity and acute changes in energy balance regulate ob gene expression and secretion is not known.

As alterations in energy balance alter leptin’s adipostatic function, this suggests that changes in energy expenditure might also influence leptin expression and secretion. However, the findings to date suggest that leptin levels are unaffected by either acute exercise (Hickey et al., 1996a) or prolonged exercise training, independent of its effect on energy balance and adiposity (Pérusse et al., 1997; Thong et al., 1997). In contrast, Hickey et al. (1997) reported a selective decrease in plasma leptin in women, but not in men, following a 12-week exercise training program. While the men in their study reported a 13.5% increase in caloric intake after exercise training, caloric intake remained unchanged in the women, suggesting that alterations in leptin secretion in women is likely due to energy imbalance rather than to exercise training itself. Furthermore, Landt et al. (1997) observed a significant reduction in leptin in response to negative energy balance induced by an ultramarathon race; however, leptin levels returned to baseline values upon rest and refeeding. There is little evidence that exercise itself alters leptin levels independently of its impact on energy balance (Landt et al., 1997).

In addition to adiposity and changes in energy balance, the regulatory role of hormones in leptin expression and secretion has also been studied. In humans, most of the studies to date suggest that chronic (but not acute) hyperinsulinemia stimulates leptin expression and secretion (Dagogo-Jack et al., 1996; Kolaczynski et al., 1996a). In rodents, however, both acute and chronic insulin administration was found to increase leptin expression and secretion (Saladin et al., 1995). The relationship between hyperinsulinemia, insulin resistance, and leptin (independent of adiposity) is not yet clear. Recent evidence suggests a strong relationship between leptin and insulin levels that is independent of adiposity, although the mechanisms underlying the relationship between adiposity and circulating leptin with insulin may be different (Schwartz et al., 1997). In addition, various hormones including glucocorticoids and catecholamines appear to regulate leptin expression and secretion in both humans and rodents (Considine et al., 1997; Slieker et al., 1996).

These findings suggest that factors other than adiposity must contribute to the regulation of leptin expression and secretion, and that the regulation of leptin may differ between rodents and humans. Regional fat distribution, changes in energy balance and hormones, including insulin and glucocorticoids, as well as reproductive hormones, are various factors that appear to contribute to the regulation of ob gene expression and leptin secretion.

LEPTIN AND REPRODUCTION

The recent findings from leptin administration to obese ob/ob mice suggest that it may provide a critical link between adipose tissue and the HPO axis that governs
reproductive function. The ob/ob mice are sterile, as a result of a functional defect in the HPO axis. Leptin administration to both male and female ob/ob mice restored fertility and stimulated the reproductive endocrine systems (Barash et al., 1996; Chehab et al., 1996; Mouzih et al., 1997). It cannot be excluded that reversal of sterility was secondary to weight loss induced by leptin. However, while weight loss induced by caloric restriction can occasionally reverse sterility in male ob/ob mice, it is ineffective in restoring reproductive function in female ob/ob mice (Chehab et al., 1996). Withdrawal of leptin on Day 14 post-copulation had no effect on continued gestation and parturition; however, despite the ability of two mothers to lactate the pups, the female ob/ob mice were unable to reproduce following withdrawal of leptin (Chehab et al., 1996).

The presence of leptin is required for proper reproductive function. In normal rodents, leptin administration accelerated the onset of puberty in the absence of weight loss, suggesting that leptin's regulatory role in reproductive function is not secondary to its effects on energy balance (Ahima et al., 1997; Chehab et al., 1997; Cheung et al., 1997). However, in malnourished rats, leptin administration only partially restored the timing of pubertal maturation, suggesting that it is neither the primary signal nor the only stimulus for the onset of puberty. Instead, under adequate nutritional status, leptin acts as a permissive factor that initiates puberty (Cheung et al., 1997).

In humans, leptin levels rise before or at the onset of puberty, then decline to a nadir in late puberty and continue to decrease in boys despite increasing BMI (Mantzoros et al., 1997a), whereas in girls, leptin levels rise continuously with the progression of puberty (Blum et al., 1997). The stimulus for this leptin surge is unknown. However, elevated leptin may provide a signal to the HPO axis that nutritional status is adequate for the onset of puberty. Following puberty, elevated leptin may no longer be required in males, but a leptin threshold may be pivotal in maintaining proper reproductive function in females (Blum et al., 1997).

