Plasma leptin in female athletes: relationship with body fat, reproductive, nutritional, and endocrine factors

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Plasma leptin was similar in the placebo and active pill phases in ROC despite a significant increase in ethinylestradiol. Leptin correlated (P < 0.05) with triiodothyronine, insulin, estradiol, and thyroid hormones. Leptin increased by 40–46% (P < 0.05) in caloric intake, insulin, estradiol, and thyroid hormones. Leptin was significantly lower in EAA (1.7 ± 0.2 ng/ml) than in ECA (2.9 ± 0.3 ng/ml), RCA (5.8 ± 0.9 ng/ml), and ROC (7.4 ± 1.3 ng/ml). Hypoleptinemia in EAA was paralleled by reductions (P < 0.05) in caloric intake, insulin, estradiol, and thyroid hormones. Leptin increased by 40–46% (P < 0.05) in the luteal phase of the menstrual cycle in RCA and ECA. Plasma leptin was similar in the placebo and active pill phases in ROC despite a significant increase in ethinylestradiol. Leptin correlated (P < 0.05) with triiodothyronine and insulin but not with estrogen, energy intake, or exercise energy expenditure. These data suggest that in female athletes 1) leptin may be a metabolic signal that provides a link between adipose tissue, energy availability, and the reproductive axis and 2) sex hormones do not directly regulate leptin secretion.

Leptin is secreted in proportion to adiposity, providing a negative-feedback signal in the regulation of body weight homeostasis (47). In addition to body fat, overall energy balance (18), insulin (37), and reproductive hormones (4) have been suggested as potential regulators of leptin (ob) gene expression and secretion. However, the exact role of leptin in human physiology, particularly in the reproductive system, remains speculative. In normal-weight sedentary women, a rise in leptin is observed in the luteal phase of the menstrual cycle despite no noticeable change in body fat (11, 27, 34). Given the high incidences of menstrual disturbances in female athletes, it is not clear whether leptin would display such menstrual phasic pattern. The postovulatory rise in leptin appears to parallel that of ovarian hormones, suggesting that a potential relationship between ovarian hormones and leptin expression might exist. However, reports on the relationship between leptin and estrogen have been conflicting. Numerous rodent and human studies suggest a regulatory role for estrogen in leptin secretion (4, 36, 39), whereas others have found no effects of oral contraceptives (5), hormone replacement therapy (12, 13), or estrogen administration to ovariectomized rodents (33) on leptin levels. To date, leptin levels in the placebo vs. the active pill phases of one continuous oral contraceptive pill cycle, as well as the relationship of leptin to the synthetic estrogen ethinylestradiol, have not been examined in female athletes.

Amenorrheic and eumenorrheic athletes frequently report lower dietary energy intake than expected for their high activity level. Yet, paradoxically, they remain relatively weight stable. Amenorrhea in female athletes is accompanied by hypoglycemia (19), hyperinsulinemia (19), hypercortisolemia (19, 25), hypothyroidism (24), reduced basal metabolic rate (BMR) (30), lower leptin levels, and an absence of the diurnal leptin rhythm (20). Suppression of the thyroidal axis and BMR suggests that counterregulatory mechanisms are invoked to conserve metabolic fuels in an energy-deprived state (19). Thyroid hormones and leptin are implicated in the regulation of energy homeostasis; however, the literature on this relationship has been inconsistent (7, 28, 46). Furthermore, the relationship between leptin and thyroid hormones in female athletes, especially in amenorrheic athletes, is not known.

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Lower leptin levels are also observed in anovulatory (42) and in normal-weight amenorrheic females (45). Leptin antiserum administration suppressed LH pulsatility, which was reversed/prevented by leptin administration (3). Leptin (ob) gene expression and secretion respond disproportionately to acute and chronic changes in energy balance relative to adiposity (18). Moreover, the starvation-induced neuroendocrine responses, including suppression of LH pulsatility, were prevented by exogenous leptin administration (1, 31). These findings support the notion that leptin may be a peripheral signal relating information on energy availability and that altered leptin levels may be associated with menstrual disturbances observed in female athletes.

