Luteal Phase Deficiency in Recreational Runners: Evidence for a Hypometabolic State

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Exercising women with amenorrhea exhibit a hypometabolic state. The purpose of this study was to evaluate the relationship of luteal phase deficient (LPD) menstrual cycles to metabolic hormones, including thyroid, insulin, human GH (hGH), leptin, and IGF-I and its binding protein levels in recreational runners. Menstrual cycle status was determined for three consecutive cycles in sedentary and moderately active women. Menstrual status was defined as ovulatory or LPD. Subjects were either sedentary (n = 10) or moderately active (n = 20) and were matched for age (27.7 ± 1.2 yr), body mass (60.2 ± 3.3 kg), menstrual cycle length (28.4 ± 0.9 d), and reproductive age (14.4 ± 1.2 yr). Daily urine samples for the determination of estrone conjugates, pregnanediol 3-glucuronide, and urinary levels of LH were collected. Blood was collected on a single day during the follicular phase (d 2–6) of each menstrual cycle for analysis of TSH, insulin, total T3, total T4, free T4, leptin, hGH, IGF-I, and IGF binding protein (IGFBP)-1 and IGFBP-3. Among the 10 sedentary subjects, 28 of 31 menstrual cycles were categorized as ovulatory (SedOvul). Among the 20 exercising subjects, 24 menstrual cycles were included in the ovulatory category (ExOvul), and 21 menstrual cycles were included in the LPD category (ExLPD). TSH, total T3, and free T4 levels were not significantly different among the three categories of cycles. Total T3 was suppressed (P < 0.035) in the ExLPD (1.63 ± 0.07 nmol/liter) and the ExOvul categories of cycles (1.75 ± 0.8 nmol/liter) compared with the SedOvul category of cycles (2.15 ± 0.1 nmol/liter). Leptin levels were lower (P < 0.001) in both the ExOvul (5.2 ± 0.4 µg/liter) and the ExLPD categories of cycles (5.1 ± 0.4 µg/liter) when compared with the SedOvul category of cycles (13.7 ± 1.7 µg/liter). Insulin was lower (P = 0.009) only in the ExLPD category of cycles (31.9 ± 2.8 pmol/liter) compared with the SedOvul (60.4 ± 8.3 pmol/liter) and ExOvul (61.8 ± 10.4 pmol/liter) categories of cycles. IGF-I, IGFBP-1, IGFBP-3, IGF-I/IGFBP-1, IGF-I/IGFBP-3, and hGH were comparable among the different categories of cycles. These data suggest that exercising women with LPD menstrual cycles exhibit hormonal alterations consistent with a hypometabolic state that is similar to that observed in amenorrheic athletes and other energy-deprived states, although not as comprehensive. These alterations may represent a metabolic adaptation to an intermittent short-term negative energy balance. (J Clin Endocrinol Metab 88: 337–346, 2003)

A MENORRHEA ASSOCIATED WITH exercise training has been observed in many female athletes across a wide spectrum of sport activities (1, 2). The prevalence of amenorrhea associated with exercise has been variously reported to range from 1–46% in the population of athletic women (2). Amenorrhea is the most severe menstrual disturbance observed in athletes; other more subtle and less severe menstrual disturbances include LPD and anovulatory cycles. LPD occurs in female athletes and active women at a much greater prevalence than amenorrhea (3). De Souza et al. (3) reported a 3-month sample prevalence and incidence rate of abnormal menstrual cycles to be 48 and 79%, respectively in recreationally active women. LPD and anovulatory cycles represent the most common menstrual cycle abnormality associated with exercise, although, many of these women go undiagnosed due to the asymptomatic nature of these disturbances (3). Typically, LPD presents without any significant change in menstrual cycle length, despite prolonged follicular phases and shortened progesterone-deficient luteal phases (3). Clinically, LPD is associated with abnormal corpus luteal function, which includes the aforementioned short luteal phases and inadequate progesterone production, and also inappropriate endometrial stimulation and maturation (4). These luteal phase alterations cause asynchronous follicular growth, compromised oocyte maturation, and differentiated (out of phase) function of the endometrium, which is associated with low rates of cycle fecundity and high rates of embryonic loss, i.e. infertility and spontaneous abortion (4).

Although the exact mechanism(s) underlying these menstrual disturbances remains unclear, the impact of energetic stress and its key components, energy intake, energy expenditure, and energy balance, are likely involved. A preponderance of data are accumulating in both animal and human models to support a chronic deficiency in energy intake resulting in negative energy balance (5–9). This is negative energy balance that is the likely cause of exercise-associated menstrual disturbances, including amenorrhea (5–9). Recently, Williams et al. (5, 6) eloquently documented the pivotal role of adequate energy availability on the induction and reversal of amenorrhea in an exercising nonhuman primate model. Williams et al. (6) demonstrated that the induction of amenorrhea was a product of the volume of calories used during exercise training, which decreased the energy available for reproductive and other necessary metabolic func-

Abbreviations: BMI, Body mass index; Cr, creatinine; E1C, estrone conjugates; ExLPD, exercising subject in LPD cycle category; ExOvul, exercising subject in ovulating cycle category; FT4, free T4; hGH, human GH; IGFBP, IGF binding protein; LPD, luteal phase deficient or luteal phase deficiency; PDG, pregnanediol 3-glucuronide; SedOvul, sedentary subject in ovulatory cycle category; VO2 peak, peak oxygen uptake.
tions; the amenorrhea was reversed simply by supplementing energy intake with no modifications in exercise volume. Shifting of metabolic fuels away from the costly function of reproduction and toward life-sustaining metabolic processes is likely a metabolic effort to conserve energy during periods of low energy availability, a situation similar to the metabolic paradigm observed in amenorrheic exercising women.

