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

MARY JANE DE SOUZA, JACI VAN HEEST, LAURENCE M. DEMERS, AND BILL L. LASLEY

Women's Exercise and Bone Health Laboratory (M.J.D.S., J.V.H.), Faculty of Physical Education and Health, University of Toronto, Toronto, Ontario, Canada M5S 2W6; Department of Kinesiology (J.V.H.), University of Connecticut, Storrs, Connecticut 06269; Penn State University (L.M.D.), Milton S. Hershey Medical Center, Hershey, Pennsylvania 17033; and Institute of Toxicology and Environmental Health (B.L.L.), University of California, Davis, California 95616

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 T_3, total T_4, free T_4, 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 T_4, and free T_4 levels were not significantly different among the three categories of cycles. Total T_3 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)

AMENORRHEA 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). It is this 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; FT_4, free T_4; 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; VO_2 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 hormone 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 status within the past 12 months; 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. Spe-

cific 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 (SedOvul; n = 28 cycles); 2) exercising women (n = 11 subjects) with ovulatory menstrual cycles (ExOvul; 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 substudy 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 prelabeled 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. Daily urine samples were analyzed for creatinine (Cr), LH, pregnanediol-3-glucoronide (PdG), and estrone conjugates (E1C).

Determination of ovulatory and menstrual cycle phases

Ovulatory 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 than 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.

The 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 in 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 nanograms 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 inter-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 anticubital 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 T_4, free T_4 (FT_4), total T_3, 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 0.01 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 T_4 assay was 6.43 nmol/liter. The intra-assay and interassay coefficients of variation for the total T_4 assay were 7.5% and 8.7%, respectively. The analytical sensitivity of the FT_4 assay was 1.93 pmol/liter. The intra-assay and interassay coefficients of variation for FT_4 were 6.7% and 9.1%, respectively. The analytical sensitivity of the total T_3 assay was 0.54 nmol/liter. The intra-assay and interassay coefficients of variation for the total T_3 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 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

VO_2 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; r = 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 metabolic hormones, 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

TABLE 1. By subject comparisons based on exercise status

	Sedentary (n = 10 subjects)	Exercising (n = 20 subjects)	*P*
Demographic			
Age (yr)	26.6 ± 1.2	28.8 ± 1.3	0.292
Height (cm)	163.3 ± 1.7	164.6 ± 1.8	0.645
Weight (kg)	62.2 ± 5.1	58.1 ± 1.6	0.339
Lean body mass (kg)	47.5 ± 1.8	47.5 ± 0.7	0.976
Fat mass (kg)	18.0 ± 1.9	11.9 ± 0.5[a]	<0.001
Body fat (%)	25.5 ± 2.5	19.8 ± 1.2	0.014
BMI (kg/m^2)	23.3 ± 0.9	21.5 ± 0.3	0.019
Reproductive characteristics			
Age of menarche (yr)	13.6 ± 0.5	12.7 ± 0.2	0.075
Reproductive age (yr)	13.0 ± 1.1	15.8 ± 1.3	0.162
Training characteristics			
VO$_2$ peak (ml/kg · min)	30.2 ± 1.4	41.4 ± 1.2[a]	<0.001
Distance run (km/wk)	0.2 ± 0.2	32.4 ± 3.5[a]	<0.001
Hours of other activity/wk	0.6 ± 0.3	5.3 ± 0.8[a]	<0.001
Training heart rate (bpm)	na	132.0 ± 4.7	na
Resting heart rate (bpm)	74.5 ± 1.7	63.8 ± 1.6[a]	<0.001

Values are mean ± SEM. na, Not available; bpm, beats per minute.
[a] Sedentary *vs.* exercising.

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 VO$_2$ 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 substudy 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 menstrual 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.

Progesterone 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 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 T_4, or FT_4 (Table 3). The ExLPD and ExOvul categories of cycles did have lower ($P = 0.035$) total T_3 levels when compared with the SedOvul category of cycles (Fig. 1).

Insulin and leptin

The results for insulin and leptin are presented in Figs. 2 and 3. The serum insulin level was lower ($P = 0.08$) in the ExLPD category of cycles compared with the SedOvul and

ExOvul categories of cycles (Fig. 2). Leptin levels were reduced ($P < 0.001$) in the ExOvul and ExLPD categories of cycles when compared with the SedOvul group (Fig. 3). An analysis of covariance, with BMI selected as a covariant, revealed that leptin levels remained significantly different between the SedOvul group compared with the ExOvul and ExLPD categories of cycles.

