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High Frequency of Luteal Phase Deficiency and Anovulation in Recreational Women Runners: Blunted Elevation in Follicle-Stimulating Hormone Observed during Luteal-Follicular Transition*

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ABSTRACT

The purposes of this investigation were to evaluate the characteristics of three consecutive menstrual cycles and to determine the frequency of luteal phase deficiency (LPD) and anovulation in a sample of sedentary and moderately exercising, regularly menstruating women. For three consecutive menstrual cycles, subjects collected daily urine samples for analysis of FSH, estrone conjugates (E1C), pregnanediol-3-glucuronide (PdG), and creatinine (Cr). Sedentary (n = 11) and exercising (n = 24) groups were similar in age (27.0 ± 1.3 yr), weight (60.3 ± 3.1 kg), gynecological age (13.8 ± 1.2 yr), and menstrual cycle length (28.3 ± 0.8 days). Menstrual cycles were classified by endocrine data as ovulatory, LPD, or anovulatory. No sedentary women (0%) had inconsistent menstrual cycle classifications from cycle to cycle, but 46% of the exercising women were inconsistent. The sample prevalence of LPD in the exercising women was 48%, and the 3-month sample incidence was 79%. In the sedentary women, 90% of all menstrual cycles were ovulatory (SedOvul; n = 28), whereas in the exercising women only 45% were ovulatory (ExOvul; n = 30); 43% were LPD (ExLPD; n = 28), and 12% were anovulatory (ExAnov; n = 8). In ExLPD cycles, the follicular phase was significantly longer (17.9 ± 0.7 days), and the luteal phase was significantly shorter (8.2 ± 0.5 days) compared to ExOvul (14.8 ± 0.9 and 12.9 ± 0.3 days) and SedOvul (15.9 ± 0.6 and 12.9 ± 0.4 days) cycles. Luteal phase PdG excretion was lower ($P < 0.001$) in ExLPD (2.9 ± 0.3 μg/mg Cr) and ExAnov (0.8 ± 0.1 μg/mg Cr) cycles compared to SedOvul cycles (5.0 ± 0.4 μg/mg Cr). ExOvul cycles also had less ($P < 0.01$) PdG excretion during the luteal phase (3.7 ± 0.3 μg/mg Cr) than the SedOvul cycles. E1C excretion during follicular phase days 2–5 was lower ($P = 0.05$) in ExOvul, ExLPD, and ExAnov cycles compared to SedOvul cycles and remained lower ($P < 0.02$) in the ExLPD and ExAnov cycles during days 6–12. The elevation in FSH during the luteal-follicular transition was lower ($P < 0.007$) in ExLPD (0.7 ± 0.1 ng/mg Cr) cycles compared to SedOvul and ExOvul cycles (1.0 ± 0.1 and 1.1 ± 0.1 ng/mg Cr, respectively). Energy balance and energy availability were lower ($P < 0.05$) in ExAnov cycles than in other menstrual cycle categories. The blunted elevation in FSH during the luteal-follicular transition in exercising women with LPD may explain their lower follicular estradiol levels. These alterations in FSH may act in concert with disrupted LH pulsatility as a primary and proximate factor in the high frequency of luteal phase and ovulatory disturbances in regularly menstruating, exercising women. (*J Clin Endocrinol Metab* **83:** 4220–4232, 1998)

INTENSE exercise training is associated with a broad spectrum of menstrual cycle alterations along a continuum ranging from ovulatory cycles to luteal insufficiency and short luteal phases in asymptomatic cycles of regular length, to menstrual irregularity, anovulation, and the most extreme disturbance, amenorrhea (1–3). Ovulatory disturbances and luteal phase deficiency (LPD) are probably the most common menstrual abnormalities associated with physical activity and exercise training and, for the most part, remain unperceived and thus undiagnosed in the majority of exercising women (4). It is not known whether LPD and ovulatory disturbances in exercising women represent an intermediary point along a continuum that has the potential to progress to an extreme end point in amenorrhea, whether they represent an end point of successful acclimation to exercise training, or whether they represent an end point in more robust women (4, 5).

The prevalence of amenorrhea has been variously reported from 5–46% in runners (6–10) to 37–44% in ballet dancers (11–13). No data are available on the prevalence or incidence of ovulatory disturbances and LPD in either competitive or recreational athletes, but as the majority of physically active women participate in either recreational or moderate levels of physical activity, it is especially important to determine the nature and frequency of menstrual phase abnormalities in that population. As recently acknowledged, scientists cannot define the scope of the potential problem (the number of women affected) or the depth of concern warranted (the health risks) for these active women without this information (14).

Attempts to characterize the actual types of LPD that occur in athletes have been few and limited to single menstrual

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cycle evaluations (1, 3, 15). To quantify the prevalence and incidence of ovulatory status and LPD, daily hormonal measurements of LH and progesterone in the blood or urine are necessary (16, 17). As these techniques are very time consuming and costly, it is not surprising that the documentation and characterization of LPD in athletes has been incomplete (1, 3, 4, 15). In the only attempt at a prospective observational evaluation, 20–27% of moderate and long distance runners experienced one or more episodes of anovulation during two menstrual cycles monitored at the beginning and end of a 12-month period (15), but these data are questionable because the menstrual cycles were minimally monitored. A few cross-sectional evaluations of a single menstrual cycle have revealed evidence of ovulatory disturbances and LPD in both high mileage (18) and recreational runners (3, 4, 19). As menstrual cycle status may not be consistent from cycle to cycle, detailed prospective hormonal evaluations of several menstrual cycles are necessary to estimate the incidence as well as prevalence of ovulatory disturbances. Furthermore, it is important to monitor factors that have been hypothesized to disrupt reproductive function in active women (*i.e.* exercise stress and energy availability).

The transduction of adverse effects at the level of the hypothalamic GnRH pulse generator to the ovary has been attributed to changes in LH pulsatility (18, 20, 21). To date, there have been no prospective observational daily evaluations of reproductive hormones, particularly FSH, across several consecutive menstrual cycles in exercising women. The importance of FSH secretion during the luteal-follicular transition on the recruitment and development of a cohort of follicles, including the dominant follicle, has been emphasized (22, 23). Alterations in daily FSH secretion during the luteal-follicular transition may impact significantly on the subsequent recruitment and maturation of tertiary follicles (22–24).

The purposes of this study were to evaluate the daily ovarian steroid and urinary FSH excretion characteristics in a small sample of regularly menstruating women participating in recreational to moderate levels of exercise and to determine the frequency (*i.e.* the prevalence and incidence) of LPD and anovulatory cycles in the sample during three consecutive menstrual cycles. We also evaluated the relationship of the observed menstrual phase alterations with specific exercise stress and energy availability characteristics.

Of course, frequencies in a small sample may not precisely estimate the true prevalence and incidence in the general population of regularly menstruating women runners, but they can indicate whether there is reason to be concerned. Furthermore, because we restricted our sample to regularly menstruating runners, and because the frequency of irregular menses is high in women runners, the frequencies observed in this study probably underestimate the actual prevalence and incidence of LPD and anovulation in the general population of all women runners.

Subjects and Methods

Subjects

Forty-six women met the following general eligibility criteria. They 1) were between the ages of 18–36 yr; 2) were in good health, as determined by a medical examination, including a normal Papanicolaou smear within the past year; 3) were free of any chronic disease including hyperprolactinemia and thyroid disease; and 4) had menstrual cycle lengths of 24–36 days. They also had 5) not experienced any recent change in menstrual status within 12 months, 6) an appropriate activity history, 7) not taken any form of hormonal therapy for at least 12 months, 8) no history of an eating disorder or depressive illness within the past 3 yr, 9) no contraindications that might preclude participation in the study, and 10) not taken any medication that would interfere in calcium metabolism within the past 3 yr. Women with oligomenorrhea (defined as irregular menstrual cycles occurring every 39–90 days) and primary or secondary amenorrhea were excluded from this study. Specific admission criteria outlined below were met by 35 of the 46 subjects screened, and these women were included in this study.