Therefore, this study was undertaken to gain insight into 1) the interrelationships among leptin, energy intake, exercise energy expenditure, insulin, thyroid hormones, and reproductive function in amenorrheic and cyclic female athletes; 2) the menstrual phasic pattern of leptin in cyclic athletes; 3) the relationship of leptin to ovarian hormones in the menstrual cycle; and 4) the effects of synthetic estrogen administration on leptin in female athletes. We hypothesized that amenorrheic athletes will consume less dietary energy despite higher exercise energy expenditure and that a collateral suppression of estrogen, thyroid hormones, insulin, and leptin would be observed compared with athletes who are cyclic or those taking oral contraceptives. In addition, in the cyclic athletes with normal ovulatory cycles, a higher leptin in the luteal phase of the menstrual cycle would be observed and the rise in leptin in the luteal phase would not be correlated with changes in sex hormones. Similarly, alterations in synthetic estrogen levels from the active to the pill-free phase of oral contraceptives would not affect leptin secretion.

**METHODS**

Subjects. Thirty-nine female athletes distinguished on the basis of both menstrual status and athleticism participated in this study: 21 cyclic athletes, 13 females athletes taking combined estrogen-progesterin pills, and 5 amenorrheic athletes. The protocol for this study was approved by the University of Guelph Human Subjects Ethics Committee and written informed consent was obtained from each subject.

The cyclic athletes comprised 8 elite (ECA) and 13 recreationally active (RCA) females. All female athletes taking oral contraceptives were recreationally active (ROC). All amenorrheic females were elite athletes (EAA). We defined elite athletes as those who compete successfully at the provincial and/or national level in their respective sports, including running and cycling, whereas recreational athletes exercised regularly (running, aerobics, cycling) but not at this competitive level. Maximal aerobic capacity (VO2max) determined by a running protocol was required to be above 55 and 48 ml·min⁻¹·kg⁻¹ for elite and recreational athletes, respectively.

The cyclic athletes were required to have menstrual cycle duration of 26–35 days, with at least 3 mo of documented menstrual cycles, and were not using the oral contraceptive pill for at least 6 mo preceding the study. Amenorrhea in the present study was defined as absence of menses for at least 6 consecutive mo before the study, but the amenorrheic subjects had a previous history of regular menstruation. The subjects in the ROC group were required to be taking the oral contraceptive pill for a minimum of 1 yr before the study. All oral contraceptive pills contained 30–35 µg ethinylestradiol as the synthetic estrogen. Subjects with a history of eating disorders or primary or postpill amenorrhea were excluded from this study. They were otherwise healthy, none was taking any medications, and all subjects were nonsmokers. Table 1 provides the physical characteristics of the subjects.

**Experimental protocol.** 
VO2max was determined by a progressive VO2max test on a motorized treadmill. The VO2max test was preceded by a 2- to 3-min warm-up period that also allowed each subject to familiarize herself with the treadmill. After a warm-up period, the workload was increased every 1–2 min until volitional exhaustion. Verbal encouragement was pro-

**Table 1. Physical, dietary, and exercise characteristics of recreationally active and elite cyclic athletes, elite amenorrheic athletes, and oral contraceptive pill users**