In amenorrheic athletes, Laughlin and Yen (10, 11) and others (7–9, 12–14) have described a constellation of hormonal and metabolic shifts that are indicative of a hypometabolic state. These metabolic disturbances include a lowering of the classic endocrine marker of metabolism, total T$_3$ (7–9, 10, 12, 13), decreased leptin (11, 15), decreased plasma glucose (9, 10), increased human GH (hGH; Refs. 9, 10, and 14) and cortisol (9, 10, 13), decreased insulin (9, 10), and a decrease in IGF-I (10). These hormonal and metabolic shifts are also observed in other nonathletic women with functional hypothalamic amenorrhea as a result of self-imposed severe nutritional restrictions, particularly of dietary fat (16, 17). Williams et al. (5) also demonstrated that total T$_3$ was reduced during the induction of exercise-associated amenorrhea and increased during the calorie-supplemented reversal of amenorrhea in nonhuman primates. These metabolic changes have been reported in association with a reduction in resting metabolic rate in amenorrheic athletes compared with eumenorrheic athletes and their sedentary counterparts (18, 19), a phenomenon referred to as energy conservation. The concept of energy conservation, in concert with metabolic substrate and hormone alterations, is supportive of a metabolic shift that repartitions metabolic fuels away from the reproductive axis and toward cellular, locomotive, and other important life-sustaining metabolic processes to conserve energy during periods of low energy availability (20).

It appears likely that exercising women with subtle disturbances in menstrual function, such as LPD, may exhibit many of the characteristic hormonal and metabolic alterations observed in amenorrheic athletes. There have, however, been no prospective evaluations of this issue, specifically with a defined sample population across a 3-month period of time. The purpose of this investigation, therefore, was to evaluate the relationship between menstrual cycle status, specifically LPD, and markers of energy metabolism, including thyroid hormones, insulin, hGH, IGF-I, IGF binding proteins (IGFBPs), and leptin levels.

Subjects and Methods

Subjects

Forty-six women initially volunteered for the study. All subjects were required to read and sign an approved consent form. This study was approved by the New Britain General Hospital Institutional Review Board and the University of Connecticut Health Center Institutional Review Board. Women were: 1) between the ages of 18 and 36 yr; 2) in good health, as determined by a medical examination, including a normal Papanicolaou smear within the past year; 3) free of any chronic disease, including hyperprolactinemia and thyroid disease; 4) had menstrual cycle lengths of 24–36 d; 5) had not experienced any recent change in menstrual bleeding; 6) had an activity history of either sedentary (exercise no more than 1 h/wk) or active (running at least 2 h/wk); 7) had not taken any form of hormonal therapy for at least 12 months; 8) had no history of eating disorders or depressive illnesses within the past 3 yr, as indicated by self report; and 9) had no other contraindications that precluded participation in the study. Specific admission criteria were met by 35 of the 46 subjects screened, and these women were included in the overall study on menstrual status (3).

Subject classification

The sample population consisted of a cohort of women who were initially classified as either 1) sedentary eumenorrheic women, performing no more than 1 h of aerobic exercise per week for the past 12 months, with a peak oxygen uptake (VO$_2$ peak) less than 35 ml/kg/min; or 2) recreational runners, running at least 2 h/wk for the past 12 months, with a VO$_2$ peak greater than 35 ml/kg/min. Each subject completed a maximal exercise test to exhaustion to document peak aerobic power (VO$_2$ peak).

All subjects were subsequently classified after a three-cycle prospective evaluation of training status (determined by analysis of training diaries) and menstrual status (determined by hormonal evaluations) during the 3-month study period. For training status, two general categories were used: sedentary and exercising. For menstrual status, two general categories were used: ovulatory and LPD, as defined below. The three combined categories of exercise and menstrual cycles evaluated were: 1) sedentary women (n = 10 subjects) with ovulatory menstrual cycles (SEDov; n = 28 cycles); 2) exercising women (n = 11 subjects) with ovulatory menstrual cycles (Excov; n = 24 cycles); and 3) exercising women (n = 9) with LPD menstrual cycles (ExLPD; n = 21). Sedentary women with LPD menstrual cycles (n = 1 individual; n = 3 cycles) and exercising women with anovulatory menstrual cycles (n = 4 individuals; n = 8 cycles) were excluded from all data analyses.

Training status

Training activities were recorded daily. Factors recorded on the training cards included distance and duration of each run. Heart rate by radial artery palpation (15 sec × 4) was also recorded after each run. Any other physical activity performed for 3 or more sustained minutes was also recorded on the training record with an associated heart rate (15 sec × 4). Training cards were monitored weekly and collected monthly. Exercise training volume was defined as the actual number of kilometers run per week, as recorded on the training logs. Exercise training hours were defined as the number of hours run per week plus the hours per week of other weight-bearing physical activity. Weight, menstrual patterns, nutritional and training habits, and any unusual stress events (self report in daily logs) that might affect ovulatory function were monitored throughout this study. Subjects were weighed at each weekly visit.

Menstrual categorization

Menstrual calendars were used 1 month before the study and for the duration of the study to record the first and last day of menses for each cycle. Women who had menstrual cycles of less than 20 d or more than 38 d during the 3-month monitoring period were excluded from the data analysis. From the initial 46 women who were screened for this study, 35 women qualified, and these data were previously published in a manuscript that focused on the reproductive hormone characteristics of their menstrual status (3). For this study on metabolic status, 10 sedentary and 20 exercising subjects were included after the exclusion of 1 woman in the sedentary group who presented with LPD and 4 in the exercising group who presented with anovulatory cycles.