Subject categorization

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 activity 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 or 16 km/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_{2peak}) and a body fat analysis.

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, three general categories were used: ovulatory, LPD, and anovulatory, as defined below.

Training status

Training activities were recorded daily. Factors recorded on the training cards included distance and duration of each run. Heart rate by radial palpation (15 s × 4) was also recorded after each run. Any other physical activity performed for 3 or more sustained min was also recorded on the training record with an associated heart rate (15 s × 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. The average exercise heart rate during each menstrual cycle served as an index of exercise intensity.

Dietary records and activity monitoring

Seven-day prospective nutritional diaries were completed every month during the study period during the early follicular phase (days 2–9). Subjects were educated on techniques to estimate food portion sizes with the use of food models and were instructed to record all food and liquid consumed throughout the week days and both weekend days. All diet records were analyzed by the same nutritionist using the Nutritionist IV Program (San Bruno, CA). During these 7-day periods, a Caltrac accelerometer (Caltronics, Vienna, VA) was worn on the nondominant hip to measure activity. The Caltrac accelerometer is a motion sensor that weighs 400 g, records vertical acceleration, and estimates energy expenditure. The 7 days of energy expenditure assessments were performed during the early follicular phase (week 1) and the luteal phase (week 3), and a cycle mean was calculated. Because there were no significant differences noted between estimated energy expenditure during weeks 1 and 3, the cycle mean was used for all analyses. The dietary intake of the menstrual cycle categories was assessed for the same 7 days corresponding to the days of energy expenditure assessments during week 1. Heart rate (15 s × 4) was also recorded upon awakening while in bed, at midday, and at midevening during these 7-day monitoring periods.

Weight, menstrual patterns, nutritional and training habits, and any unusual stressors 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 study and for the duration of the study to record the first and the last day of menses for each cycle. Women who had cycles less than 20 days or greater than 38 days during the 3-month monitoring period were excluded from the data analysis. Three women in the exercising group were excluded from the analyses, one for pregnancy, one for a 19-day cycle followed by a 39-day cycle, and one for a 42-day cycle. For six women in the exercising group, data were collected for only two cycles; three of the women had consistent cycles that were ovulatory, one had consistent cycles that were anovulatory, one woman had consistently LPD cycles, and two women had inconsistent cycles with one ovulatory cycle and one LPD cycle [for group comparisons, these two women were included in the exercising LPD (ExLPD) group]. For two women in the sedentary group, data were collected for only two cycles, both were consistent and ovulatory.

Experimental protocol

Volunteers were initially interviewed by phone. If eligible, they reported to the office of the principal investigator to read and sign the informed consent form, which was approved by the institutional review boards at the University of Connecticut Health Center and New Britain General Hospital.

Subjects were then required to maintain a menstrual record and to collect timed 8-h urine samples beginning on day 2, 3, or 4 of the menstrual cycle (day 1 was defined as the first day of menstrual bleeding) until the onset of the next menses every day for two or three consecutive study cycles. Timed 8-h urine samples were initiated each night upon retiring, continued throughout the night, and terminated with the first morning void. The 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, total FSH, LH, pregnanediol-3-glucuronide (PdG), and estrone conjugates (E1C).

Determination of menstrual phase characteristics

Ovulatory status was determined for all subjects using the following criteria. The day of the LH surge was identified by the LH peak and by the concurrence of the day of or the day after the midcycle E1C peak. There is a delay between the plasma and urinary peaks of LH, and the urinary peak was used as the day of the LH surge, because the urinary peak is temporally closer than the plasma peak to the actual release of the oocyte (1). The menstrual cycle length was defined as the number of days from day 1 of menses to the day before day 1 of the next menses. The follicular phase length was defined as the number of days from day 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 the follicular phase length. LPD was defined as short when a luteal phase length was less than 10 days or inadequate when peak PdG excretion was less than 3 μg/mg creatinine (Cr) for 3 or more midluteal phase days (25, 26). 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.

Some data were analyzed by subject groups and others by individual menstrual cycle categories. Groups and individual menstrual cycles were classified as 1) sedentary ovulatory (SedOvul), 2) exercising ovulatory (ExOvul), 3) exercising LPD (ExLPD), and 4) exercising anovulatory (ExAnov). For group comparisons, women were classified according to the menstrual cycle category they displayed in at least two of the three cycles observed. All hormonal data were analyzed by cycle category.

The urinary metabolites, E1C and PdG, were compared among the cycles by the integrated area under the curve (AUC) and by the method of mean steroid levels recently described by Winters *et al.* (25). E1C excretion was assessed during three different periods; 1) during follicular recruitment, defined as days 2–5; 2) during growth and development of steroidogenically active follicles, defined as days 6–12; and 3) during corpus luteum activity, defined as the day after the LH surge to the day before the next menses.

PdG excretion was assessed during two different periods: 1) during adrenal excretion, defined as days 6–10; and 2) during corpus luteum activity, 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 the method of Jordon *et al.* (26), including the sum of the 3-day midluteal peak PdG (sum of midluteal peak PdG $\pm$ 1 day) and the peak PdG.

Urinary analyses

The validity of the urinary techniques as representative of the 24-h pattern of excretion of LH, E1C, and PdG has been presented by other investigators (1, 25, 27). Refrigerated 10-mL aliquots of urine were delivered to the laboratory, aliquoted into polyethylene tubes, and frozen at -80 C. Samples for urinary LH were analyzed by RIA prepared in a 1:2 dilution (Diagnostic Products Corp., Los Angeles, CA). The sensitivity of the assay was 1.2 mIU/L. The inter- and intraassay variances were less than 3.4% and less than 4.7%, respectively. RIA determination of urinary LH was 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.* (27). E1C and PdG were both indexed to Cr excretion in the same sample to control for variations in urine volume. E1C and PdG are expressed as nanograms and micrograms per mg Cr, respectively. Urine samples in which the Cr level was less than 0.2 mg/mL were considered too dilute to yield accurate measurements; levels for these measurements were treated as missing values. The sensitivity of the E1C and PdG assays were 7.8 ng/mL and 0.15 μg/mL, respectively. Values below the sensitivity were reported at the minimum detection limits. The interassay 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.

Urine samples were analyzed for FSH by enzyme immunoassay as described by Qiu *et al.* (28). FSH was also indexed to Cr excretion in the same sample to control for variations in urine volume. FSH is expressed as nanograms per mg Cr. Urine samples in which the Cr level was less than 0.2 mg/mL were also considered too dilute to yield accurate measurements, and these measurements, too, were treated as missing values. The sensitivity of the assay, calculated at 3 SD above the zero standard, was 0.32 ng/mL. Values below the sensitivity were reported at the minimum detection limits. The intraassay coefficients of variation for FSH were 3.0% and 2.3% for the 1.3 and 4.5 ng/mL internal controls, respectively (28). All assays were performed in duplicate at the Institute for Toxicology and Environmental Health, University of California-Davis.

Peak exercise testing

The 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 subjects' training history, and the VO_2 peak was determined by previously published methods and criteria (5). 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 via skinfold measurements (29) at various sites, including the tricep, subscapula, illiac crest, abdomen and thigh with a constant pressure skinfold caliper (Holtain, Ltd., UK). All measurements were made in triplicate on the right side of the body by one investigator (test-retest, $r = 0.96$).

Energy balance and energy availability

Energy balance was estimated by subtracting energy expenditure (estimated by 24-h Caltrac monitoring) from dietary energy intake. Energy availability was estimated by subtracting exercise energy expenditure from dietary energy intake (30). Exercise energy expenditure was estimated by evaluating activity logs. The energy cost for each specific physical activity was calculated by multiplying the estimated caloric value per minute by the number of minutes engaged in a specific activity (31). Energy expenditure due to exercise was accumulated over the 24-h period for each cycle on each of the 7 days observed. The average energy expenditure was then used as the daily exercise energy expenditure. All energy parameters were analyzed both in absolute quantities and normalized by body weight.