<table>
<thead>
<tr>
<th></th>
<th>RCA (n = 13)</th>
<th>ECA (n = 8)</th>
<th>EAA (n = 5)</th>
<th>ROC (n = 13)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, yr</strong></td>
<td>23.6 ± 0.8</td>
<td>22.9 ± 2.2</td>
<td>22.0 ± 0.7</td>
<td>24.4 ± 0.7</td>
</tr>
<tr>
<td><strong>Age at menarche, yr</strong></td>
<td>13.5 ± 0.4</td>
<td>13.8 ± 0.5</td>
<td>14.8 ± 1.2</td>
<td>13.7 ± 0.6</td>
</tr>
<tr>
<td><strong>Gynecologic age, yr</strong></td>
<td>10.1 ± 1.0</td>
<td>9.1 ± 1.9</td>
<td>7.2 ± 1.1</td>
<td>10.7 ± 1.1</td>
</tr>
<tr>
<td><strong>Weight, kg</strong></td>
<td>57.8 ± 2.3</td>
<td>52.9 ± 1.8</td>
<td>52.6 ± 3.4</td>
<td>58.8 ± 2.0</td>
</tr>
<tr>
<td><strong>BMI, kg/m²</strong></td>
<td>20.9 ± 0.4 ±</td>
<td>19.3 ± 0.4</td>
<td>18.9 ± 0.7</td>
<td>20.7 ± 1.9 ±</td>
</tr>
<tr>
<td><strong>Body fat, %</strong></td>
<td>21.3 ± 0.7 ±</td>
<td>15.2 ± 1.3</td>
<td>14.6 ± 0.8</td>
<td>21.1 ± 1.3 ±</td>
</tr>
<tr>
<td><strong>LBM, kg</strong></td>
<td>45.4 ± 1.6</td>
<td>44.6 ± 1.3</td>
<td>44.8 ± 2.6</td>
<td>46.2 ± 1.2</td>
</tr>
<tr>
<td><strong>V̇O2max, ml·kg⁻¹·min⁻¹</strong></td>
<td>52.6 ± 1.1</td>
<td>62.8 ± 1.0</td>
<td>68.2 ± 2.1</td>
<td>52.7 ± 2.1</td>
</tr>
<tr>
<td><strong>Energy intake, kcal/day</strong></td>
<td>2,041.0 ± 94.7</td>
<td>2,277.3 ± 109.5</td>
<td>1,672.9 ± 32.0</td>
<td>2,039.5 ± 120.2</td>
</tr>
<tr>
<td><strong>Dietary protein, g</strong></td>
<td>65.5 ± 5.8</td>
<td>78.3 ± 5.1</td>
<td>54.9 ± 7.3</td>
<td>68.1 ± 3.1</td>
</tr>
<tr>
<td><strong>Dietary carbohydrate, g</strong></td>
<td>305.7 ± 22.7</td>
<td>358.0 ± 29.6</td>
<td>390.1 ± 28.7</td>
<td>320. ± 24.4</td>
</tr>
<tr>
<td><strong>Dietary fat, g</strong></td>
<td>62.3 ± 6.05</td>
<td>63.3 ± 5.25</td>
<td>38.5 ± 5.9</td>
<td>56.3 ± 4.7</td>
</tr>
<tr>
<td><strong>%Calories as protein</strong></td>
<td>13 ± 1</td>
<td>14.1 ± 1.1</td>
<td>12 ± 1.2</td>
<td>13 ± 1.1</td>
</tr>
<tr>
<td><strong>%Calories as carbohydrate</strong></td>
<td>62 ± 8</td>
<td>61 ± 2</td>
<td>71 ± 1</td>
<td>62 ± 2</td>
</tr>
<tr>
<td><strong>%Calories as fat</strong></td>
<td>25 ± 1</td>
<td>25 ± 2</td>
<td>17 ± 1</td>
<td>24 ± 1</td>
</tr>
<tr>
<td><strong>Exercise energy expenditure, kcal/day</strong></td>
<td>676.5 ± 35.1</td>
<td>954.6 ± 54.7</td>
<td>970.3 ± 32.0</td>
<td>579.9 ± 59.6</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of subjects. RCA, recreationally active cyclic athletes; ECA, elite cyclic athletes; EAA, amenorrheic elite athletes; ROC, recreationally active women taking oral contraceptives; BMI, body mass index; LBM, lean body mass; VO2max, maximal aerobic power. † P < 0.05 vs. RCA; ‡ P < 0.05 vs. ROC. § P < 0.05 vs. ECA. P < 0.05 vs. EAA.
vided by the investigators. Expired gas samples were analyzed for O2 and CO2 fractions with an applied Electrochemi-
cal S-3A O2 analyzer and a Sensormedics LB-2 CO2 analyzer, respectively. Expired volume was determined with a Parkin-
son-Cowan volumeter. The analyzers were calibrated with gases of known concentrations. The volumeter was calibrated by using a calibrated syringe.

Body composition was determined by underwater weigh-
ting. The average of the last 3 of 10 measures was used to
determine true underwater weight. Percent body fat was
calculated by using the Siri equation (40). Fat mass was
calculated and was used to determine lean body mass (LBM).