Subjects were required to maintain a detailed menstrual record and to collect timed 8-h urine samples beginning on d 2, 3, or 4 of the menstrual cycle (d 1 defined as the first day of menstrual bleeding) until the onset of their next menses every day for 2–3 consecutive study cycles. Timed 8-h urine sample collections were initiated each night upon retiring, continued throughout the night, and terminated after the first morning void. Date and time of sample collection were recorded. Subjects were provided with a toilet-type urine catch kit with prelabeled urine containers for each study cycle. All subjects were required to place an aliquot (10 ml) of each urine sample in a prefilled labeled tube and to store the tubes in a refrigerator. Samples were delivered to the laboratory on a weekly basis. At this visit, urine collection tubes were given to the subject for the next week. By urine samples were analyzed for creatinine (Cr), L.H, pregnanediol-3-glucoronide (PdG), and estrone conjugates (EIC).
Determinant of ovulatory and menstrual cycle phases

Ovarian status was determined for all subjects using the following criteria. The day of the LH surge was identified by a LH peak and by the concurrence of the day of or the day after the midcycle E1C peak. Because there is a delay between the plasma and urinary peaks of LH, the urinary peak was used as the day of ovulation, since the urinary peak is temporally closer to the plasma peak to the actual release of the oocyte (21). Menstrual cycle length was defined as the number of days from d 1 of menses to the day before d 1 of the next menses. The follicular phase length was defined as the number of days from d 1 of menses up to and including the day of the LH surge. The luteal phase length was defined as the difference between the cycle length and follicular phase length. LPD was defined as short when a luteal phase length was less than 10 d, or inadequate when peak PdG excretion was less than 1.5 µg/mg Cr for 3 or more midluteal phase days (22–27). An anovulatory cycle was defined as a cycle in which no increase in E1C was observed in concurrence with a failure of LH to rise at midcycle.

Urinary metabolites, E1C and PdG, were compared among the cycles by the method of mean steroid levels recently described by Winters et al. (23). E1C excretion was assessed during the follicular phase and during the luteal phase. Peak E1C was also assessed.

PdG excretion was assessed during the luteal phase, defined as the day after the LH surge to the day before the onset of the next menses. Luteal phase adequacy was further examined using several methods, as modified from Jordan et al. (22) in serum and from others in urine (23–27), including the sum of the 3-d midluteal peak PdG (sum of midluteal peak PdG < 1 d) and the peak PdG.

Urine hormone measurements

The validity of the urine technique as representative of the 24-h pattern of E1C and PdG excretion has been reported by other investigators (21, 23). Refrigerated 10-ml aliquots of urine were delivered to the laboratory, aliquoted into polyethylene tubes, and frozen at −80°C. Samples for urine LH were determined by RIA using a 1-in-2-dilution (Diagnostic Products Corp., Los Angeles, CA). The sensitivity of the assay is 1.2 mIU/liter. The interassay and intra-assay coefficients of variation were less than 3.4% and less than 4.7%, respectively. RIA measurements of urine LH were performed in duplicate at the Reproductive Endocrinology Laboratory at New Britain General Hospital (New Britain, CT).

Urine samples were analyzed for E1C and PdG by enzyme immunoassay as described by Munro et al. (24). E1C and PdG concentrations were both normalized to Cr excretion in the same sample to control for variations in urine output and volume. E1C and PdG levels are expressed as nano-grams per milligram and micrograms per milligram of Cr, respectively. Urine samples in which the Cr level was less than 0.2 mg/ml were considered too dilute to yield accurate measurements; therefore, the values for these samples were treated as missing results. The sensitivities of the E1C and PdG assays were 7.8 ng/ml and 0.15 µg/ml, respectively. Values below the sensitivity of the assay were reported at the minimum detection limit. The intra-assay coefficients of variation for high and low internal controls in 111 individual assays were 14.7% and 13.1% for E1C and 15.6% and 12.9% for PdG, respectively. All E1C and PdG assays were performed in duplicate at the Institute for Toxicology and Environmental Health, University of California (Davis, CA).

Blood sampling for metabolic hormones

Blood samples were obtained during the early follicular phase (d 2–6) of each menstrual cycle monitored. Sample values that were used were from all cycles included in this metabolic study. Blood was obtained from the antecubital vein and collected into plain red-top tubes. Samples were allowed to clot and then centrifuged for 15 min. The serum was aliquoted into 2-ml polyethylene storage tubes and stored frozen at −80°C until analysis. The serum samples were analyzed for TSH, total T4, free T4 (FT4), total T3, insulin, leptin, hGH, IGF-I, IGFBP-1, and IGFBP-3.

Thyroid hormone measurements

The thyroid hormones were analyzed using a chemiluminescence-based immunoassay analyzer (Immulite, Diagnostic Products Corp.). The functional sensitivity of the TSH assay on this instrument is 0.1 mIU/liter. The intra-assay and interassay coefficients of variation for the TSH assay were 5.1% and 9.0%, respectively. The analytical sensitivity of the total T4 assay was 6.43 nmol/liter. The intra-assay and interassay coefficients of variation for the total T4 assay were 7.5% and 8.7%, respectively. The analytical sensitivity of the FT4 assay was 1.93 pmol/liter. The intra-assay and interassay coefficients of variation for the FT4 assay were 6.7% and 9.1%, respectively. The analytical sensitivity of the total T3 assay was 0.54 nmol/liter. The intra-assay and interassay coefficients of variation for the total T3 assay were 9.7% and 8.7%, respectively. Thyroid hormone analysis was performed by the Reproductive Endocrinology Laboratory at New Britain General Hospital (New Britain, CT).

Insulin analysis

Insulin was analyzed using a chemiluminescence-based immunoassay analyzer (Immulite, Diagnostic Products Corp.). The analytical sensitivity of the insulin assay was 13.89 pmol/liter. The intra-assay and interassay coefficients of variation were 4.8% and 5.8%, respectively.

Leptin analysis

Leptin was determined using an ELISA method (Linco Research, Inc., St. Charles, MO). The sensitivity of the leptin assay was 0.05 µg/liter. The intra-assay and interassay coefficients of variation were 3.8% and 4.4%, respectively. Leptin analysis was performed by the Core Endocrine Laboratory at the Penn State Milton Hershey Medical Center (Hershey, PA).