Statistical methods

All demographic data among the four subject groups were analyzed via a one-way ANOVA. All training logs, menstrual cycle characteristics, and hormonal parameters were also analyzed via a one-way ANOVA. In an effort not to violate the assumption of independence on the grouping fixed factor, subjects with three varying menstrual cycles were placed in a single menstrual category where the most frequent category was observed. The third differing cycle was deleted from the analysis. This resulted in the deletion of 12 menstrual cycles. For the urinary hormonal data, the AUC was calculated by the trapezoidal method. ANOVA was then performed on the AUC. A significance level of 0.05 was used to detect the presence of significant differences. *Post-hoc* analyses (least significant squares) were performed where significant F ratios were found. Contingency tests (χ^2) were performed on categorical menstrual cycle data. Tests of monotone alternatives (32) were used when the direction of the monotonic progression of values was justified by previous information (*i.e.* peak LH).

As the direction of interest in group differences was known in advance from previous research, all *post-hoc* statistical comparisons were one sided. In planned *post-hoc* comparisons of sedentary and exercising women, the number of observations in the study provided more than a 99.95% probability of detecting differences larger than 2 SD (*i.e.* outside the so-called normal range) and more than an 85% probability of detecting differences larger than 1 SD (*i.e.* beyond the central 68% of the normal range), with a tolerable type I error rate of 5%.

In planned *post-hoc* comparisons of ovulatory cycles in sedentary and exercising women and of ovulatory and LPD cycles in exercising women, the number of observations in this study provided more than a 99.95% probability of detecting differences larger than 2 SD (*i.e.* outside the so-called normal range) and an 80% probability of detecting differences larger than 0.8 SD with a tolerable type I error rate of 5%. In planned *post-hoc* comparisons of ovulatory cycles or LPD cycles to anovulatory cycles, the number of observations in this study provided more than a 99% probability of detecting differences larger than 2 SD and an 80% probability of detecting differences larger than 1.1 SD, with a tolerable type I error rate of 5%.

Results

Group comparisons

Demographic characteristics. The demographic characteristics of the study participants are presented in Table 1. Thirty-five women participated in this prospective evaluation of menstrual status. Eleven women were sedentary, and 24 women were runners performing exercise volumes of approximately 32.4 km/wk. The sedentary (SedOvul) and exercising subject groups were similar with respect to age, height, and weight. Within the exercising group, there were no differences among the predominately ovulatory (ExOvul), luteal deficient (ExLPD), and anovulatory (ExAnov) subjects with respect to demographic, reproductive, and training characteristics. Therefore, these data on exercising women were collapsed across menstrual categorization. The sedentary subjects had significantly more ($P < 0.001$) fat mass and a higher percentage of body fat 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).

Exercise training and activity. Within the exercising group, there were no significant differences in training characteristics among the predominately ovulatory, luteal deficient, and anovulatory women (Table 1), including distance run and time spent in other weight-bearing physical activity per week (stairmaster, walking/running, resistance training, hiking, tennis, and racquetball). The volume of exercise performed by these subjects is characteristic of moderate or recreational running. As expected, these training parameters as well as aerobic capacity and resting heart rate were significantly different ($P < 0.05$) from those of the sedentary subjects and were representative of a moderately exercise-trained population.

TABLE 1. By subject comparisons based on exercise status

	Sedentary (n = 11)	Exercising (n = 24)	Probability
Demographic			
Age (yr)	26.2 ± 1.2	27.8 ± 1.3	0.422
Ht (cm)	163.9 ± 1.5	164.6 ± 1.5	0.793
Wt (kg)	61.8 ± 4.7	58.8 ± 1.5	0.429
Lean body mass (kg)	47.5 ± 1.8	47.3 ± 0.7	0.873
Fat mass (kg)	18.0 ± 1.9	11.9 ± 0.5	<0.001
Body fat (%)	25.8 ± 2.2	20.1 ± 1.0	0.011
Reproductive characteristics			
Menstrual cycle length (days)	28.8 ± 0.6	27.7 ± 0.9	0.394
Age of menarche (yr)	13.5 ± 0.5	12.8 ± 0.2	0.088
Reproductive age (yr)	12.6 ± 1.1	14.9 ± 1.2	0.264
Training characteristics			
VO_2 peak (mL/kg · min)	29.1 ± 1.8	41.5 ± 1.1	<0.001
Distance run (km/wk)	0.2 ± 0.2	32.4 ± 3.5	<0.001
Hours of other activity/week	0.5 ± 0.3	5.0 ± 0.7	<0.001
Training heart rate (beats/min)		132.2 ± 4.9	
Resting heart rate (beats/min)	75.0 ± 1.6	63.3 ± 1.4	<0.001

Values are the mean ± SEM.

Frequency and sample prevalence and incidence. Subjects were categorized according to the predominant type of menstrual cycle as presented in Table 2A. Among the sedentary women, 91% (10 of 11) were ovulatory and 9% (1 of 11) demonstrated LPD (short luteal phase). Among the exercising women, 42% (10 of 24) were ovulatory, 42% (10 of 24) demonstrated LPD (short and/or inadequate luteal phase), and 16% (4 of 24) were anovulatory. A χ^2 analysis revealed that the frequency of LPD and anovulation was much greater ($P < 0.006$) in the exercising women than in the sedentary women. Menstrual cycle status from cycle to cycle was very consistent in the sedentary women and very inconsistent in the exercising women. Among the sedentary women, 91% (10 of 11) were ovulatory during all 3 of the cycles observed, whereas only 54% (13 of 24) of the exercising women were either consistently ovulatory (21%, 5 of 24) or consistently demonstrating LPD or anovulation (33%, 8 of 24). In addition to the 33% of runners whose menstrual cycles were consistently abnormal, another 46% (11 of 24) displayed inconsistent menstrual cycle status from cycle to cycle. A χ^2 analysis again revealed that the frequency of inconsistent LPD and anovulation was much greater ($P < 0.008$) in the exercising women than in the sedentary women.

On the average, studies examining only a single menstrual cycle would observe only one third of these inconsistently abnormal cycles. In this study, we measured the 3-month sample incidence of LPD and anovulation in exercising women to be 79% (33% + 46%), and the sample prevalence to be 48% [33% + (46%)/3-cycle observance period]. Therefore, studies monitoring only a single menstrual cycle would probably underestimate the occurrence of LPD and anovulation in runners by about 38% (100 [(33% + 46%) − (33% + [46%/3])]/(33% + 46%)).

Cycle comparisons

Overall comparison by menstrual cycle status. In the sedentary women, 28 of 31 (90%) menstrual cycles monitored were ovulatory, whereas only 30 of 66 (45%) menstrual cycles monitored were ovulatory in the exercising women. One sedentary woman had short luteal phases during all 3 of the menstrual cycles monitored. Among the exercising women, 55% of the 66 menstrual cycles monitored demonstrated either LPD (43%; n = 28) or anovulation (12%; n = 8). Among the LPD cycles, 47% (n = 17) displayed short luteal phases, 14% (n = 5) were inadequate (low progesterone concentrations), and 25% (n = 9) were short and inadequate. These data are shown in Table 2b. Characteristic menstrual cycles in consistently ovulatory and LPD exercising women are shown in Fig. 1. Characteristic inconsistent consecutive menstrual cycles of an exercising woman are also shown in Fig. 1.

Menstrual cycle parameters. Only the sedentary women who were ovulatory were included in the evaluation of the individual menstrual cycles; one sedentary woman tested had a LPD and was not included in the menstrual cycle comparisons. Two women in the exercising group displayed menstrual cycle lengths during the study outside our admission criteria (one <20 days and one >38 days), and they, too, were dropped from the analyses.

Menstrual cycle parameters are presented in Table 3. The ExOvul and SedOvul cycles were similar for all menstrual parameters presented in Table 3. The lengths of the SedOvul, ExOvul, ExLPD, and ExAnov cycles were similar ($P = 0.12$). The ExLPD cycles had significantly longer follicular phases (17.9 ± 0.7 days) compared to the ExOvul (14.8 ± 0.9 days) and SedOvul (15.9 ± 0.6 days) cycles. As anticipated, luteal phase length was significantly shorter in the ExLPD cycles (8.2 ± 0.5 days). The ExAnov cycles were excluded from the statistical analyses on luteal and follicular phase lengths.