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

The results for IGF-I, IGFBP-1, IGHBP-3, and hGH are presented in Table 3. 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 T_3 (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

	SedOvul (n = 28 cycles)	ExOvul (n = 24 cycles)	ExLPD (n = 21 cycles)	P
Menstrual cycle length (d)	28.8 ± 0.6	27.8 ± 1.0	26.1 ± 0.8	0.138
Follicular phase length (d)	15.9 ± 0.6	14.8 ± 0.9	17.9 ± 0.7^c	<0.001
Luteal phase length (d)	12.9 ± 0.4	12.9 ± 0.3	8.2 ± 0.5^c	<0.001
Estrogen excretion[a]				
E1C peak	82.9 ± 6.3	89.1 ± 5.2	85.7 ± 5.3	0.798
E1C follicular phase	43.5 ± 2.6	42.1 ± 2.6	41.6 ± 3.4	0.501
E1C luteal phase	49.8 ± 3.3	45.2 ± 3.6	50.3 ± 3.7	0.063
Progesterone excretion[b]				
Peak luteal PdG	8.5 ± 0.5	6.8 ± 0.5^d	$4.4 \pm 0.5^{d,e}$	0.007
PdG luteal phase	5.0 ± 0.4	3.7 ± 0.3^d	2.9 ± 0.3^d	0.004
Sum of 3-d midluteal PdG	21.7 ± 1.4	17.8 ± 2.3	$10.9 \pm 1.2^{c,e}$	0.006
LH excretion				
Peak LH (mIU/liter)	97.0 ± 8.5	95.3 ± 7.5	74.5 ± 8.1	0.267

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

	SedOvul (n = 28 cycles)	ExOvul (n = 24 cycles)	ExLPD (n = 21 cycles)	P
TSH (μIU/ml)	2.3 ± 0.4	2.2 ± 0.2	1.8 ± 0.2	0.901
Total T_4 (nmol/liter)	88.8 ± 2.6	84.9 ± 2.6	84.9 ± 2.6	0.717
FT_4 (pmol/liter)	16.7 ± 1.3	15.4 ± 1.3	15.4 ± 1.3	0.906
IGF-I (nmol/liter)	28.0 ± 2.0	33.5 ± 2.5	39.5 ± 2.9	0.111
IGFBP-1 (nmol/liter)	16.8 ± 1.5	21.0 ± 2.3	19.2 ± 2.1	0.109
IGFBP-3 (μmol/liter)	162.0 ± 4.0	171.0 ± 1.0	182.0 ± 7.0	0.487
IGF-I/IGFBP-1[a]	3.8 ± 0.7	3.2 ± 0.4	4.5 ± 0.8	0.428
IGF-I/IGFBP-3[a]	46.3 ± 2.9	52.8 ± 2.4	58.0 ± 3.0	0.114
hGH (μg/liter)	1.9 ± 0.7	1.2 ± 0.4	4.0 ± 1.5	0.173

Values are mean ± SEM.
[a] Ratios were calculated with original concentrations in metric units.