While the mechanism by which leptin affects reproductive function is not fully understood, the evidence to date suggests that its effects can be mediated both centrally and peripherally. In addition to the hypothalamus, functional leptin receptors have also been identified in human ovaries (Karlsson et al., 1997) and pre-ovulatory follicles (Cioffi et al., 1997). Leptin could regulate the HPO axis at the level of the hypothalamus or the pituitary by modulating GnRH and LH/FSH secretion, as well as directly at the level of the ovaries. Leptin administration increased LH levels in female ob/ob mice and in fasted normal mice (Ahima et al., 1997; Chehab et al., 1996; 1997). In male rodents, leptin stimulated in vitro GnRH release from hypothalamic explants and FSH and LH release from anterior pituitaries (Yu et al., 1997).

Reports on the relationship between leptin and ovarian steroids have been inconsistent. While leptin appears to have stimulatory effects on the reproductive axis in vivo, it inhibited in-vitro insulin-induced peripheral ovarian steroidogenesis in bovine follicles (Spicer and Francisco, 1997, 1998). In both humans and rodents, estrogen increased leptin secretion from isolated adipocytes (Casabelli et al., 1998; Sliker et al., 1996), while progesterone had no effect on leptin secretion (Casabelli et al., 1998). In addition, estrogen selectively increased leptin secretion from adipocytes obtained from women, but not from men (Casabelli et al., 1998). Ob gene expression was decreased following ovariectomy in rodents, but was in-
Figure 1. Schematic diagram of leptin's putative role as a peripheral signal of nutritional status and the proposed mechanism by which exercise affects reproductive function (see text for explanation). GnRH = gonadotropin-releasing hormone; TRH = TSH-releasing hormone; CRH = corticotropin-releasing hormone; LH = luteinizing hormone; FSH = follicle-stimulating hormone; TSH = thyrotropin; ACTH = adrenocorticotropic hormone; E_2 = estradiol; P_4 = progesterone; T_3 = 3,5,5'-triiodothyronine; T_4 = thyroxine.
increased in subcutaneous adipose tissue following estradiol supplementation (Shimizu et al., 1997). Thus, leptin could exert its regulatory effects at any level of the HPO axis (see Figure 1). The relationship between leptin and estrogen may be bidirectional, and estrogen may contribute to the gender-dependent regulation of leptin secretion (Casabiell et al., 1998). In men, leptin levels may be a more accurate reflection of total adiposity compared to women, whose circulating leptin levels reflect both adiposity and the responsiveness of adipose tissue to hormones such as estrogen (Casabiell et al., 1998).

To date, correlational studies of the relationship between leptin and ovarian hormones in humans have reported contradictory findings. While Sumner et al. (1998) and Havel et al. (1996) found no difference in leptin concentrations between pre- and postmenopausal women, Rosenbaum et al. (1996) reported significantly lower leptin levels in postmenopausal women. Rosenbaum’s results were likely confounded by higher adiposity in the premenopausal women. A positive correlation was found between leptin and estradiol in premenopausal women (Paolisso et al., 1998), but this relationship has not been consistently verified (Lukaszuk et al., 1998; Sumner et al., 1998; Thong, 1998). Leptin concentrations in postmenopausal women receiving hormone replacement therapy (HRT) were similar to those in untreated post- and premenopausal women, suggesting that neither natural menopause nor exogenous estrogen affects leptin levels (Haffner et al., 1997; Havel et al., 1996; Hickey et al., 1998).

Oral contraceptive treatment in hyperandrogenic obese (Nadar et al., 1997) and normal-weight premenopausal women (Castracane et al., 1998) did not alter circulating leptin levels. In menstrual cycles that were superovulated with FSH, resulting in a continuous rise in estradiol to levels exceeding physiological values, there was no effect on leptin secretion, which suggests there may be a limit to the stimulatory effect of estradiol on leptin secretion (Messinis et al., 1998). Since oral contraceptive steroids induce a pharmacological rather than a physiological state, the relationship between leptin and contraceptive steroids may be unlike the one between leptin and ovarian sex hormones at various phases of the menstrual cycle. Furthermore, it is frequently reported that healthy premenopausal women taking oral contraceptives can develop metabolic disturbances such as hyperglycemia, hyperinsulinemia, and increased insulin resistance (Watanabe et al., 1994). As insulin has been shown to regulate leptin expression and secretion, the relationship between leptin and the metabolic disturbances arising from oral contraceptive use remains to be examined.