IGF-I, IGFBP-1, and IGFBP-3 analysis

IGF-I was determined by acid ethanol extraction RIA (Nichols Institute Diagnostics, San Juan Capistrano, CA). The analytical sensitivity of the IGF-I assay was 25 µg/liter, and the intra-assay and interassay coefficients of variation were 3.0% at a concentration of 207 µg/liter and 6.0% at a concentration of 127 µg/liter, respectively. IGFBP-1 was determined by an IRMA method (Radioassay Systems Laboratory, Webster, TX). The analytical sensitivity of the IGFBP-1 assay was 1.0 µg/liter. The intra-assay coefficients of variation were 5.2% at a concentration of 5.2 µg/liter, 4.6% at a concentration of 50.2 µg/liter, and 2.7% at a concentration of 144.6 µg/liter. The inter-assay coefficients of variation were 3.5% at a concentration of 5.2 µg/liter, 6.0% at a concentration of 47.1 µg/liter, and 3.6% at a concentration of 4.97 nmol/liter. IGFBP-3 was determined by RIA (Nichols Institute Diagnostics). The analytical sensitivity of the IGFBP-3 assay was 1.0 µmol/liter. The intra-assay coefficients of variation were 8.0% at a concentration of 20.0 µmol/liter, 3.4% at a concentration of 60.0 µmol/liter, and 3.8% at a concentration of 120.0 µmol/liter. The interassay coefficients of variation were 5.3% at a concentration of 20 µmol/liter, 6.0% at a concentration of 165 µmol/liter, and 6.3% at a concentration of 60 µmol/liter. IGF-I, IGFBP-1, and IGFBP-3 analyses were performed by the Core Endocrine Laboratory at the Penn State Milton S. Hershey Medical Center.

hGH analysis

hGH was determined via RIA (Diagnostic Products Corp.) The sensitivity of the hGH assay was 1.2 µg/liter. The intra-assay coefficients of variation were 2.8% at a concentration of 1.7 µg/liter, 1.6% at a concentration of 5.3 µg/liter, and 1.5% at a concentration of 10.9 µg/liter. The interassay coefficients of variation were 10.4% at a concentration of 3.6 µg/liter, 7.6% at a concentration of 9.7 µg/liter, and 8.0% at a concentration of 19.7 µg/liter. hGH determinations were performed at the Core Endocrine Laboratory at the Penn State Milton S. Hershey Medical Center.

Peak exercise testing

VO2 peak was determined by measurement of expired metabolic gases during a progressive treadmill test to volitional exhaustion. The treadmill test was a continuous graded test that was modified according to each subject’s training history. During the test, the subjects breathed continuously through a Hans Rudolph valve and corrugated plastic tubing connected to a pneumotach. Expired air samples were measured using an on-line Medical Graphics Exercise System 2000 (Medical Graphics, St. Paul, MN).
Body composition testing

Body fat was determined using skinfold measurements (28) at various sites, including the tricep, subscapula, iliac crest, abdomen, and thigh, with a constant pressure skinfold caliper (Holtain, Ltd., Crymych, Dyfed, UK). All measurements were made in triplicate on the right side of the body by one investigator (test–retest; τ = 0.96). It should be noted that body fat percentage was not determined for six subjects (two sedentary and four exercising).

Statistical methods

Demographic data were analyzed using an independent sample Student’s t test (sedentary vs. exercising women). All training parameters, menstrual cycle characteristics, and hormone parameters were analyzed among the categories of menstrual cycles identified based on exercise and menstrual cycle status (i.e., SedOvul, ExOvul, and ExLPD categories of menstrual cycles). When comparing training parameters, menstrual cycle characteristics, and hormone parameters, a mixed model ANOVA, accounting for multiple cycles from individuals in a single experimental category, was used to compare menstrual cycle category means. A significance level of 0.05 was used to detect the differences for all statistical procedures. When a significant main (fixed) effect was observed, Student’s t tests were used with a Bonferroni correction for multiple comparisons to determine where the significant differences existed. Pearson-product moment correlation analysis was used to examine relationships among variables. Data were analyzed using the Statistica (Statsoft, Tulsa, OK) software package.

Results

Demographic characteristics

The demographic characteristics of the study participants are presented in Table 1. Because the exercising subjects in both menstrual categories [ExOvul (n = 11) and ExLPD (n = 9)] were similar on all demographic characteristics, their data were collapsed across menstrual categories. The groups were similar with respect to age, height, weight, and lean body mass. The sedentary subjects had significantly more (P < 0.001) fat mass, a higher (P = 0.014) percentage of body fat, and a higher body mass index (BMI; P = 0.019) than the exercising group. The body weight of the subjects did not differ (P < 0.05) from week to week across the menstrual cycles monitored (data not shown). The subject groups were similar with respect to reproductive characteristics, including age of menarche and reproductive maturity (Table 1).

Training characteristics

Within the exercise groups of women, there were no significant differences in training characteristics (Table 1), including distance run and time spent in other weight-bearing physical activities per week (Stairmaster, walking/running, resistance training, hiking, tennis, and racquetball). The volume of exercise performed by these subjects was typical of moderate or recreational runners. As expected, the training parameters, including aerobic capacity and resting heart rate, were different (P < 0.001) from the sedentary group and representative of a moderately exercise-trained population. The sedentary group had a significantly lower VO₂ peak and resting heart rate, ran fewer kilometers per week, and engaged in fewer hours of other activity.

Menstrual cycle categorization and characteristics

The menstrual cycle parameters are presented in Table 2. Thirty-five women participated in the prospective evaluation of menstrual status, which was previously published (3). For this study on metabolic status, 10 sedentary and 20 exercising subjects were included; 1 woman in the sedentary group presented with LPD, and 4 in the exercising group presented with anovulatory cycles and were excluded from this investigation. For menstrual category comparisons, women were classified according to the predominant menstrual category (ovulatory or LPD) they displayed in at least two of three menstrual cycles evaluated. The sedentary group included 10 women; 9 had 3 of 3 and 2 had 2 of 2 ovulatory menstrual cycles. These 28 menstrual cycles comprised the SedOvul categorization of menstrual cycles. The exercising women included 11 women with 24 menstrual cycles that comprised the ExOvul category of menstrual cycles; 3 had 3 of 3, 2 had 2 of 2, 5 had 2 of 3, and 1 had 1 of 2 ovulatory menstrual cycles. The exercising women also included 9 women with 21 menstrual cycles that comprised the ExLPD category of cycles; 4 had 3 of 3, 1 had 2 of 2, 3 had 2 of 3, and 1 had 1 of 2 LPD menstrual cycles.