FIG. 1. Total T_3 (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.
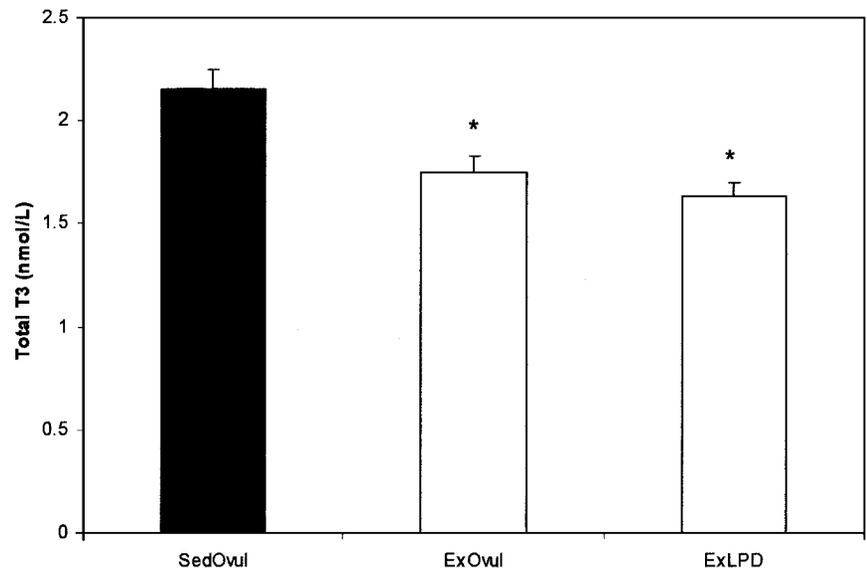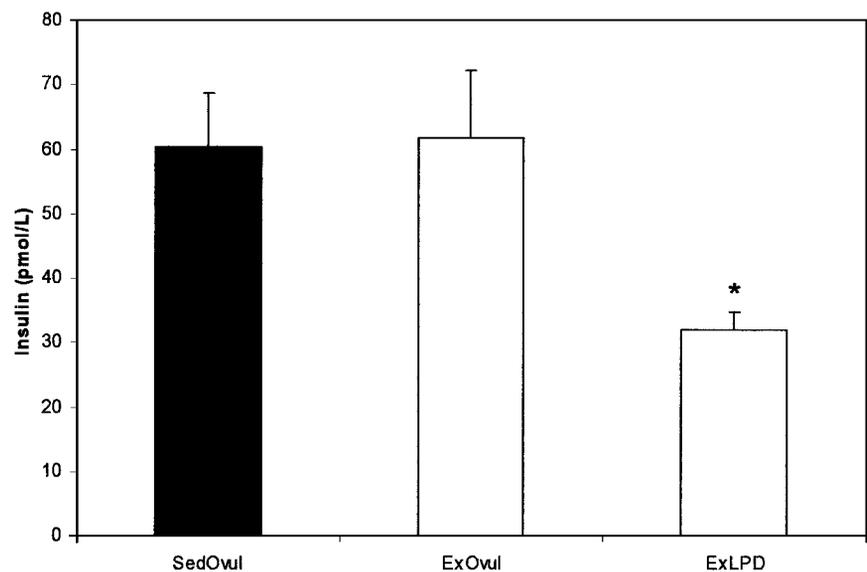

FIG. 2. Insulin (picomoles per liter) across menstrual and training status groups. Data are presented as mean ± SEM. *, Mean concentration is significantly ($P = 0.018$) lower than the SedOvul and ExOvul.

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 T_3. 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. Data from the 1980s (29–31) are supportive of the concept that short-term changes in energy status can induce alterations in menstrual cyclicity. In a series of prospective experiments in healthy women with normal body weights,
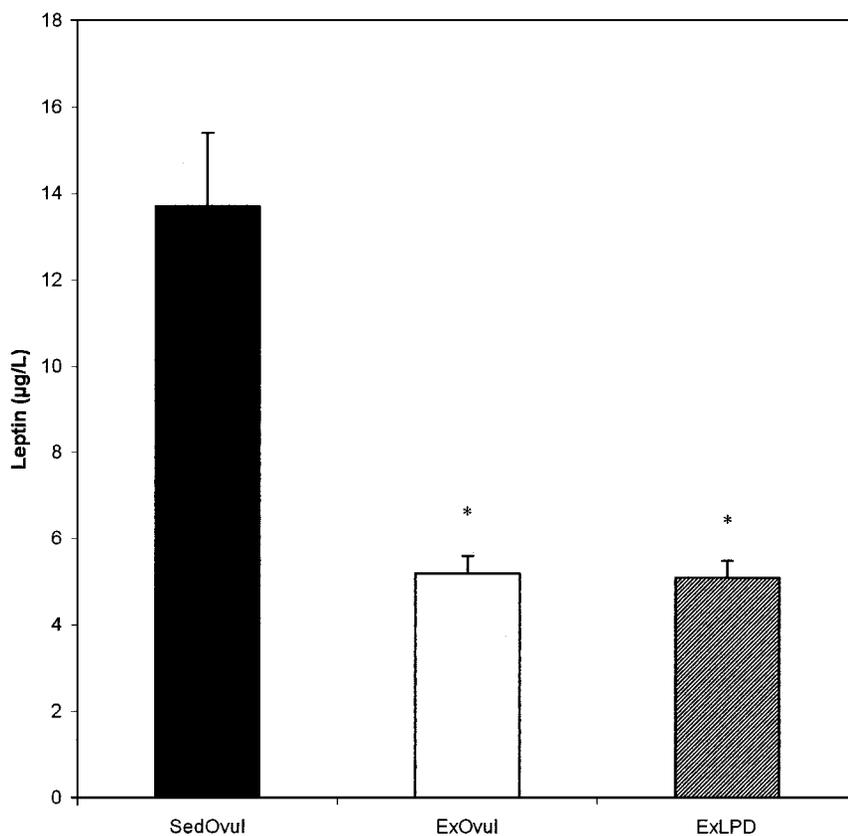
FIG. 3. Leptin (micrograms per liter) across menstrual and training status groups. Data are presented as mean ± SEM. *, Mean concentration is significantly ($P < 0.001$) less than the SedOvul.