Endocrine analyses. Statistical analyses were performed
by using SAS statistical package (Cary, NC). Leptin concen-
trations were log transformed to normalize the distribution.
Between-group comparisons of anthropometric, dietary, and
exercise parameters; thyroid hormones; and insulin were
made with a one-way ANOVA. Between-group differences in
leptin, E, and P over time were assessed with a two-way
repeated-measures ANOVA. Analysis of covariance was used to
assess group differences in plasma leptin independent of
body fat. Within-group differences were assessed with a one-way
repeated-measures ANOVA. A Tukey post hoc comparison was
made where applicable. Regression analyses, Pearson product-
moment correlation and partial correlation coefficients were used
to evaluate associations among different variables. Statistical
significance was accepted at P < 0.05. All data are presented
as means ± SE unless otherwise indicated.

RESULTS

Anthropometrics and dietary results. All female ath-
letes were similar in age, age at menarche, body
weight, and LBM (Table 1). The elite athletes (ECA
and EAA) had similar percent body fat, but their levels
were significantly lower than those of the recreational ath-
letes (RCA and ROC). The elite athletes had higher
V˙O2max (P < 0.05) than did RCA and ROC, and V˙O2max
was also higher in EAA compared with ECA (P < 0.05;
Table 1). The elite athletes reported higher exercise
energy expenditure (P < 0.05) compared with the
recreational athletes (Table 1). The number of kilome-
ters ran per week was ~106 ± 8 for EAA, 102 ± 9 for
ECA, and 46 ± 4 for the recreational athletes (P < 0.001 vs. the elite athletes).

As indicated in Table 1, the average daily caloric
intake and dietary macronutrient composition of the
diets were similar among ECA, RCA, and ROC. Despite
expend ing 970 kcal/day on exercise, EAA reported
significantly lower daily caloric intakes compared with
ECA, RCA, and ROC and were only meeting
70% of their recommended daily caloric intake. EAA also con-
sumed significantly less dietary fat (grams and as a
percentage of total calories) compared with ECA, RCA,
and ROC. Similarly, EAA also consumed significantly
lower dietary protein (grams) than did ECA. Although
the total amount of carbohydrate (grams) consumed by
EAA was similar to that consumed by RCA, ECA,
and ROC, a significantly higher percentage of carbohydrate
(percentage of total calories) comprised the diet of EAA
compared with RCA, ECA and ROC.

Endocrine analyses. The second blood samples ob-
tained from two subjects in the RCA group were
excluded from statistical comparisons because onset of
menses occurred later than 35 days. However, samples
obtained in the follicular phase of these subjects were
included in the regression and correlational analyses
between leptin and various variables.

As shown in Fig. 1, plasma leptin levels in the elite
athletes were significantly lower than in the follicular
phase of the recreational athletes. Despite similar body
fat between the elite athletes, plasma leptin was lower
(P < 0.05) in EAA compared with ECA. Similarly,
plasma leptin was significantly lower in EAA compared
with the recreational athletes (RCA and ROC). This
relationship remained significant even after normaliza-
Significant differences were found in total T4 and total T3 compared with those of RCA, ECA, and ROC. No significant differences in T3 were found between RCA and ECA, but they were significantly lower in EAA compared with those of ROC (Table 2). Elevated T4 and total T3 levels in EAA were lower (Fig. 1). Plasma T4 in ROC could be related to an estrogen-induced decrease in EAA (P < 0.05) compared with the cyclic athletes and those taking oral contraceptives (Table 2). Serum E and plasma P were significantly higher in the luteal compared with the follicular phases of all cyclic athletes. Serum E and plasma P in the follicular and luteal phases did not differ between RCA and ECA (Table 2).