Menstrual cycle length was comparable in the three menstrual categories. As anticipated, the luteal phase length was significantly shorter in the ExLPD category of cycles (8.2 ± 0.5) when compared with the SedOvul (12.9 ± 0.4) and ExOvul (12.9 ± 0.3) category of cycles. In concert with this finding, the ExLPD category of cycles had a longer (P = 0.015) follicular phase than the SedOvul and ExOvul categories of cycles.

Estrogen production. The excretion patterns for E1C are presented in Table 2. There were no significant differences noted among the different categories of cycles with respect to peak E1C excretion, mean follicular phase E1C excretion, or mean luteal phase E1C excretion.

Progestrone excretion. The pattern of PdG excretion is presented in Table 2. The ExLPD category of cycles was characterized by significantly less PdG during the luteal phase when compared with the SedOvul and ExOvul categories of cycles. PdG excretion was lower in the ExLPD category of cycles.

### Table 1. By subject comparisons based on exercise status

<table>
<thead>
<tr>
<th></th>
<th>Sedentary (n = 10 subjects)</th>
<th>Exercising (n = 20 subjects)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographic</td>
<td></td>
<td></td>
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<tr>
<td>Age (yr)</td>
<td>26.6 ± 1.2</td>
<td>28.8 ± 1.3</td>
<td>0.292</td>
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<tr>
<td>Height (cm)</td>
<td>163.3 ± 1.7</td>
<td>164.6 ± 1.8</td>
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<tr>
<td>Weight (kg)</td>
<td>62.2 ± 5.1</td>
<td>58.1 ± 1.6</td>
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</tr>
<tr>
<td>Lean body mass (kg)</td>
<td>47.5 ± 1.8</td>
<td>47.5 ± 0.7</td>
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<td>Fat mass (kg)</td>
<td>18.0 ± 1.9</td>
<td>11.9 ± 0.5 *                &lt;-0.001</td>
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<tr>
<td>Body fat (%)</td>
<td>25.5 ± 2.5</td>
<td>19.8 ± 1.2</td>
<td>0.014</td>
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<td>BMI (kg/m²)</td>
<td>23.3 ± 0.9</td>
<td>21.5 ± 0.3</td>
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<td>Reproductive</td>
<td></td>
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<tr>
<td>characteristics</td>
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<td>Age of menarche (yr)</td>
<td>13.6 ± 0.5</td>
<td>12.7 ± 0.2</td>
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<td>Reproductive age (yr)</td>
<td>13.0 ± 1.1</td>
<td>15.8 ± 1.3</td>
<td>0.162</td>
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<td>Training characteristics</td>
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<tr>
<td>VO₂ peak (mL/kg · min)</td>
<td>30.2 ± 1.4</td>
<td>41.4 ± 1.2 *              &lt;-0.001</td>
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<tr>
<td>Distance run (km/wk)</td>
<td>0.2 ± 0.2</td>
<td>32.4 ± 3.5 *              &lt;-0.001</td>
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<tr>
<td>Hours of other activity/wk</td>
<td>0.6 ± 0.3</td>
<td>5.3 ± 0.8 *              &lt;-0.001</td>
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<tr>
<td>Training heart rate</td>
<td>na</td>
<td>132.0 ± 4.7</td>
<td>na</td>
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<tr>
<td>Resting heart rate</td>
<td>74.5 ± 1.7</td>
<td>63.8 ± 1.6 *              &lt;-0.001</td>
<td></td>
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</tbody>
</table>

Values are mean ± SEM. na, Not available; bpm, beats per minute. * Sedentary vs. exercising.
cycles when assessed by peak PdG (P = 0.007), mean luteal phase PdG (P = 0.004), and the sum of the 3-d mid-luteal phase PdG (P = 0.006). The ExOvul category of cycles also exhibited reduced corpus luteal function. This category of cycles had significantly less PdG excreted during the luteal phase and a significantly lower peak PdG when compared with the SedOvul category of cycles.

**LH excretion.** There were no significant differences noted among the individual categories of cycles with respect to peak LH excretion (Table 2).

**Thyroid hormones**

The results of the thyroid hormone assays are presented in Table 3 and Fig. 1. There were no differences (P > 0.05) noted among the categories of menstrual cycles with respect to TSH, total T4, or FT4 (Table 3). The serum insulin level was lower (P < 0.001) in the ExOvul and ExLPD categories of cycles when compared with the SedOvul category of cycles (Fig. 1). There were no differences (P > 0.05) noted in IGF-I, IGFBP-1, IGFBP-3, and the ratio of IGF-I/IGFBP-1, IGF-I/IGFBP-3, and hGH among the categories of cycles.