Further studies could clarify the relationship between leptin and ovarian hormones, particularly estrogen, in healthy premenopausal women. In particular, since estrogen and progesterone receptors have been found in adipose tissue and leptin receptors have been identified in human ovaries, the question of whether there is a bidirectional/feedback regulatory loop between adipose tissue and the ovaries remains to be addressed.

In eumenorrheic women, circulating leptin displays a menstrual phasic rhythm, with levels rising during the follicular to peri-ovulatory phase, peaking in the luteal phase and declining to baseline values from the luteal to the follicular phase of one spontaneous menstrual cycle (Hardie et al., 1997; Lukaszuk et al., 1998; Shimizu et al., 1997; Thong, 1998). Peak leptin levels paralleled those of plasma progesterone, and since peak estradiol occurs during the ovulatory phase
of the menstrual cycle, the preovulatory rise in estradiol may have stimulated leptin release during the luteal phase (Hardie et al., 1997). Midfollicular nocturnal rise in leptin was found to be associated temporally with LH pulsatile parameters (Licinio et al., 1998). It seems plausible that follicular development is dependent on the synchronized pulse parameters of both leptin and LH. This hypothesis is supported by the observation that intra-cerebroventricular administration of leptin antiserum to normal female rats impaired LH pulsatility and induced anestruis (Carro et al., 1997).

The physiological significance of a post-ovulatory rise in leptin despite relatively immutable body fatness in eumenorrheic women remains unknown. It is widely accepted that an entire female mammalian reproductive cycle, from ovulation to pregnancy to lactation, is energetically costly; thus reproductive function is governed by energetic constraints (Bronson and Manning, 1991). If a reproductive cycle began with insufficient energy stores to support it, there may be a risk to both the mother and the fetus (Flier, 1998). It seems plausible that a post-ovulatory rise in leptin despite no noticeable changes in fat size may be necessary to induce a temporary “leptin-resistance” period in order to initiate the accumulation of energy stores needed to support the ensuing pregnancy and lactation.

Since the integral function of the HPO axis under conditions of energy equilibrium appears to be dependent on leptin, it is not unreasonable to suggest that reproductive dysfunction during periods of energy imbalance, such as amenorrhea in female athletes, must also be linked to leptin. Recent findings appear to support the hypothesis that a defect in the GnRH pulse generator, resulting in amenorrhea, is linked to leptin. Plasma leptin was found to be significantly lower in female athletes with anovulatory cycles compared to eumenorrheic athletes matched for body fatness (Tataranni et al., 1997). McLean and Graham (personal communication, August 1996) observed an absence of a post-ovulatory rise in leptin in a woman with an anovulatory cycle, suggesting that alterations in menstrual cyclicity are likely related to leptin secretion. These findings further suggest that reproductive dysfunction is associated with leptin, independent of adiposity.

Two recent studies (Laughlin and Yen, 1997; Tataranni et al., 1997) reported that plasma leptin levels were similar between eumenorrheic and amenorrheic athletes, but were significantly decreased compared to sedentary eumenorrheic women. Similarly, circulating leptin levels were decreased in normal-weight women with hypothyroidic amenorrhea compared to eumenorrheic women matched for body fatness (Miller et al., 1998). Decreased adiposity in the female athletes could have accounted for decreased leptin, yet leptin levels were lower than could be explained by body fat alone. Furthermore, amenorrhea in the female athletes was associated with a loss of leptin’s diurnal rhythm. Thus, reproductive dysfunction is due to a loss of leptin’s diurnal rhythm rather than to a decline in leptin expression and secretion (Laughlin and Yen, 1997).