Table 2. Hormone concentrations in cyclic athletes, amenorrheic athletes, and athletes taking oral contraceptives

<table>
<thead>
<tr>
<th></th>
<th>Plasma T4, nmol/l</th>
<th>Plasma T3, nmol/l</th>
<th>Serum Insulin, µU/ml</th>
<th>Serum E, pg/ml</th>
<th>Plasma P, pg/ml</th>
<th>Serum EE, pg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>EAA</td>
<td>59.7 ± 3.5ab</td>
<td>1.1 ± 0.04ab</td>
<td>8.8 ± 0.2ab</td>
<td>24.9 ± 1.6ab</td>
<td>0.8 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>RCA</td>
<td>77.2 ± 4.3bc</td>
<td>1.3 ± 0.06bc</td>
<td>13.3 ± 0.8c</td>
<td>44.5 ± 8.5bc</td>
<td>0.7 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>Luteal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ECA</td>
<td>75.1 ± 3.5bc</td>
<td>1.3 ± 0.04bc</td>
<td>14.6 ± 0.8c</td>
<td>38.6 ± 4.9bc</td>
<td>0.7 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>Luteal</td>
<td></td>
<td></td>
<td></td>
<td>97.8 ± 11.7d</td>
<td>9.5 ± 1.7d</td>
<td></td>
</tr>
<tr>
<td>ROC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Active</td>
<td>107.8 ± 5.0bc</td>
<td>1.6 ± 0.05bc</td>
<td>13.3 ± 0.5c</td>
<td>10.0 ± 0.8d</td>
<td>0.5 ± 0.1d</td>
<td>398.1 ± 64.5</td>
</tr>
<tr>
<td>Placebo</td>
<td></td>
<td></td>
<td></td>
<td>27.4 ± 4.6bc</td>
<td>0.5 ± 0.1d</td>
<td>8.3 ± 3.6c</td>
</tr>
</tbody>
</table>

Values are means ± SE. T4 = total thyroxine; T3 = total triiodothyronine; E = estradiol; P = progesterone; EE = 17α-ethinylestradiol. aP < 0.05 vs. RCA and ECA. bP < 0.05 vs. ROC. cP < 0.05 vs. EAA. dP < 0.05 vs. follicular phase (RCA and ECA). eP < 0.05 vs. active pill phase (ROC).
of the diet, or exercise energy expenditure. No correlations were found between leptin and any of these parameters despite normalization for body fat (Table 3).

**DISCUSSION**

In this study, we examined plasma leptin concentrations in elite amenorrheic athletes, in the follicular and luteal phases of menstrual cycles of elite and recreational cyclic athletes, and in the placebo and active pill phases in recreational female athletes taking oral contraceptive steroids. To date, plasma leptin in the phases in recreational female athletes taking oral contraceptives (ROC). Values are means ± SE. *P < 0.05 vs. follicular phase (RCA and ECA).

**Table 3. Partial correlations**

<table>
<thead>
<tr>
<th>Variable: Plasma Leptin vs.</th>
<th>Partial Correlation Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>r = 0.04 (P = 0.82)</td>
</tr>
<tr>
<td>Age at menarche</td>
<td>r = 0.15 (P = 0.75)</td>
</tr>
<tr>
<td>VO2max</td>
<td>r = -0.12 (P = 0.48)</td>
</tr>
<tr>
<td>Caloric intake</td>
<td>r = -0.04 (P = 0.81)</td>
</tr>
<tr>
<td>Macronutrient content</td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>r = 0.06 (P = 0.73)</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>r = -0.24 (P = 0.20)</td>
</tr>
<tr>
<td>Fat</td>
<td>r = 0.07 (P = 0.69)</td>
</tr>
<tr>
<td>Exercise energy expenditure</td>
<td>r = -0.16 (P = 0.44)</td>
</tr>
<tr>
<td>Estradiol</td>
<td>r = -0.02 (P = 0.89)</td>
</tr>
<tr>
<td>Progesterone</td>
<td>r = 0.21 (P = 0.23)</td>
</tr>
<tr>
<td>17α-Ethynylestradiol</td>
<td>r = -0.18 (P = 0.58)</td>
</tr>
<tr>
<td>Thyroxine</td>
<td>r = 0.25 (P = 0.13)</td>
</tr>
<tr>
<td>Triiodothyronine</td>
<td>r = 0.32 (P &lt; 0.05)</td>
</tr>
<tr>
<td>Insulin</td>
<td>r = 0.33 (P &lt; 0.05)</td>
</tr>
</tbody>
</table>

Partial correlations were determined for leptin-to-percent body fat relationship. Plasma leptin was measured from blood samples obtained in follicular and placebo pill phases for cyclic athletes and those taking oral contraceptives, respectively.