Ovarian steroid characteristics. Estrogen excretion: Suppressive effects on the ovary were observed in the exercising women. The ExOvul, ExLPD, and ExAnov cycles demonstrated significant suppression of estradiol excretion during the early follicular phase (days 2–5 of the menstrual cycle) compared to the SedOvul cycles (Table 4). During days 6–12, E1C excretion was significantly less in the ExLPD and ExAnov cycles than in the ExOvul and SedOvul cycles. Over the entire

TABLE 2. Overall by subject comparisons of menstrual status among the sedentary and exercising women (A) and overall by menstrual cycle characteristics of the individual menstrual cycles (B)

A	Sedentary women (n = 11)	Exercising women (n = 24)	Probability
Ovulatory	91	42	0.006 ($\chi^2 = 7.468$)
Luteal phase abnormality	9	42	
Anovulatory	0	16	
Consistently ovulatory	91	21	0.008 ($\chi^2 = 6.993$)
Consistently LPD/anovulatory	9	33	
Inconsistently ovulatory	0	21	
Inconsistently LPD/anovulatory	0	25	

B	Ovulatory (n = 58)	Short (n = 17)	Inadequate (n = 5)	Short and inadequate (n = 9)	Anovulatory (n = 8)
Sedentary (n = 31)	90	10	0	0	0
Exercising (n = 66)	45	21	8	14	12

	Luteal phase abnormality		
Sedentary (n = 31)	90	10	0
Exercising (n = 66)	45	43	12

Values are given as percentages.

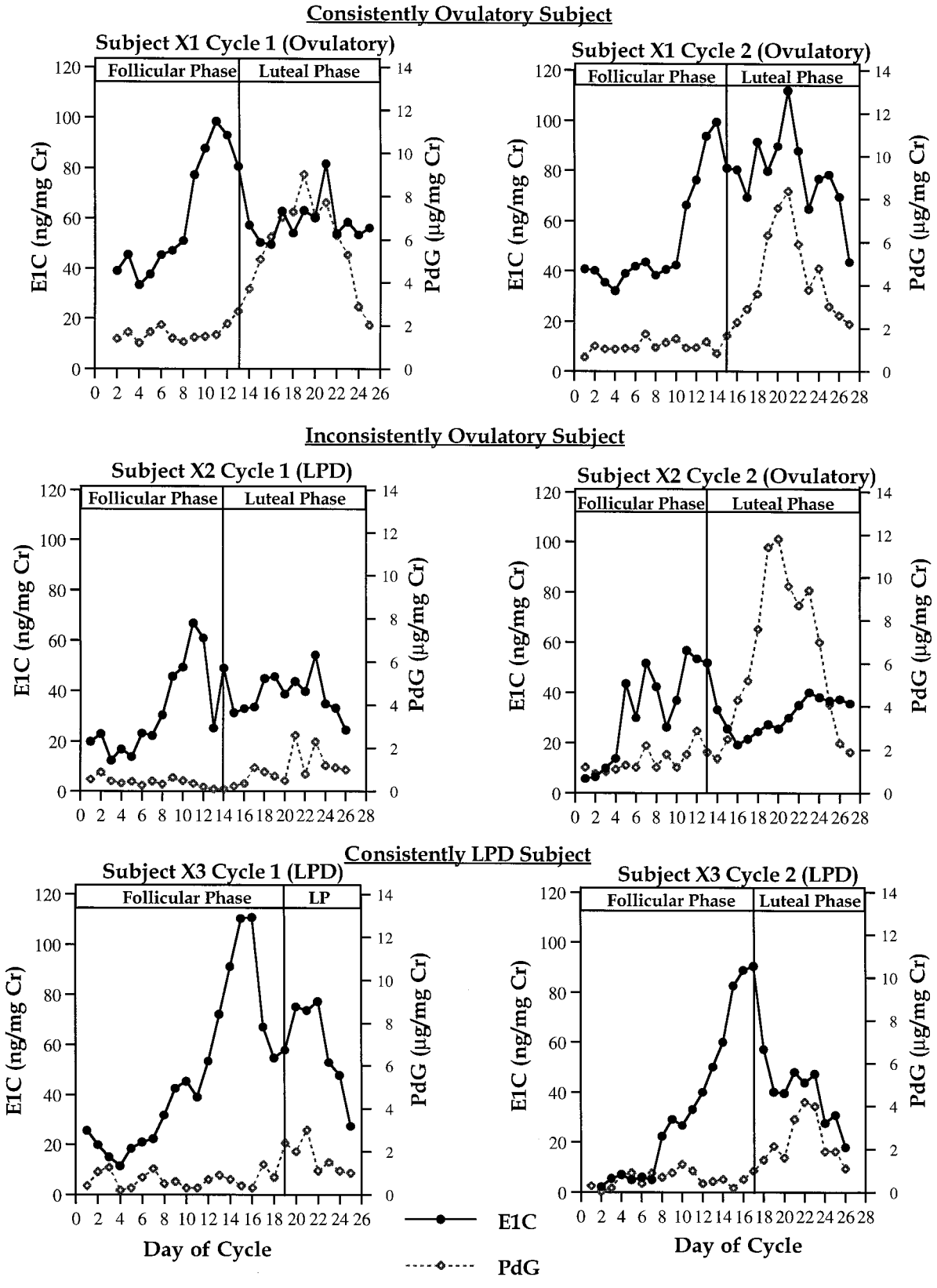

FIG. 1. *Top*, Characteristic consecutive menstrual cycles in a consistently ovulatory woman. *Middle*, Characteristic consecutive menstrual cycles in a inconsistently ovulatory woman. *Bottom*, Characteristic consecutive menstrual cycles in a consistently LPD woman. Daily urinary excretion is shown for E1C (nanograms per mg Cr) on the *left y*-axis and PdG (micrograms per mg Cr) on the *right y*-axis. Data are plotted relative to the first day of menses.

TABLE 3. Menstrual cycle parameters

	SedOvul (n = 28)	ExOvul (n = 24)	ExLPD (n = 21)	ExAnov (n = 8)	Probability
Menstrual cycle length (days)	28.8 ± 0.6	27.8 ± 1.0	26.1 ± 0.8	26.5 ± 1.6	0.120
Follicular phase length (days)	15.9 ± 0.6	14.8 ± 0.9	17.9 ± 0.7[a]		0.015
Luteal phase length (days)	12.9 ± 0.4	12.9 ± 0.3	8.2 ± 0.5[a]		<0.001

Values are the mean ± SEM. SedOvul, Sedentary/ovulatory; ExOvul, exercise/ovulatory; ExLPD, exercise/luteal phase deficiency; ExAnov, exercise/anovulatory.
[a] SedOvul and ExOvul *vs.* ExLPD.

TABLE 4. Estrogen excretion characteristics

	SedOvul (n = 28)	ExOvul (n = 24)	ExLPD (n = 21)	ExAnov (n = 8)	Probability
Estrogen excretion					
Peak E1C[a]	82.9 ± 6.3	88.1 ± 5.9	85.7 ± 5.3	58.1 ± 9.1	0.093
Peak E1C day	15.0 ± 0.6	13.8 ± 0.9	16.4 ± 0.7	14.4 ± 1.0	0.111
E1C days 2–5	27.6 ± 2.0	20.8 ± 2.4[b]	21.3 ± 2.4[b]	18.9 ± 2.6[b]	0.050
E1C days 6–12	38.4 ± 2.8	37.5 ± 2.8	29.7 ± 2.8[c]	24.1 ± 2.8[d]	0.016
E1C follicular phase[e]	43.5 ± 2.6	40.9 ± 2.7	41.6 ± 3.4	25.0 ± 3.0[f]	0.011
E1C luteal phase[e]	49.8 ± 3.3	48.0 ± 5.9	50.3 ± 3.7	33.3 ± 4.1[f]	0.045
Area under the curve analysis					
E1C follicular phase AUC[e]	539 ± 37	469 ± 38	588 ± 35[h]	267 ± 49[f]	<0.001
E1C luteal phase AUC[e]	629 ± 50	539 ± 45	385 ± 56[c]	389 ± 47[g]	0.003