In both the eumenorrheic and amenorrheic athletes, hypoleptinemia was associated with reduced caloric intake, despite the athletes’ increased energy expenditure compared to their sedentary counterparts (Laughlin and Yen, 1997). Caloric intake was also reduced in the normal-weight women with hypothalamic amenorrhea (Miller et al., 1998). In addition, the amenorrheic women reported 50% lower fat intake. Hypoinsulinemia and hypercortisolemia were observed in the eumenorrheic and amenorrheic women, indicative of energy deficiency (Laughlin
and Yen, 1997; Miller et al., 1998). These metabolic changes were exaggerated in the amenorrheic athletes, suggesting that energy available for locomotion and reproduction is compromised to a greater extent by exercise energy expenditure. These physiological changes are likely elicited as adaptive mechanisms to conserve metabolic fuels during periods of prolonged undernutrition. Although the mechanism by which low energy availability inhibits reproductive function remains unclear, falling leptin levels, serving as a peripheral signal of nutritional status, may contribute to these metabolic adaptations (Figure 1).

This hypothesis is supported by recent findings that in addition to its adipostatic function of regulating body weight homeostasis, leptin may be responsible for coordinating the compensatory changes in response to starvation that are fundamental to survival (Flier, 1998). In patients suffering from anorexia nervosa, both circulating (Ferron et al., 1997; Grinspoon et al., 1996; Köpp et al., 1997) and cerebrospinal fluid (Mantzoros et al., 1997b) leptin are decreased. In the three former studies, the anorexic patients also reported absence of menstrual function, or amenorrhea. Hypoleptinemia in anorexic patients was also associated with a loss of leptin’s diurnal rhythm (Balligand et al., 1998).

In response to chronic negative energy balance, shifts in leptin expression may contribute to the development of reproductive dysfunction. Administration of leptin to fasting rodents suppressed starvation-induced activation of the HPA axis; diminished the starvation-induced delayed onset of estrous in female mice; and prevented a decline in circulating LH and thyroxine (Ahima et al., 1996). It appears that, at least in rodents, a decline in leptin expression and secretion during periods of starvation may be an adaptive mechanism to an environment in which food is limited. Such a decline in leptin levels and the subsequent physiological changes will prolong survival during periods of starvation (Flier, 1998).

As a corollary, in female athletes whose energy intake is insufficient to meet increased energy demands, a decrease in leptin expression and secretion provides a signal that coordinates the ensuing physiological responses to undernutrition. These changes include suppression of the energetically costly reproductive function, while simultaneously decreasing thyroid hormone and basal metabolic rate and increasing glucocorticoids. Each of these responses bestows survival value to the individual during periods of prolonged starvation (Figure 1) (Flier, 1998). However, since ancillary hormones such as insulin and cortisol, and metabolic fuels including glucose, respond simultaneously to changes in energy balance, it cannot be excluded that they may be responsible for suppressing leptin secretion, which in turn disrupts the GnRH pulse generator. Insulin is a potential leptin secretagogue, thus hypoinsulinemia observed in amenorrheic women may inhibit leptin secretion. It is unlikely that increased cortisol is responsible for decreasing leptin, as cortisol stimulates leptin secretion.

The response of reproductive function to energy insufficiency has been proposed to occur on a graded continuum and to depend on the magnitude of undernutrition (Cameron et al., 1993). The initial physiological changes may serve as a signal to initiate suppression of reproductive function, resulting in less severe ovulatory disturbances such as luteal phase defect. As the energy insufficiency increases, however, these signals become amplified, resulting in greater suppression or complete cessation of reproductive function, such as amenorrhea (Cameron et al., 1993). As leptin secretion responds to changes in energy equilibrium without
any noticeable changes in body fat size, it is plausible that alterations in circulating leptin provide an indication to the neural network of the magnitude of energy imbalance (Figure 1). Absence of a post-ovulatory rise and decreased leptin levels in anovulatory athletes may suggest that energy reserves are barely adequate to support pregnancy and lactation. Although ovulatory function is suppressed, it is not completely abolished.