Recent evidence suggests that menstrual disturbances in female athletes are related to low energy availability and that an as-yet-unidentified peripheral/central signal suppresses the GnRH pulse generator (26). The underlying mechanism by which low energy availability suppresses the reproductive axis remains unclear. However, increasing evidence suggests the dependence of LH pulsatility on energy availability is mediated by leptin (8, 14, 20). In addition to its adipostatic function, leptin has been proposed to act as a peripheral signal of energy deprivation and may provide a potential mechanism to initiate and coordinate the complex starvation-induced neuroendocrine response (9). In rodents, in response to fasting, activation of the adrenal axis and suppression of the reproductive and thyroidal axes are accompanied by a reduction in circulating leptin. These starvation-induced responses are attenuated by leptin administration (1).

In the present study, we have found lower plasma leptin in the elite athletes compared with both groups of recreational athletes regardless of menstrual status. Significantly lower body fat can account for the difference in leptin levels between the elite cyclic and recreational athletes. However, in the amenorrheic athletes, plasma leptin remained significantly lower than can be solely accounted for by adiposity. Hypoleptinemia in amenorrheic athletes paralleled suppressed estrogen, thyroid hormones, and insulin and markedly lower caloric and fat intake than expected for their high level of activity. These findings are consistent with those reported in numerous studies (15, 19, 20).

The primary regulator of leptin (ob) gene expression and secretion is adiposity. However, acute (fasting) and chronic changes (overfeeding) in energy balance can disproportionately down- or upregulate leptin secretion, respectively (18). Furthermore, the diurnal rhythm of leptin is not dependent on energy intake or expenditure, but rather on energy and/or carbohydrate availability (14). Thus it is possible that negative energy balance in amenorrheic athletes triggers a mechanism that suppresses leptin (ob) gene expression in, and secretion from, the adipose tissue. Increasing evidence supports the notion that insulin is a critical regulator of ob gene expression and is a leptin secretagogue (37). Thus insulin may provide a mechanism by which adipose tissue detects changes in overall energy balance and in turn, up- or downregulates ob gene expression accordingly. In accordance with other studies, we found a significant correlation between leptin and insulin (20, 38). Moreover, insulin levels were significantly lower in the amenorrheic athletes in the present study, which is also consistent with numerous studies (19, 20). Furthermore, the diurnal rhythm of leptin in cyclic and amenorrheic athletes appears to be directly related to the amplitude of feeding-induced hyperinsul-
lactemia (20). It seems possible that suppressed leptin secretion in amenorrheic athletes may occur via an insulin-dependent mechanism.

The selective presence of hypothyroidism and reduced metabolic rate in amenorrheic athletes suggests an adaptive mechanism is likely responsible for conserving metabolic fuels in an energy-deprived state (19). In the present study, lower circulating leptin was shown to parallel lower thyroid hormones in amenorrheic athletes. Although we did not measure BMR in the female athletes, it is likely reduced in the amenorrheic athletes in our study. We estimated energy availability by subtracting estimated energy expenditure via exercise from dietary intake in our female athletes. Our results show energy availability of ~16 kcal·day⁻¹·kg LBM⁻¹ in the amenorrheic athletes that was ~50% of the elite (30 kcal·day⁻¹·kg LBM⁻¹) and recreational (33 kcal·day⁻¹·kg LBM⁻¹) female athletes. In exercising women, suppression of T₃ production (low-T₃ syndrome) and disruption of LH pulsatility occur abruptly when energy availability falls below the critical threshold of 20–25 kcal·day⁻¹·kg LBM⁻¹ (23). Furthermore, lower T₃ in EAA was not specific to athletic training, as indicated by the absence of similar aberration in matched athletic women who are regularly menstruating (ECA). Although energy availability is estimated in our study, it nonetheless provides a qualitative indication, and it is reasonable to speculate that these amenorrheic athletes may be in states of energy deficiency and that menstrual disturbances are likely related to low energy availability.