Values are the mean ± SEM. See Table 3 for abbreviations.
[a] All E1C concentrations are expressed as nanograms per mg creatinine.
[b] SedOvul *vs.* ExOvul, ExLPD, and ExAnov.
[c] SedOvul and ExOvul *vs.* ExLPD.
[d] SedOvul and ExOvul *vs.* ExAnov.
[e] Follicular and luteal phase values for ExAnov group represent days 1–13 (follicular) and day 14 to last day of cycle (luteal).
[f] SedOvul, ExOvul, and ExLPD *vs.* ExAnov.
[g] SedOvul *vs.* ExAnov.
[h] ExOvul *vs*. ExLPD.

follicular and luteal phases, E1C excretion did not differ among the SedOvul, ExOvul, or ExLPD cycles, but less was excreted during the ExAnov cycles ($P < 0.05$). For the ExAnov cycles, the follicular phase was assumed to be the first half of the menstrual cycle, as a true follicular phase could not be discerned. The AUC for E1C ($P < 0.001$) during the follicular phase was lower in the ExAnov cycles than in the other cycle categories. During the luteal phase, however, the E1C AUC was lower ($P < 0.004$) in both the ExLPD and ExAnov cycles than in the ovulatory cycles. Figure 2 displays the E1C excretion of the groups across the menstrual cycle.

Progesterone excretion: PdG excretion characteristics are presented in Table 5. For the ExAnov cycles, the luteal phase was assumed to be the second half of the menstrual cycle, as a true luteal phase could not be discerned. In the ExLPD and ExAnov cycles, significantly less progesterone was excreted than in the SedOvul and ExOvul cycles, as assessed by PdG AUC during the luteal phase and other measures of PdG excretion, including mean luteal phase PdG, sum of the 3-day midluteal PdG peak, and peak PdG. Moreover, for all parameters of PdG excretion, except for follicular phase PdG during days 6–10, there was significantly less PdG excreted during the ExAnov cycles than during the ExLPD cycles. Even the ExOvul cycles demonstrated some suppression of corpus luteal progesterone excretion compared to the SedOvul cycles ($P < 0.05$). The ExOvul cycles had significantly lower progesterone excretion during the luteal phase as assessed by all parameters of progesterone excretion. Figure 3 displays PdG excretion of the groups across the menstrual cycle.

Gonadotropin excretion characteristics. FSH excretion across the menstrual cycle, assessed via daily urinary FSH excretion, is presented in Table 6. Mean FSH during the last 5 days of the menstrual cycle differed ($P < 0.05$) by cycle categories. The ExLPD cycles excreted significantly less FSH than the SedOvul and ExOvul cycles ($P < 0.007$). Paradoxically, FSH excretion during the last 5 days of the menstrual cycle was significantly greater in the ExAnov cycles than in the SedOvul, ExOvul, and ExLPD cycles. Urinary FSH quantified during days 2–5 and days 6–12 of the menstrual cycle was similar ($P > 0.05$) among the cycle categories.

Monotone alternatives analysis (32) revealed a significant linear monotone decline in peak urinary LH ($P < 0.05$) from the SedOvul cycles, through the ExOvul and ExLPD cycles, to the ExAnov cycles.

Energy expenditure. In the cycles categorized as ExAnov, subjects expended significantly more energy per 24-h day than in the cycles categorized as ExOvul and ExLPD. However, when normalized by body weight, the ExAnov cycles were similar to the ExOvul and ExLPD cycles. As anticipated, in cycles categorized as SedOvul, subjects expended significantly less energy in exercise compared to cycles categorized as ExOvul, ExLPD, and ExAnov (Table 7).

Dietary energy and macronutrient intake. Dietary energy and macronutrient intake data are also summarized in Table 7. In cycles categorized as ExAnov, dietary energy intake and dietary energy normalized for body weight were less than those in other cycle categories. There were no significant

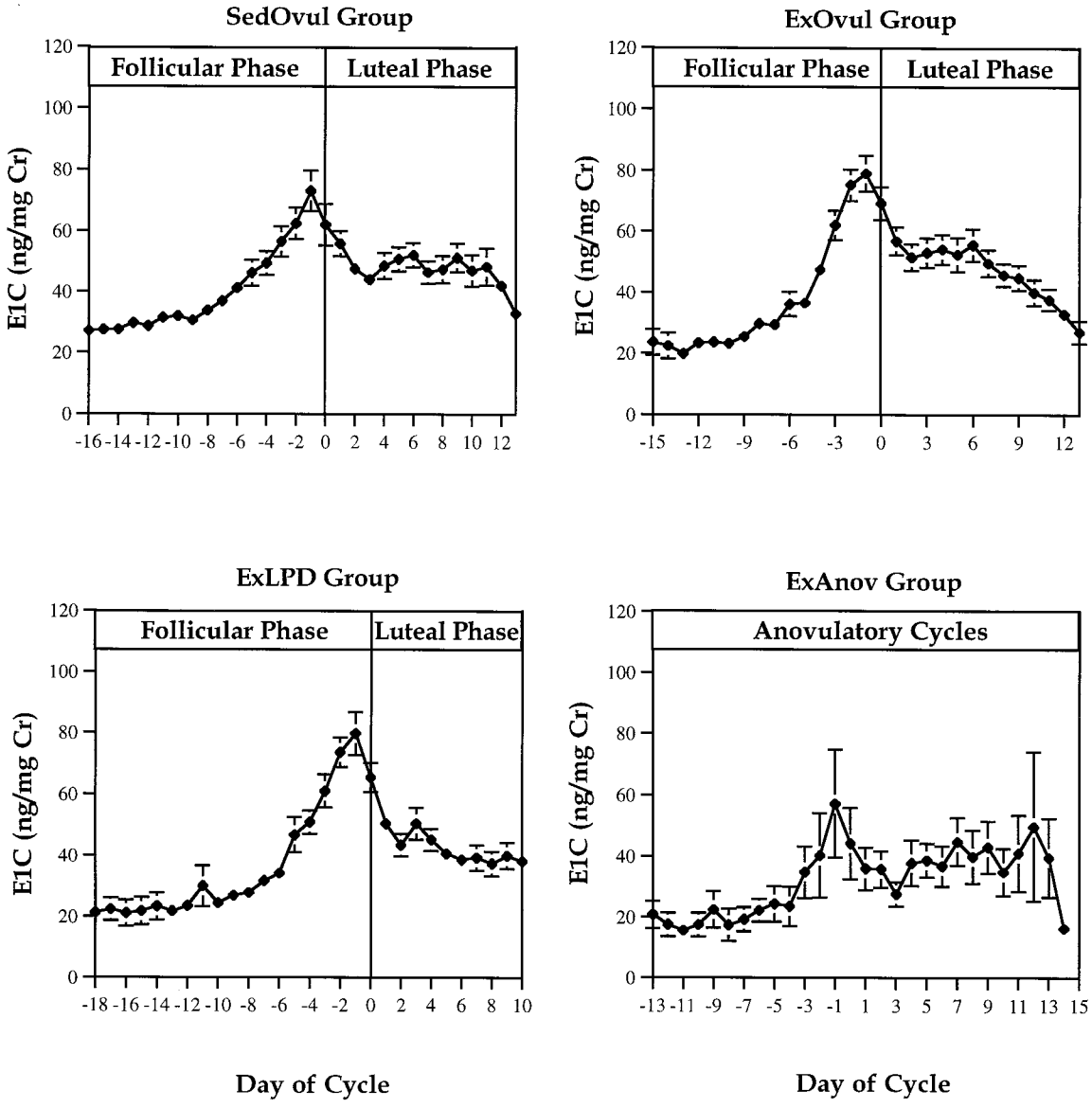

FIG. 2. Daily urinary excretion during the menstrual cycle for E1C (nanograms per mg Cr) for four categories of menstrual cycles. Values (mean ± SE) are shown for the SedOvul cycles (*top left*; n = 28), the ExOvul cycles (*top right*; n = 24), the ExLPD cycles (*bottom left*; n = 21), and the ExAnov cycles (*bottom right*; n = 8). Data are plotted relative to the LH peak.

differences in protein energy intake among the menstrual cycle categories, but differences ($P < 0.05$) in fat and carbohydrate energy intake were noted (see Table 7).