**Discussion**

Amenorrheic athletes have been shown to have lower resting metabolic rates (18, 19), in concert with shifts in hormone and metabolic substrates, including low plasma glucose levels (10), low T3 (7–10, 12), low insulin (9, 10), low IGF-I (9) and IGF-I/IGFBP-1 (10), increased levels of hGH (9, 10, 14), and mild hypercortisolism (9, 10, 13). This pattern of hormone and metabolic substrates emerges in the face of what is likely a chronic deficiency of caloric intake in

### Table 2. Menstrual cycle parameters

<table>
<thead>
<tr>
<th></th>
<th>SedOvul (n = 28 cycles)</th>
<th>ExOvul (n = 24 cycles)</th>
<th>ExLPD (n = 21 cycles)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Menstrual cycle length (d)</td>
<td>28.8 ± 0.6</td>
<td>27.8 ± 1.0</td>
<td>26.1 ± 0.8</td>
<td>0.138</td>
</tr>
<tr>
<td>Follicular phase length (d)</td>
<td>15.9 ± 0.6</td>
<td>14.8 ± 0.9</td>
<td>17.9 ± 0.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Luteal phase length (d)</td>
<td>12.9 ± 0.4</td>
<td>12.9 ± 0.3</td>
<td>8.2 ± 0.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Estrogen excretion[]^a</td>
<td>E1C peak</td>
<td>82.9 ± 6.3</td>
<td>89.1 ± 5.2</td>
<td>85.7 ± 5.3</td>
</tr>
<tr>
<td></td>
<td>E1C follicular phase</td>
<td>43.5 ± 2.6</td>
<td>42.1 ± 2.6</td>
<td>41.6 ± 3.4</td>
</tr>
<tr>
<td></td>
<td>E1C luteal phase</td>
<td>49.8 ± 3.3</td>
<td>45.2 ± 3.6</td>
<td>50.3 ± 3.7</td>
</tr>
<tr>
<td>Progesterone excretion[]^b</td>
<td>Peak luteal PdG</td>
<td>8.5 ± 0.5</td>
<td>6.8 ± 0.5f</td>
<td>4.4 ± 0.5f</td>
</tr>
<tr>
<td></td>
<td>PdG luteal phase</td>
<td>5.0 ± 0.4</td>
<td>3.7 ± 0.3f</td>
<td>2.9 ± 0.3f</td>
</tr>
<tr>
<td></td>
<td>Sum of 3-d midluteal PdG</td>
<td>21.7 ± 1.4</td>
<td>17.8 ± 2.3</td>
<td>10.9 ± 1.2f</td>
</tr>
<tr>
<td>LH excretion</td>
<td>Peak LH (mIU/liter)</td>
<td>97.0 ± 8.5</td>
<td>95.3 ± 7.5</td>
<td>74.5 ± 8.1</td>
</tr>
</tbody>
</table>

Values are mean ± SEM.

\[\]^a All E1C concentrations are expressed as nanograms per milligram Cr.

\[\]^b All PdG concentrations are expressed as micrograms per milligram Cr.

\[\]^c ExLPD vs. SedOvul and ExOvul.

\[\]^d ExLPD and ExOvul vs. SedOvul.

\[\]^e ExOvul vs. ExLPD.

### Table 3. Metabolic hormone levels

<table>
<thead>
<tr>
<th></th>
<th>SedOvul (n = 28 cycles)</th>
<th>ExOvul (n = 24 cycles)</th>
<th>ExLPD (n = 21 cycles)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSH (µIU/ml)</td>
<td>2.3 ± 0.4</td>
<td>2.2 ± 0.2</td>
<td>1.8 ± 0.2</td>
<td>0.901</td>
</tr>
<tr>
<td>Total T3 (nmol/liter)</td>
<td>88.8 ± 2.6</td>
<td>84.9 ± 2.6</td>
<td>84.9 ± 2.6</td>
<td>0.717</td>
</tr>
<tr>
<td>FT4 (pmol/liter)</td>
<td>16.7 ± 1.3</td>
<td>15.4 ± 1.3</td>
<td>15.4 ± 1.3</td>
<td>0.906</td>
</tr>
<tr>
<td>IGF-I (nmol/liter)</td>
<td>28.0 ± 2.0</td>
<td>33.5 ± 2.5</td>
<td>39.5 ± 2.9</td>
<td>0.111</td>
</tr>
<tr>
<td>IGFBP-1 (nmol/liter)</td>
<td>16.8 ± 1.5</td>
<td>21.0 ± 2.3</td>
<td>19.2 ± 2.1</td>
<td>0.109</td>
</tr>
<tr>
<td>IGFBP-3 (µmol/liter)</td>
<td>162.0 ± 4.0</td>
<td>171.0 ± 1.0</td>
<td>182.0 ± 7.0</td>
<td>0.487</td>
</tr>
<tr>
<td>IGF-I/IGFBP-1[]^a</td>
<td>3.8 ± 0.7</td>
<td>3.2 ± 0.4</td>
<td>4.5 ± 0.8</td>
<td>0.428</td>
</tr>
<tr>
<td>IGF-I/IGFBP-3[]^a</td>
<td>46.3 ± 2.9</td>
<td>52.8 ± 2.4</td>
<td>58.0 ± 3.0</td>
<td>0.114</td>
</tr>
<tr>
<td>hGH (µg/liter)</td>
<td>1.9 ± 0.7</td>
<td>1.2 ± 0.4</td>
<td>4.0 ± 1.5</td>
<td>0.173</td>
</tr>
</tbody>
</table>

Values are mean ± SEM.

\[\]^a Ratios were calculated with original concentrations in metric units.
amenorrheic athletes and is suggestive of a hypometabolic state that helps to conserve energy. Strong support for this notion has recently been documented by Williams et al. (5, 6), who demonstrated that both the induction and reversal of amenorrhea was intimately related to energy availability in an exercise training nonhuman primate model. The present study investigated whether a hypometabolic pattern of hormones is found in exercising women with a less severe reproductive dysfunction, specifically LPD. Unique to this study is the 3-month observational period that accurately assessed ovulatory status and follicular and luteal phase dynamics while documenting the 3-month pattern of metabolic hormones that impact metabolism and energy regulation.

This study documents two important findings. Firstly, exercising women with LPD exhibit the classic endocrine sign of a reduced metabolic rate; namely, a decrease in total T₃. Moreover, both insulin and leptin levels are lower compared with sedentary ovulatory women, and, in the case of insulin, compared with ovulatory exercising women. These findings are consistent with and suggestive of a compromised metabolic state, presumably indicating that unfavorable combinations of dietary intake and physical exercise lead to compromised luteal function. The second important observation is that changes in energy status that lead to LPD in many recreational runners occur intermittently. Data indirectly supportive of this are the high percentage of inconsistent presentations of menstrual cycle status in our previous work. De Souza et al. (3) reported that among exercising women, 46% presented with inconsistent menstrual cycles, including both LPD and anovulatory perturbations, during a three consecutive menstrual cycle evaluation. Because we observed in the current study that different menstrual cycle categories were associated with altered metabolic profiles, it is likely that individuals who present with inconsistent cycle types also experience transient fluctuations in metabolic status.