We found a significant correlation between plasma leptin and thyroid hormones in the female athletes. Although the relationship between total T₄ and leptin is not independent of body fat, plasma leptin remained significantly correlated with total T₃ despite normalization for body fat. Although speculative, it is possible that a collateral decrease in leptin and T₃ may interact in a synergistic manner to suppress BMR in female athletes. Decreased leptin levels may also be responsible for initiating the ensuing adaptive responses to an energy-deprived state, including suppression of the energetically costly reproductive function as well as activation of the beneficial adrenal axis to conserve metabolic fuels (9). These observations are consistent with a “threshold model” for the effects of leptin on energy homeostasis (35). Thus when leptin falls below a certain “critical threshold level” in an energy-deprived state, the metabolic responses that characterize individuals in a starvation period are invoked to conserve metabolic fuel (35). Further research is warranted to establish whether leptin and T₃ utilize common and/or separate pathways, which may converge during periods of energy imbalance, to regulate energy homeostasis.

The exact mechanism by which leptin may mediate or modulate the reproductive axis is intriguing. Leptin may act directly, indirectly or in conjunction with and/or metabolic signals to influence the reproductive system. Alteration in glucose availability is thought to control GnRH secretion, indicating it may be a critical metabolic signal (44). In humans, fasting-induced suppression of leptin was prevented or temporarily reversed by glucose infusion (10). Moreover, exogenous leptin administration could not restore reproductive function in the presence of decreased glucose availability (8). A recent study by Hilton and Loucks (14) suggests LH pulsatility may not be dependent on energy availability per se but rather on glucose availability. These recent experimental findings suggest leptin might mediate its effects on reproduction through influencing glucose availability.

In normal-weight sedentary women, a rise in leptin during the midluteal phase is observed (11, 34) and midfollicular nocturnal rise in leptin was found to be associated temporally with LH pulsatile parameters and was synchronous to estradiol (21), suggesting that the ability of leptin to modulate the reproductive axis is not secondary to its adipostatic function. It is not known whether leptin secretion would display this menstrual phasic change in female athletes, a population with high incidences ofovulatory and menstrual disturbances. In the present study, we have found a 40–46% rise in leptin during the luteal phase of the menstrual cycle in a group of elite and recreational female athletes, which is comparable to that reported in normal-weight sedentary women (33–60%) (11, 27, 34). However, the absolute rise in leptin in the midluteal phase was much smaller and was not consistently found in all the cyclic athletes. In three female athletes, plasma leptin in the luteal phase was actually lower compared with the follicular phase. We also observed a wide interindividual variation in the relative postovulatory rise in plasma leptin in the cyclic athletes, and, in several cyclic athletes, plasma leptin only rose by ~4–7% in the luteal phase. It is not clear why either a lack or a considerably lesser postovulatory rise in leptin was observed in these female athletes. It is plausible that menstrual disturbances could be present in some of our athletes that were undetectable by a single blood measurement. Preovulatory follicles have been suggested as a potential source of leptin during the luteal phases (6), thus abnormal ovarian follicular development might also account for these findings.

The physiological significance of a rise in leptin in the luteal phase of the menstrual cycle is unclear, but it may be a teleological mechanism to prepare energy reserves to support the ensuing pregnancy and lactation by decreasing sensitivity to leptin via the induction of a transient leptin-resistant state (9). In addition, a postovulatory rise in leptin may act in a coordinated manner with other regulatory ovarian factors to signal or prepare for the fertilized egg (6). Functional leptin receptors are expressed in human ovaries (16), and estrogen receptors have been identified in human adipose tissues (32). Although the physiological significance of these findings remains to be ascertained, the existence of a feedback regulatory mechanism between adipose tissue and and the gonadal axis cannot be excluded. Estrogen administration either stimulated (5) or had no effect on leptin (ob) gene expression and secretion in postmenopausal women (12, 13) or after correction for estrogen-induced fat loss in ovariectomized rats (33). Conversely,
leptin inhibited insulin-induced estrogen production by granulosa cells but had no effect on basal estrogen production in vitro (41). Moreover, numerous studies by use of correlational methods have found either a positive (36) or no (5, 12, 13) relationship between leptin and estrogen. Although the literature on the relationship between leptin and estrogen is inconsistent, it appears to be a complex one.