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 de-

scribed 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 associated with exercise training but 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

amenorrhea was not associated with weight loss, but tightly linked to a reduction in total T_3. 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 T_3, leptin, and insulin. Serum total T_3 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 T_3, but not to the same degree as that observed in the LPD runners. The suppression of total T_3 is referred to as low T_3 syndrome and is indicative of inadequate calorie intake (7, 8, 12). Because T_3 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 T_4 to T_3, 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 T_3 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 T_3 syndrome. Interestingly, Williams and Cameron (43) have also shown that following an infusion of exogenous T_3 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 T_3 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 T_3 (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 T_3, insulin, and leptin, and are suggestive of an intermittent short-term negative energy balance that is likely a product of an unfavorable balance between caloric intake and energy expenditure. The data from the present study support 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

References

1. **De Souza MJ, Nulsen JC** 1992 Effects of exercise training on sex steroids: endocrine profile and clinical implications. Infertil Reprod Med Clin North Am 3:129–148
2. **Loucks AB** 1990 Effects of exercise training on the menstrual cycle: existence and mechanisms. Med Sci Sports Exerc 22:275–280
3. **De Souza MJ, Miller BM, Loucks, AB, Luciano AS, Pescatello LS, Campbell CG, Lasley BL** 1998 High frequency of LPD and anovulation in recreational women runners: blunted elevation in FSH observed during luteal-follicular transition. J Clin Endocrinol Metab 83:4220–4232
4. **Ginsburg KA** 1992 Luteal phase defect: etiology diagnosis and management. In: Moghissi KS, ed. Endocrinology and metabolism clinics of North America. Philadelphia: WB Saunders Co; 85–104
5. **Williams NI, Helmreich DL, Parfitt DB, Caston-Balderrama A, Cameron JL** 2001 Evidence for a causal role of low energy availability in the induction of menstrual cycle disturbances during strenuous exercise training. J Clin Endocrinol Metab 86:5184–5193
6. **Williams NI, Caston-Balderrama, Helmreich DL, Parfitt DB, Nosbisch C, Cameron JL** 2001 Longitudinal changes in reproductive hormones and menstrual cyclicity in cynomolgus monkeys during strenuous exercise training: abrupt transition to exercise-induced amenorrhea. Endocrinology 142:2381–2389
7. **Loucks AB, Callister R** 1993 Induction and prevention of low-T3 syndrome in exercising women. Am J Physiol 264:R924–R930
8. **Loucks AB, Heath EM** 1994 Induction of low-T3 syndrome in exercising women occurs at a threshold of energy availability. Am J Physiol R817–R823
9. **Loucks AB, Verdun M, Heath EM** 1998 Low energy availability, not stress of exercise, alters LH pulsatility in exercising women. J Appl Physiol 84:37–46
10. **Laughlin GA, Yen SS** 1996 Nutritional and endocrine metabolic aberrations in amenorrheic athletes. J Clin Endocrinol Metab 81:4301–4309
11. **Laughlin GA, Yen SS** 1997 Hypoleptinemia in women athletes: absence of a diurnal rhythm with amenorrhea. J Clin Endocrinol Metab 82:318–321
12. **Loucks AB, Laughlin GA, Mortola JF, Girton L, Nelson JC, Yen SSC** 1992 Hypothalamic-pituitary-thyroidal function in eumenorrheic and amenorrheic athletes. J Clin Endocrinol Metab 75:514–518