FIG. 1. Total T₃ (nanomoles per liter) across menstrual and training status groups. Data are presented as mean ± SEM. *, Mean concentrations in ExOvul and ExLPD are significantly (P = 0.035) less than SedOvul.

FIG. 2. Insulin (picomoles per liter) across menstrual and training status groups. Data are presented as mean ± SEM. *, Mean concentration is significantly (P = 0.018) lower than the SedOvul and ExOvul.
caloric restriction alone during one menstrual cycle induced LPD (29–31).

The evidence presented in this study of a hypometabolic state in exercising women with LPD is similar to the hypometabolic state observed in amenorrheic athletes (9, 10, 12), but it is not as comprehensive. Amenorrheic athletes exhibit a greater array and more advanced hormonal and metabolic substrate alterations indicative of a compromised metabolism that are similar in breadth and magnitude to what is observed in other energy-deprived situations, including patients with anorexia nervosa (18, 19, 32), women with functional hypothalamic amenorrhea who self-impose severe restrictions of food intake and dietary fat (16, 17), women with weight-loss associated menstrual irregularities (29–31, 33), and amenorrheic athletes who have energy deficits as a result of disordered eating and compulsive excessive exercise (34, 35).

Menstrual cycle disturbances have been described previously as existing along a spectrum or continuum of reproductive disorders, ranging from ovulatory cycles and subtle LPD to overt amenorrhea (1, 3, 36, 37). The possibility that the same woman may express many of these disturbances intermittently may be more common than previously realized (3). Modulation of an individual’s position on this continuum is essentially affected by caloric intake and caloric expenditure; that is, energy balance (38). As the data by De Souza et al. (3) suggest, exercising women who exhibit mild to moderate menstrual cycle abnormalities revert back and forth from ovulatory cycles, LPD cycles, and anovulation rather frequently and intermittently, and this is probably modulated by changes in energy balance. The LPD women described in this study are most likely in a transitional state in their metabolic and endocrine physiology whereby their metabolic hormonal balance is less homeostatic than in those individuals who are either consistently ovulatory or consistently amenorrheic. It is therefore probable that these exercising women with LPD are in a state of intermittent short-term negative energy balance and are likely adapting to unfavorable combinations of dietary intake and physical exercise that lead to compromised luteal function.

In this study, the stress of the likely intermittent short-term negative energy balance associated with their caloric intake and training and exercise programs is not evident from the subjects’ stable body weights across the 3-month evaluation period, but is manifested by its impact on their hormonal status and luteal function. There are several examples in the literature of alterations in menstrual function that are not necessarily accompanied by significant weight loss (3, 10, 37–39). In a prospective study that induced menstrual disturbances (37), 2 months of rigorous exercise training in a previously sedentary population resulted in LPD and anovulation, whether or not weight was maintained and whether or not the women remained in neutral energy balance. The nature of the ovarian suppression was, however, most profound in the weight loss group. Ellison and Lager (38) found lower salivary progesterone profiles in recreational runners in the absence of differences in body weight and weight for height when compared with sedentary controls. In a nonhuman primate model, Williams et al. (5, 6) demonstrated that during exercise training, the transition from ovulatory normal cycles to

![Fig. 3. Leptin (micrograms per liter) across menstrual and training status groups. Data are presented as mean ± SEM. * Mean concentration is significantly (P < 0.001) less than the SedOvul.](image-url)
amenorrhea was not associated with weight loss, but tightly linked to a reduction in total T3. Thus, varying degrees of adaptive shifts in metabolic homeostasis that alter an individual’s allocation of energy resources in the face of an intermittent short-term negative energy balance may occur and may be associated with luteal phase perturbations (20, 36).

A comment is warranted on the interaction of initial body composition and weight loss to ovarian function. It has been shown that lean women who lose weight experience more severe degrees of ovarian suppression than women of normal weight for height (39). LPD is observed in women of normal weight who lose only 1 kg during the course of caloric restriction over one menstrual cycle (29–31). It is likely that the exercising women in this study are not unlike the vast majority of exercising women who practice moderate dietary restriction and increased energy expenditures, some in the face of cognitive dietary restraint (40), in their efforts to attain targeted health and fitness goals. Thus, they experience ongoing and varying degrees of energetic stress that impacts their hormonal metabolic status and reproductive function as evidenced by our findings.

The hormonal changes observed in this study demonstrate that LPD runners, whether experiencing a short luteal phase or inadequate progesterone production, have suppressed levels of total T3, leptin, and insulin. Serum total T3 was reduced in the LPD runners to the same magnitude as that reported in amenorrheic athletes (7, 8, 12). This pattern was also observed in the ovulatory runners who exhibited a decline in total T3, but not to the same degree as that observed in the LPD runners. The suppression of total T3 is referred to as low T3 syndrome and is indicative of inadequate calorie intake (7, 8, 12). Because T3 is the primary regulator of metabolic rate in humans and is tightly coupled to energy balance (41), it is conceivable that counter-regulatory mechanisms are activated in these runners to conserve metabolic fuel during what is likely an energy-deprived state, or simply, that the conversion of T4 to T3, which takes place in the liver, is mildly compromised as it is in the euthyroid sick syndrome. The women runners in this study clearly showed a reduction in total T3 as a result of their probable intermittent short-term negative energy balance, and it appears that the thyroid hormone axis might be influenced early in response to the altered metabolic energy state in these women with LPD.