Energy availability and energy balance. There were no differences ($P > 0.05$) observed in energy balance when comparing the SedOvul and ExLPD cycles (Table 7), but the ExAnov cycles did display a substantial deficit ($P < 0.001$) in energy balance (−16.1 ± 2.2 Cal/kg BW·day) compared to the SedOvul (−0.8 ± 0.8 Cal/kg BW·day), ExOvul (−3.9 ± 1.7 Cal/kg BW·day), and ExLPD (−1.4 ± 1.4 Cal/kg BW·day) cycles. The ExOvul cycles displayed a greater ($P < 0.05$) deficit in energy balance than the SedOvul cycles. Energy availability was lower ($P < 0.05$) in the cycles categorized as ExOvul, ExLPD, and ExAnov than in the cycles categorized as SedOvul, both when availability was expressed as total energy per day and when it was normalized by body weight. In addition, cycles categorized as ExAnov were significantly lower in energy availability normalized by body weight than in the other menstrual cycle categories.

Discussion

Frequency of menstrual abnormalities

This is the first detailed prospective observational study to document the frequency of luteal and ovulatory abnormalities in consecutive, asymptomatic, regular menstrual cycles of normal length in moderately active women. The exercising women in this study averaged 32 km/wk of running, a volume representative of a moderate or recreational level exercise training. None of the runners in our study trained excessively or was amenorrheic. This study clearly showed

TABLE 5. Progesterone excretion characteristics

	SedOvul (n = 28)	ExOvul (n = 24)	ExLPD (n = 21)	ExAnov (n = 8)	Probability
Progesterone excretion					
Peak PdG[a]	8.5 ± 0.5	6.8 ± 0.5[b]	4.4 ± 0.5[c]	1.4 ± 0.3[d]	<0.001
Peak PdG day	23.0 ± 0.7	22.0 ± 0.7	22.0 ± 0.7	21.7 ± 1.0	0.659
PdG days 6–10	0.8 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.5 ± 0.1	0.347
PdG luteal phase[e]	5.0 ± 0.4	3.7 ± 0.3[b]	2.9 ± 0.3[c]	0.8 ± 0.1[d]	<0.001
Sum of 3-day midluteal PdG	21.7 ± 1.4	17.8 ± 2.3[b]	10.9 ± 1.2[c]	3.3 ± 0.7[d]	<0.001
Area under the curve analysis					
PdG luteal phase AUC[e]	58 ± 4	46 ± 4[b]	24 ± 3[c]	10 ± 2[d]	<0.001

Values are the mean ± SEM. See Table 3 for abbreviations.
[a] All PdG concentrations are expressed as micrograms per mg creatinine.
[b] SedOvul *vs.* ExOvul.
[c] SedOvul and ExOvul *vs.* ExLPD.
[d] SedOvul, ExOvul, and ExLPD *vs.* ExAnov.
[e] Luteal phase PdG values for ExAnov group represent days 14 to last day of menstrual cycle.

that menstrual cycle length is not an accurate marker of ovarian function in this population. Even though all the women in this study presented with repeatable menstrual cycle lengths in the normal range, their ovarian function was highly variable and frequently abnormal. We found the sample prevalence and sample incidence of abnormal ovarian function in our subjects to be 48% and 79%, respectively. Such high frequencies in asymptomatic women are remarkable. Investigators need to carefully consider this issue when designing studies in which ovarian function is important.

Another compelling finding of this study is the high frequency of inconsistent menstrual status; almost half (46%) of the exercising women presented with inconsistent menstrual status from cycle to cycle. By contrast, none of the sedentary women presented with inconsistent menstrual status from cycle to cycle; that is, they were either consistently ovulatory (91%) or consistently LPD (9%). The monitoring of only one menstrual cycle in exercising women underestimates the frequency of ovarian disturbances. On the average, studies examining a single menstrual cycle will observe only one third of the inconsistently abnormal cycles. As abnormal ovarian function was displayed by 33% of our subjects consistently and by another 46% inconsistently, earlier studies of only a single menstrual cycle (3, 25) probably underestimated the 3-month sample incidence of LPD among regularly menstruating athletes by about 100[(33% + 46%) − (33%+[46%/3])]/(33% + 46%) = 38%. Therefore, investigators of ovarian function or parameters, such as infertility, that are affected by ovarian function may miss or confound important findings if they classify exercising women by the menstrual status observed during a single cycle.

The only other attempt at a prospective observational evaluation of menstrual cycles in athletes reported that 20–27% of moderate and long distance runners experienced one or more episodes of an anovulatory cycle among two menstrual cycles monitored at the beginning and end of a 12-month period (15). As has been discussed in detail previously (33), that study did not differentiate between LPD and anovulatory cycles, due to questionable methodologies used for menstrual categorization. The accuracy in the estimation of anovulatory frequency is suspect because the menstrual cycles were assessed by pooling only a single hormonal measurement during the follicular and another during the luteal phase. This method was probably not representative of actual ovarian function, and it probably grossly underestimated the incidence of LPD. By contrast, our investigation carefully monitored daily urinary hormone levels, including the direct documentation of a LH surge, to assess ovarian function during each menstrual cycle.

Other investigators (3, 18, 19, 25) have reported the occurrence of LPD in cross-sectional evaluations of one menstrual cycle in exercising women. Loucks *et al.* (18) found shorter luteal phases with reduced progesterone concentrations in regularly menstruating competitive runners and triathletes compared to sedentary women. Ellison (19) found similar results in recreational runners who ran only 12 miles/week. Broocks *et al.* (3) observed luteal phase inadequacies in 7 of 17 recreational joggers who ran 12–18 miles/wk. These women showed evidence of disturbed folliculogenesis (determined via transvaginal follicular monitoring) and luteal phase inadequacy (determined via daily progesterone levels). Another cross-sectional evaluation of daily E1C and PdG (25) reported that 4 of 10 high mileage (42.4 miles/wk) runners and 5 of 10 active (12.3 miles/wk) women displayed anovulatory cycles or luteal phase abnormalities. Thus, our multicycle data confirm and strengthen the earlier single cycle findings of widespread luteal and ovulatory abnormalities among entirely asymptomatic, regularly menstruating women who engage in only moderate levels of physical activity.

Hormonal characteristics

Before this study, the transduction of the adverse effects of exercise training at the level of the hypothalamic GnRH pulse generator has been attributed to changes in LH pulsatility (18, 20, 21). In this study, we discovered a second gonadotropin abnormality that may act in concert with the disruption of LH pulsatility to impair ovarian function in exercising women. Like LH, alterations in FSH secretion may also significantly impact ovarian function by altering folliculogenesis (22–24, 34). Typically, elevations in FSH are observed during the luteal-follicular transition and at midcycle, and declines are usually observed during the late follicular and early luteal phases (22, 23, 35). We found that the characteristic rise in FSH release during the luteal-follicular tran-