We found no correlation between leptin and sex hormones, and the rise in circulating leptin in the luteal phase is not correlated with rise in estrogen and progesterone. Thus the rise in circulating leptin in the luteal phase does not appear to result from the stimulation of leptin secretion by the adipose tissue by either estrogen or progesterone. Similarly, the transition from the placebo pill to the active pill phase of oral contraceptives had no effect on plasma leptin despite a significant increase in EE concentration. However, oral contraceptives induce a pharmacological rather than a physiological state, with estrogen concentrations 10-fold higher compared with those of menstrual cycle phases. Thus the relationship between leptin and sex hormones may be distinct in the menstrual cycle vs. exogenous administration of synthetic estrogen. Moreover, the hormonal milieu fluctuates throughout the menstrual cycle and is not characterized solely by variation in estrogen level. In this regard, the nocturnal rise in leptin was reported to be synchronous with estradiol and LH in the midfollicular phase of menstrual cycles in healthy women and the effects of a nocturnal rise of leptin on estradiol may be dependent on a threshold effect of baseline estradiol concentrations (21). Thus the synchronous release of leptin, LH and estradiol, rather than the absolute levels of each, may be more critical in maintaining the functional integrity of the hypothalamo-pituitary-ovarian axis. Furthermore, there may be an upper limit to the stimulatory effects of estrogen on leptin secretion because menstrual cycles superovulated with follicle-stimulating hormone, which resulted in supraphysiological estrogen levels, did not affect leptin levels (29). Thus the relationship between leptin and the reproductive axis, particularly estrogen, may be dependent on the physiological context (13). Moreover, to ascribe a regulatory role to leptin in estrogen production or vice versa on the basis of correlation is difficult and may be misleading.

In summary, amenorrhea in female athletes was accompanied by suppressed plasma leptin, markedly lower caloric and fat intake, estrogen, thyroid hormones, and insulin. Our findings are consistent with the hypothesis that the dependence of reproductive function on energy availability may be mediated by leptin. Suppressed insulin in amenorrheic athletes may provide a mechanism by which adipose tissue detects energy deficiency and, in turn, downregulates leptin (ob) gene expression and secretion. The observation of a concomitant suppression of leptin and $T_3$ suggests they likely function in a synergistic manner to suppress basal metabolic rate. Our findings are consistent with the hypothesis that leptin provides an additional level of communication between adipose tissue, nutritional status, and the functional integrity of the reproductive axis in humans. Leptin may coordinate the myriad neuroendocrine responses associated with energy deprivation, including activation of the beneficial adrenal axis and suppression of the energetically costly reproductive system in an effort to conserve metabolic fuels. The existence of an afferent system that senses energy availability, which in turn provides this information to the neural network, as well as integrating the appropriate responses to metabolic perturbations, would be of important survival value to the individual. We also found a 40–46% rise in leptin in the luteal phase of the menstrual cycle in female athletes, which does not appear to be related to stimulation of adipose tissue by sex hormones. Estrogen in the form of oral contraceptive steroids was found to have no effect on circulating leptin levels. These findings support the hypothesis that estrogen does not have a direct regulatory role on leptin secretion from adipose tissue. However, it cannot be excluded that leptin and estrogen may interact in an indirect manner. Further research is warranted to determine whether leptin has the potential to function as a signal relating information on energy status to the hypothalamic regulators of reproduction or whether it impacts on the reproductive system only to the extent that it can influence glucose availability. Further investigation of the physiological significance of a rise in leptin in the luteal phase of the menstrual cycle and the functional role of leptin receptors in the ovaries is also warranted.

The authors gratefully acknowledge the technical assistance provided by Premila Sathasivam and the exceptional cooperation of the subjects who volunteered in this study.

This work was supported by the Natural Science and Engineering Research Council of Canada (NSERC). F. Thong is a recipient of a postgraduate NSERC award and a Student Research Award from Gatorade Sports Science Institute (GSSI). C. McLean was a recipient of an Ontario Graduate Scholarship and a Student Research Award from GSSI.

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Received 3 September 1999; accepted in final form 16 February 2000.

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