In 1995, Williams et al. (42) demonstrated that increased exercise in cycling women reduced LH pulse frequency only when the exercise was unaccompanied by an increase in caloric intake. Loucks et al. (7–9) then linked the reduced LH pulse frequency during exercise in the face of low energy availability to a low T3 syndrome. Interestingly, Williams and Cameron (43) have also shown that following an infusion of exogenous T3 in male rhesus monkeys, the fasting-induced reduction in LH pulse frequency was in fact not prevented. Thus, these and other data (44) provide evidence that T3 probably does not directly control the neuroendocrine axis that maintains the hypothalamic-pituitary-gonadal axis; rather it appears that many of the aforementioned metabolic substrates and hormones may play a key role in serving as a metabolic cue to the functionality of the reproductive axis (20, 45–47).

One such peripheral metabolic signal to the reproductive axis in both animal and human models that has been proposed is leptin, a hormone secreted by adipocytes that acts as a chemical signal of satiety (20, 45–47). Leptin receptors have been localized at all levels of the hypothalamic-pituitary-gonadal axis, including the GnRH neurons of the arcuate nucleus and ventromedial hypothalamus, the ovary, and other neuronal cells that impinge on GnRH neurons (48–50). It has been suggested that leptin might act as a communication link between adipocytes and the hypothalamus, providing the hypothalamus with peripheral cues regarding energy balance and metabolic status to control reproductive integrity (45–47).

Our study clearly demonstrates that exercising women with LPD menstrual cycles have suppressed levels of circulating leptin when compared with sedentary ovulatory women. Leptin levels were also suppressed to a comparable magnitude in the exercising ovulatory women. Laughlin and Yen (11) reported reduced 24-h levels of leptin in both exercising cyclic and amenorrheic women compared with sedentary women. Hilton and Loucks (15) have, in turn, demonstrated that low energy availability profoundly suppressed the 24-h diurnal rhythm of leptin secretion in healthy cyclic women.

It is notable that because leptin is a product of adipose tissue that fluctuates with fat stores and it is a potential regulator of metabolic rate (45–47), it is possible that the lower leptin levels observed in the exercising women in our study, including both the ovulatory and LPD categories, were the result of a lower body fat percentage in these women. In this study and similar to that of Laughlin and Yen (11), leptin was significantly correlated to body fat (r = 0.74, P < 0.01) and BMI (r = 0.69, P < 0.01). Body fat percentage was not measured in all subjects in the present study, although each subject completed weight and height assessments during each of the three menstrual cycles monitored. Therefore, the leptin data were reanalyzed using BMI as a covariant. Similar to Laughlin and Yen (11), the differences between the groups remained significant. Leptin was also significantly correlated to insulin (r = 0.62, P < 0.01) and total T3 (r = 0.56; P < 0.01). Together, these data support the paradigm that the hormonal environment in LPD women is indicative of energetic stress and, in particular, energy deprivation.

Another metabolic hormone indicative of altered metabolism is insulin. The exercising women with LPD menstrual cycles had lower insulin levels compared with both the sedentary and ovulatory cycles. Hypoinsulinemia is typically observed in cross-sectional reports of amenorrheic vs. eumenorrheic athletes (10), but also in response to a standardized meal (10). Loucks et al. (9) have also demonstrated that an acute exposure to a combined regime of exercise training and caloric restriction significantly reduces circulating insulin levels. It appears that the exercising women with LPD do present with a relative hypoinsulinemia, supporting the hypometabolic shift toward energy conservation, but it is unlikely that the degree of disturbance is as severe as that observed in amenorrheic athletes.

The relationship between insulin levels and IGF-I and its binding proteins is quite complex. In the present study, the exercising LPD women exhibited similar levels of IGF-I, IGFBP-1, and IGFBP-3 compared with the ovulatory sedentary and exercising women. These findings are similar to data reported by Laughlin and Yen (11) in which they also observed similar IGF-I and IGFBP-3 levels in amenorrheic and
cyclic athletes compared with sedentary women. IGFBP-1, which is insulin dependent, was strikingly elevated in their amenorrheic vs. cyclic athletes and controls, consistent with the negative regulation of IGFBP-1 by insulin (11, 51). Thus, the exercising LPD women in this study did not display the advanced perturbation in the IGF-I axis, consistent with amenorrheic athletes and other energy-deprived conditions.

It has been reported that the hGH-IGF-I axis is altered in amenorrheic women (14, 52, 53). Similarly, in our studies, the hGH levels were mildly but not significantly elevated in the exercising LPD women compared with the other two groups. The lack of a significant change may simply relate to the poor sensitivity of the hGH assay in detecting subtle changes in concentration. Although it is well known that exercise is a stimulus for GH release (52, 53), the exact mechanism for the enhanced release of GH with exercise, however, has yet to be delineated. Waters et al. (14) reported an increased GH mass per pulse and a decrease in GH pulse frequency in amenorrheic women. One could therefore speculate that our data reflect a shorter period of time when the pulse mass is elevated in the exercising LPD women compared with normally ovulating individuals. It is unclear, however, whether the hGH level measured in the present study reflects an alteration in response to energy changes in the exercising LPD women. The present study is the first prospective study to evaluate the influence of LPD in exercising women on the hormones and growth factors associated with energy regulation and metabolism. It is obvious from this investigation that exercising women with LPD menstrual cycles exhibit some metabolic hormone changes that are consistent with those observed in amenorrheic athletes. However, the extent of the effects is not as comprehensive in the exercising LPD women. These metabolic alterations include a decrease in total T3, insulin, and leptin, and a decrease in GH pulse frequency in amenorrheic athletes and other energy-deprived conditions.

The present study supports a spectrum of metabolic hormone alterations that occur in exercising women with LPD, which includes ovarian suppression associated with no change in menstrual cycle length or body weight. These hormonal alterations can be reflected by perturbations in the hormones that regulate energy metabolism and suppressed reproductive hormones. It is important that future studies address the issues of energy balance, exercise energy expenditure, and caloric requirements in relationship to ovarian suppression and resting metabolism to fully understand the causes of LPD in active women.

Acknowledgments

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