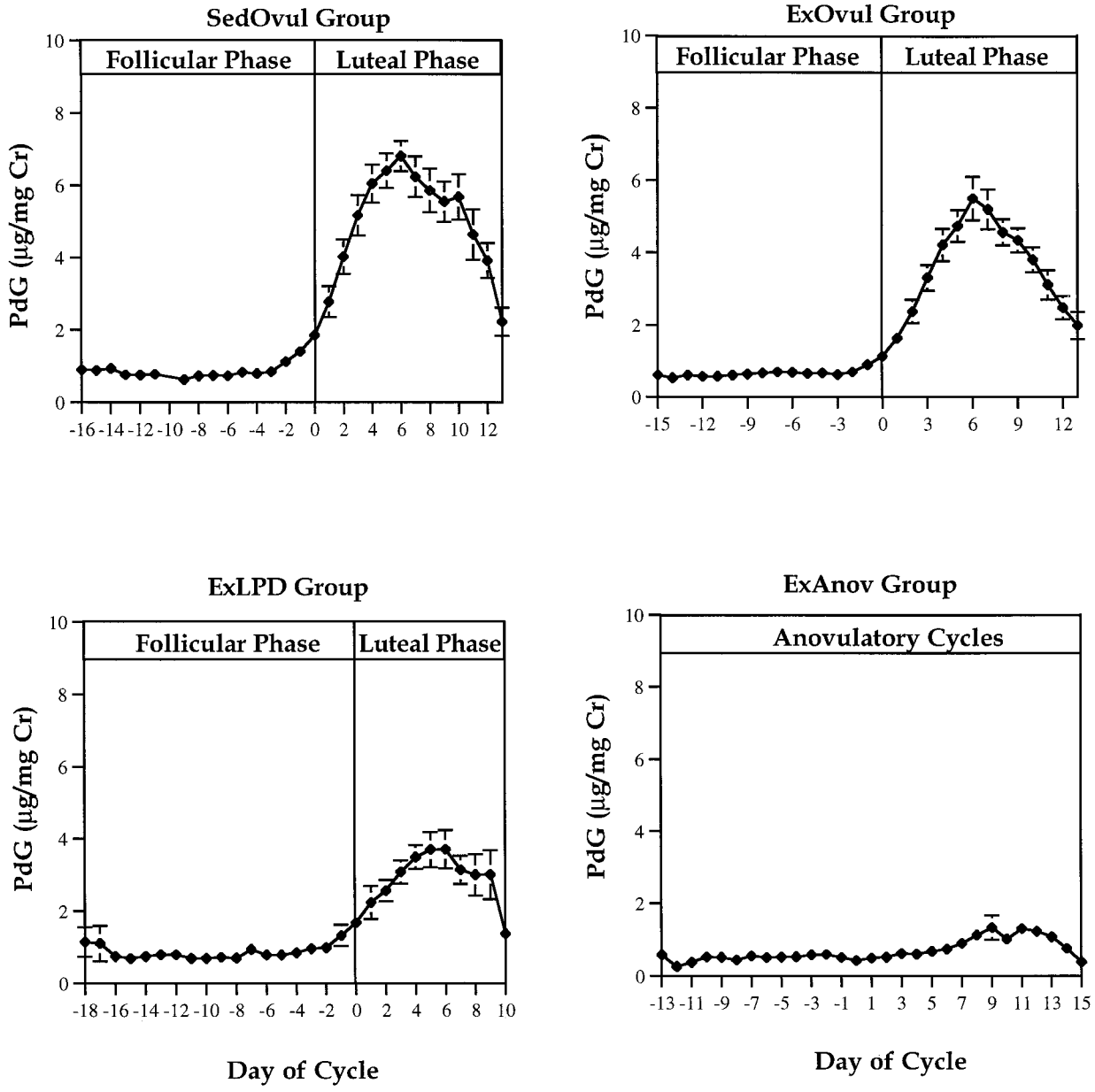

FIG. 3. Daily urinary excretion during the menstrual cycle for PdG (micrograms per mg Cr) for four categories of menstrual cycles. Values (mean ± SE) are shown for the SedOvul cycles (*top left*; n = 28), the ExOvul cycles (*top right*; n = 24), the ExLPD cycles (*bottom left*; n = 21), and the ExAnov cycles (*bottom right*; n = 8). Data are plotted relative to LH peak.

sition appears to be blunted in exercising women with LPD. This is the first report of an abnormality in the monthly pattern of FSH excretion in such women.

FSH characteristics during the luteal-follicular transition in clinical presentations of LPD have not yet been described, but a blunted elevation in FSH during the luteal-follicular transition may in part explain the decreased estradiol excretion that we observed during the early to midfollicular phase of cycles with ovarian abnormalities. Recently, a delay in the rise in urinary FSH during the midluteal phase of conceptive cycles compared to nonconceptive cycles has been described and has permitted the detection of pregnancy 10 days after the midcycle LH surge (36).

The characteristic rise in FSH during the luteal-follicular transition is strongly related to the preceding LH surge, indicating a high degree of neuroendocrine hypothalamic regulation during this critical transition period of the menstrual cycle (23). GnRH pulse frequency may play a key role in the etiology of the rise of FSH during the luteal-follicular transition and may be critical to normal folliculogenesis (23).

A progressive decline in peak LH along the continuum from ovulatory cycles to anovulatory cycles in this study resembled similar findings reported in infertile women with LPD (37). Luteal phase defects in 63 normally cycling infertile women were associated with smaller dominant follicles and a lower midcycle ovulatory LH surge compared to women without evidence of LPD (37).

The exercising women in this study also exhibited a pro-

TABLE 6. Gonadotropin excretion characteristics

	SedOvul (n = 28)	ExOvul (n = 24)	ExLPD (n = 21)	ExAnov (n = 8)	Probability
FSH excretion					
Mean FSH[a] last 5 days of cycle	1.0 ± 0.1	1.1 ± 0.1	0.7 ± 0.1[b]	1.5 ± 0.5[c]	0.007
Mean FSH days 2–5	1.7 ± 0.1	2.1 ± 0.2	1.8 ± 0.1	1.7 ± 0.2	0.103
Mean FSH days 6–12	1.5 ± 0.1	2.0 ± 0.1	1.7 ± 0.1	1.8 ± 0.2	0.090
LH excretion					
Peak LH (mIU/L)	97.0 ± 8.5	95.3 ± 7.5	74.5 ± 8.1		0.121

Values are the mean ± SEM. See Table 3 for abbreviations.
[a] All FSH concentrations are expressed as nanograms per mg creatinine.
[b] SedOvul, ExOvul, and ExAnov *vs.* ExLPD.
[c] SedOvul and ExOvul *vs.* ExAnov.

TABLE 7. By cycle comparison of energy intake, energy expenditure, and energy balance and energy availability

	SedOvul (n = 28)	ExOvul (n = 24)	ExLPD (n = 21)	ExAnov (n = 6)[a]	Probability
Energy intake					
Total Cal/day	1804.7 ± 64.6	1837.0 ± 79.6	1992.6 ± 84.8[b]	1325.5 ± 110.0[c]	0.011
Cal/kg BW · day	30.0 ± 1.2	31.6 ± 1.6	35.0 ± 1.7[b]	20.4 ± 2.1[c]	0.003
24-h energy expenditure					
Total Cal/day	1861.0 ± 52.6	2074.7 ± 57.9[d]	2031.3 ± 52.5[d]	2405.2 ± 157.0[c,d]	<0.001
Cal/kg BW · day	30.8 ± 0.9	35.5 ± 1.0[d]	35.8 ± 1.1[d]	36.4 ± 2.2[d]	<0.001
Energy balance (intake − 24-h expenditure)					
Total Cal/day	−56.3 ± 51.0	−237.7 ± 91.8[e]	89.1 ± 77.2	−1079.6 ± 160.3[c]	<0.001
Cal/kg BW · day	−0.8 ± 0.8	−3.9 ± 1.7[e]	−1.4 ± 1.4	−16.1 ± 2.2[c]	<0.001
Exercise energy expenditure					
Total Cal/day	0	479.5 ± 53.0[f]	494.3 ± 64.1[f]	272.0 ± 78.3[f]	<0.001
Cal/kg BW · day	0	8.3 ± 1.0[f]	8.6 ± 1.1[f]	4.3 ± 1.2[f]	<0.001
Energy availability (intake − exercise expenditure)					
Total Cal/day	1804.7 ± 64.6	1357.5 ± 84.4[d]	1521.6 ± 97.7[d,g]	1166.9 ± 145.4[d]	<0.001
Cal/kg BW · day	30.0 ± 1.2	23.3 ± 1.6[d]	26.5 ± 1.8[d,g]	18.8 ± 3.2[c,d]	0.001
Total energy intake, composition					
Protein calories	265.4 ± 10.6	270.7 ± 10.6	280.7 ± 14.8	229.9 ± 25.9	0.270
CHO calories	951.8 ± 37.8	1137.5 ± 60.6[h]	1152.3 ± 64.8[h]	915.3 ± 90.8[i]	0.013
Fat calories	582.2 ± 35.2	489.8 ± 32.4[j]	575.6 ± 56.5	324.6 ± 55.9[k]	0.002