13. **De Souza MJ, Maguire MS, Maresh CM, Kraemer WJ, Rubin KR, Loucks AB** 1991 Adrenal activation and the prolactin response to exercise in eumenorrheic and amenorrheic runners. J Appl Physiol 70:2378–2387
14. **Waters DL, Qualls CR, Dorin R, Veldhuis JD, Baumgartner RN** 2001 Increased pulsatility, process regularity and nocturnal through concentrations of growth hormone in amenorrheic compared to eumenorrheic athletes. J Clin Endocrinol Metab 86:1013–1019
15. **Hilton LK, Loucks AB** 2000 Low energy availability, not exercise stress, suppresses the diurnal rhythm of leptin in healthy young women. Am J Physiol Endocrinol Metab 273:E43–E49
16. **Laughlin GA, Dominguez CE, Yen SSC** 1998 Nutritional and endocrine-metabolic aberrations in women with functional hypothalamic amenorrhea. J Clin Endocrinol Metab 83:25–32
17. **Berga SL, Mortola L, Girton B, Suh B, Laughlin G, Pharm P, Yen SS,** Neuroendocrine aberrations in women with functional hypothalamic amenorrhea. J Clin Endocrinol Metab 68:301–308
18. **Myerson M, Gutin B, Warren MP, May MT, Contento I, Lee M, Pi-Sunyer FX, Pierson RN, Brooks-Gunn J** 1991 Resting metabolic rate and energy balance in amenorrheic and eumenorrheic runners. Med Sci Sports Exerc 23:15–22
19. **Lebenstedt M, Platte P, Pirke K** 1999 Reduced resting metabolic rate in athletes with menstrual disturbances. Med Sci Sports Exerc 31:1250–1256
20. **Wade GN, Schneider JE** 1992 Metabolic fuels and reproduction in female mammals. Neurosci Biobehav Rev 16:235–272
21. **Beitins IZ, McArthur JW, Turnbull BA, Skrinar GS, Bullen BA** 1991 Exercise induces two types of human luteal dysfunction: confirmation by urinary free progesterone. J Clin Endocrinol Metab 72:1350–1358
22. **Jordan J, Craig K, Clifton DK, Soules MR** 1994 Luteal phase defect: the sensitivity and specificity of diagnostic methods in common clinical use. Fertil Steril 62:54–62
23. **Winters KM, Adams WC, Meredith CN, Van Loan MD, Lasley BL** 1996 Bone density and cyclic ovarian function in trained runners and active controls. Med Sci Sport Exerc 28:776–785
24. **Munro CJ, Stabenfeldt GH, Cragun JR, Addiego LA, Overstreet JW, Lasley BL** 1991 Relationship of serum estradiol and progesterone concentrations to the excretion profiles of their major urinary metabolites as measured enzyme immunoassay and radioimmunoassay. Clin Chem 37:838–844
25. **Blackwell LF, Brown JB, Cooke D** 1998 Definition of the potentially fertile period from urinary steroid excretion rates. Part II. A threshold value for pregnanediol glucuronide as a marker for the end of the potentially fertile period in the human menstrual cycle. Steroids 63:5–13
26. **Magini A, Pinzani P, Bolelli GF, Bassi F, Salerno R, Messeri G, Pazzagli M** 1989 Measurement of estrone-3-glucuronide and pregnanediol-3 α-glucuronide in early morning urine samples to monitor ovarian function. J Biolumin Chemilumin 4:567–574
27. **Leslie PW, Campbell KL, Little MA, Kigondu CS** 1996 Evaluation of reproductive function in Turkana women with enzyme immunoassays of urinary hormones in the field. Hum Biol 68:95–117
28. **Jackson AS, Pollock ML, Ward A** 1980 Generalized equations for predicting body density in women. Med Sci Sports Exerc 12:175–182
29. **Pirke KM, Schweiger U, Strowitzki T, Tuschl RJ, Laessle RG, Broocks A, Huber B, Middendorf R** 1989 Dieting causes menstrual irregularities in normal weight young women through impairment of episodic luteinizing hormone secretion. Fertil Steril 51:263–268
30. **Schweiger U, Laessle R, Pfister H, Hoehl C, Schwingen-Schloegel M,**

Schweiger M, Pirke KM 1987 Diet-induced menstrual irregularities: effects of age and weight loss. Fertil Steril 48:746–751

31. Pirke KM, Schweiger U, Lemmel W, Krieg JC, Berger M 1985 The influence of dieting on the menstrual cycle of healthy young women. J Clin Endocrinol Metab 60:1174–1179