In female athletes who may be in a more severe negative energy balance (Table 1), reproductive function is completely abolished, as in the case of amenorrheic athletes and anorexia nervosa patients. “Low T, syndrome” is selectively observed in amenorrheic but not in eumenorrheic women, indicating that energy availability is compromised to a greater extent in amenorrheic athletes (Loucks and Callister, 1993). As in other female mammals, when the energy conditions improve, reproductive function in these athletes may resume (Wade et al., 1996). In all these cases it seems plausible that falling leptin levels serve as a gauge of energy availability, informing the neural networks governing reproductive function of the energy status needed to support locomotion and reproduction (Figure 1). Disruption of the GnRH pulse generator and loss of LH pulsatility may be related to loss of leptin’s diurnal rhythm in amenorrheic athletes. However, this does not preclude additional potential signaling processes, including insulin or metabolic fuels such as glucose, which in addition to leptin may be key components of a complex feedback loop between adipose tissue and the hypothalamic regulators of reproduction.

While correlations between leptin and reproductive function have been established, a direct regulatory role and the mechanism by which leptin modulates the HPO axis is not yet clear. In particular, the mechanism by which leptin coordinates the physiological responses to starvation, including suppression of reproduction remains unclear. Further studies are needed to determine whether a certain critical level of leptin is required for proper functioning of the GnRH pulse generator. Studying the effects of administration of leptin and leptin antagonist on reproductive function in humans would shed light on leptin’s role in modulating reproduction. In particular, investigation of leptin as the as-yet unidentified peripheral signal of nutritional status may verify the “low energy availability” hypothesis that prolonged exercise, independent of its effects on energy balance, has no disrupting effects on reproductive function in female athletes. Accordingly, a concomitant increase in caloric intake to meet the energy demands of exercise without compromising the athletes’ exercise training regimen might prevent reproductive disorders and the resulting ill effects (Loucks et al., 1998).

**Conclusion**

Exercise-associated reproductive disorders are frequently observed in highly trained and recreationally active female athletes. The etiology of these reproductive disorders remains unknown, although recent evidence suggests that exercise adversely affects the integral function of the HPO axis through its impact on energy balance. While there is no evidence of a direct causal role for a precise amount of body fat needed for reproduction to occur, reproductive function is invariably related to energy availability. As adipose tissue stores represent a vital component of whole-body energy balance, it can indirectly modulate reproduction (Bronson and Manning, 1991).
During periods of limited energy availability, female mammals allocate dietary energy to processes needed to survive, while temporarily sacrificing less crucial functions such as reproduction (Wade et al., 1996). This provided impetus for the "low energy availability" hypothesis as an etiology of exercise-associated reproductive disorders in female athletes. Support for this hypothesis has been derived from reports that female athletes frequently consume fewer calories than expected given their high activity level, signs that female athletes are in hypometabolic states, and from similarities between exercise-associated amenorrhea in female athletes and in anorexia nervosa patients (Loucks et al., 1998).

The existence of an afferent system that senses energy availability, which in turn provides this information to the neural network as well integrating the appropriate responses, would be of important survival value. The identity of such a peripheral signal and/or a component of this complex system remains unknown. The adipocyte-derived hormone, leptin, has been recently proposed as a potential peripheral signal, playing an integral part in the interaction between nutrition and reproduction. Leptin-mediated effects on several hypothalamic-pituitary-periphery axes are consistent with the alternate paradigm in which leptin, in addition to regulating body-weight homeostasis and preventing obesity, signals a depletion of energy stores and initiates the appropriate physiological responses.

Consistent with this hypothesis is that when energy deficiency in female athletes is such that leptin falls below a critical level, it coordinates the compensatory responses including suppression of reproduction and thyroid-induced thermogenesis, while simultaneously activating the beneficial adrenal axis. From this perspective, a potential mechanism by which exercise disrupts reproductive function in female athletes is through its impact on energy balance. The proposed leptin threshold is likely regulated or influenced by other metabolic, endocrine, and possibly genetic factors. Thus leptin integrates adipose tissue stores, energy homeostasis, and the hypothalamic regulators of reproduction, and as such it provides a critical link between fat and fertility.

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**Acknowledgments**

This work was supported by grants from Natural Science and Engineering Research Council of Canada (NSERC). Farah Thong is a recipient of a postgraduate scholarship from NSERC and a Student Research Award from Gatorade Sports Science Institute.

*Received December 16, 1998; accepted in final form, April 21, 1999.*