Values are the mean ± SEM. See Table 3 for abbreviations.
[a] Complete energy data were only available for six of the eight ExAnov cycles.
[b] SedOvul *vs.* ExLPD.
[c] SedOvul, ExOvul, and ExLPD *vs.* ExAnov.
[d] SedOvul *vs.* ExOvul, ExLPD, and ExAnov.
[e] SedOvul *vs.* ExOvul.
[f] SedOvul *vs.* ExOvul, ExLPD, and ExAnov.
[g] ExLPD *vs.* ExAnov.
[h] SedOvul *vs.* ExOvul and ExLPD.
[i] ExOvul and ExLPD *vs.* ExAnov.
[j] SedOvul and ExLPD *vs.* ExOvul.
[k] SedOvul and ExLPD *vs.* ExAnov.

gressive suppression of estradiol excretion during the follicular phase, in that E1C on days 2–5 was lower in all exercise groups compared to that in the sedentary group and remained low in the ExLPD and ExAnov women on days 6–12 compared to that in the SedOvul and ExOvul groups. Days 2–5 are reflective of follicular recruitment and increased estradiol excretion (25). A delay in follicle recruitment probably explains the delay in estradiol excretion and the long follicular phases (>18 days) and short luteal phases (<10 days) in our exercising LPD women. The delay in estradiol excretion during the window of days 6–12 probably reflects a delay in the growth and maturation of steroidogenically active follicles and a subsequent delay in follicular dominance and subsequent ovulation (25). Winters *et al.* (25) also reported lower E1C excretion during the early follicular phase in runners. Thus, the suppressed follicular phase levels of estradiol observed in this study suggest a progressive suppression in follicular maturation as menstrual dysfunction progresses along the continuum from ovulatory to anovulatory cycles.

The characteristics most significantly differentiating the disturbed ovarian function (ExLPD and ExAnov) cycles from the ovulatory (SedOvul and ExOvul) cycles were those associated with the luteal phase. The length of the luteal phase was significantly shorter in association with the delayed follicular maturation and long follicular phases in the ExLPD cycles. Similarly, a monotonic suppression in corpus luteal progesterone production was observed as menstrual dysfunction became more extreme along the continuum from ovulatory to anovulatory cycles. The ExLPD and ExAnov cycles excreted significantly less progesterone than both the sedentary (SedOvul) and exercising (ExOvul) ovulatory cycles. Even the ExOvul cycles demonstrated suppressed corpus luteal progesterone excretion compared to the SedOvul

cycles. These findings support previous work by other investigators (1–3, 18, 19) who found that regularly menstruating athletes displayed shorter luteal phases with decreased progesterone compared to sedentary women. Our data and those of previous investigators (1–3, 18, 19) support the concept that in exercising women, both luteal phase progesterone excretion and early follicular phase estrogen excretion decrease concomitantly and proportionally. Of course, a cross-sectional comparison such as this cannot distinguish whether ovulatory disturbances progress to an extreme end point (amenorrhea), whether they represent successful acclimations that could be achieved by all women, or whether they are end points in robust women.

Energy intake, expenditure, availability, and balance

The hypothalamic gonadal axis has been proposed to be very sensitive to both stress (38) and energy availability (39), although the mechanisms of these factors have not yet been determined. The role(s) of these two factors in the menstrual disturbances observed in exercising women remains controversial. In this context, energy availability may be usefully defined as dietary energy intake minus exercise energy expenditure (30, 40). This permits the stress of exercise to be independently defined as everything associated with exercise except its energy cost. So defined, exercise stress and energy availability become susceptible to carefully designed experimental investigations of their independent effects on reproductive function (40, 41). By contrast, in observational studies such as this one, the effects observed in subjects (*i.e.* on progesterone secretion) have already occurred some time in the past, whereas the hypothetical causal factors (*e.g.* exercise stress and energy availability) are confounded in the habitual behavior of the subjects.

We found no association between menstrual status and the distance our exercising subjects ran or the amount of time they spent in other weight-bearing physical activity. By contrast, we found several associations with energy-related parameters.

Low energy availability has been reported to induce low T_3 syndrome (30), to slow LH pulse frequency, and to increase LH pulse amplitude in sedentary women during acute short term experiments in which exercise stress and energy availability were independently controlled (40, 41). In those 4-day experiments in habitually sedentary women, exercise had no suppressive effect on thyroid metabolism or LH pulsatility beyond the impact of its energy cost on energy availability. Energy balance under the low energy availability conditions in those experiments was approximately −22 Cal/kg BW·day.

In our study, we estimated dietary intake from 7-day prospective diet records, total energy expenditure from 24-h Caltrac monitoring, and exercise energy expenditure from an analysis of daily activity logs. By these methods, we were unable to associate differences in exercise energy expenditure with differences in ovarian function, but significant differences in energy intake, total energy expenditure, energy availability, and energy balance were observed. In the ExAnov cycles, subjects expended more energy and also consumed less than in the SedOvul, ExOvul, and ExLPD cycles. This resulted in a lower energy availability normalized by body weight and a more negative energy balance compared to those in the SedOvul, ExOvul, and ExLPD cycles. The energy availability in the ExOvul and ExLPD cycles was intermediate between those in the SedOvul and ExAnov cycles.

In short term studies examining the acute independent effects of exercise stress and energy availability, Loucks *et al.* were able to prevent the disruption of thyroid metabolism and LH pulsatility in exercising women by supplementing their dietary energy intake (30, 40). We do not know whether dietary supplementation would have prevented disturbances of reproductive function in our exercising women, but our observations are consistent with the hypothesis that energy deficiency is the factor that disrupts reproductive function in exercising women. It should be noted that the sedentary subjects in the studies of Loucks *et al.* (30, 40, 41) were placed in an acute state of low energy availability for only 4 days, and the independent effects of chronic low energy availability and exercise stress have yet to be determined in prospective experiments.

We can speculate that individuals with an even more severe reduction in energy availability may experience the pathological extreme end point of the menstrual cycle continuum, that of amenorrhea. Despite high levels of physical activity, athletes report energy intakes similar to those of sedentary women (18, 21). Most women involved in exercise programs for health, fitness, and weight control practice moderate dietary restriction and increased energy expenditure as the means to attain their goals. The exercising women in our study are probably typical of this population. The effects of dietary restriction and exercise energy expenditure are probably additive in contributing to the abnormalities we observed. Therefore, more research is needed to determine whether particular degrees of menstrual dysfunction are causally related to particular degrees of energy deficiency, and whether chronic dietary restriction and exercise energy expenditure are, indeed, additive in their effect.

Additional studies are also needed to investigate the potential clinical consequences of ovarian disturbances less extreme than amenorrhea. It has been proposed that women with ovulatory disturbances or LPD may have a lower bone mineral density (15), but recently we reported that LPD associated with decreased progesterone does not appear to adversely affect bone mass, provided normal estradiol status is maintained (33). Other potential health risks of LPD appear to be related to infertility and an increased incidence of tubal ectopic pregnancy, because women with LPD and anovulation are at a greater risk for infertility and recurrent habitual abortion, and these women also have a higher incidence of ectopic pregnancy (42–44). During the luteal phase, the endometrium is under direct stimulation by progesterone. A rapid decline in progesterone or an inadequate progesterone concentration during this period results in a degenerative endometrium, which is not receptive for implantation of a fertilized ovum or maintenance of early pregnancy (42). And finally, some epidemiological evidence has led to a preliminary hypothesis that there may be an association between a short luteal phase, low progesterone levels, and increased breast cancer risk (45–47). Given the large numbers affected,

more research is necessary to further elucidate the clinical consequences of LPD and anovulation in regularly menstruating exercising women.

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