32. Warren MP, Voussoughian F, Geer EB, Hyle EP, Adberg CL, Ramos RH 1999 Functional hypothalamic amenorrhea: hypoleptinemia and disordered eating. J Clin Endocrinol Metab 84:873–877

33. Vigersky RA, Anderson AE, Thompson RH, Loriaux DL 1977 Hypothalamic dysfunction in secondary amenorrhea associated with simple weight loss. N Engl J Med 297:1141–1145

34. De Souza MJ, Metzger DA 1991 Reproductive dysfunction in amenorrheic athletes and anorexic patients: a review. Med Sci Sports Exerc 23:995–1007

35. Otis CL, Drinkwater B, Johnson M, Loucks AB, Wilmore J 1997 ACSM position stand on the female athlete triad. Med Sci Sports Exerc 29:i-ix

36. Ellison PT, Panter-Brick C, Lipson SF, O'Rourke MT 1993 The ecological context of human ovarian function. Hum Reprod 8:2248–2258

37. Bullen BA, Skrinar GS, Beitins IZ, von Mering G, Turnbull BA, McArthur JW 1985 Induction of menstrual disorders by strenuous exercise in untrained women. N Engl J Med 312:1349–1353

38. Ellison PT, Lager C 1986 Moderate recreational running is associated with lowered salivary progesterone profiles in women. Am J Obstet Gynecol 154:1000–1003

39. Olson BR, Catledge C, Sebring N, Defensor R, Nieman L 1995 Short-term fasting affects luteinizing hormone secretory dynamics but not reproductive function in normal-weight sedentary women. J Clin Endocrinol Metab 80:1187–1193

40. Stunkard AJ, Messick S 1985 The three-factor eating questionnaire to measure dietary restraint disinhibition and hunger. J Psychosom Res 29:71–83

41. Larsen PR, Berry MJ 1995 Nutritional and hormonal regulation of thyroid hormone deiodinases. Annu Rev Nutr 15:323–352

42. Williams NI, Young JC, McArthur JW, Bullen B, Skrinar GS, Turnball B 1995 Strenuous exercise with caloric restriction: effect on luteinizing hormone secretion. Med Sci Sports Exerc 27:1390–1398

43. Williams NI, Cameron JL, Stimulation of luteinizing hormone (LH) secretion in male rhesus monkeys by food intake: role of circulating triiodothyronine (T3). Proc of the 10th International Congress of Endocrinology, San Francisco, CA, 1996, p 531 (Abstract P2–505)

44. Mentoer SM, Woodfill CJI, Karsch FJ 1991 Role of the thyroid gland in seasonal reproduction: thyroidectomy blocks seasonal suppression of reproductive neuroendocrine activity in ewes. Endocrinology 128:1337–1344

45. Moschos S, Chan JL, Mantzoros CS 2002 Leptin and reproduction: a review. Fertil Steril 77:433–444

46. Wade GN, Schneider JE, Li HY 1996 Control of fertility by metabolic cues. Am J Physiol 270:E1–E19

47. Magni P, Motta M, Martini L 2000 Leptin: a possible link between food intake, energy expenditure and reproductive function. Regul Pept 92:51–56

48. Zhang Y, Procenca R, Maffei M, Baroni M, Leopold L, Friedman JM 1994 Positional cloning of the mouse obese gene and its human homologue. Nature 372:425–432

49. Magni P, Vettor R, Pagano C, Calcagno A, Beretta E, Messi E, Zanisi M, Martini L, Motta M 1999 Expression of a leptin receptor in immortalized gonadotropin-releasing hormone-secreting neurons. Endocrinology 140:1581–1585

50. Karlsson C, Lindell K, Svensson E, Berga C, Lind P, Billig H 1997 Expression of functional leptin receptors in the human ovary. J Clin Endocrinol Metab 82:4144–4148

51. Suikkari AM, Koivisto VA, Koistinen R, Seppala M 1989 Dose-response characteristics for suppression of low molecular weight plasma insulin-like growth factor-binding protein by insulin. J Clin Endocrinol Metab 68:135–140

52. DeCree C 1998 Sex steroid metabolism and menstrual irregularities in the exercising female. Sports Med 25:369–406

53. Jenkins P 1999 Growth hormone and exercise. Clin Endocrinol (Oxf) 50:683–689