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Bone Health Is Not Affected by Luteal Phase Abnormalities and Decreased Ovarian Progesterone Production in Female Runners*

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ABSTRACT

The primary purpose of this study was to determine whether decreased ovarian progesterone production, associated with short and inadequate luteal phases in exercising women, was associated with decreased bone mineral density (BMD) and altered bone metabolism. Thirty-three eumenorrheic menstruating women participated in this study for 3 months. Subjects were required to collect daily urine samples for three consecutive menstrual cycles and have blood and urine collected weekly. Daily urine samples were analyzed for free LH, estrone conjugates (E1C), and pregnanediol 3-glucuronide (PdG), adjusted for creatinine, whereas weekly blood and urine samples were analyzed for bone markers, estradiol, progesterone, FSH, and LH. Based on the analyses of these samples, subjects were divided into three groups: sedentary ovulatory (SedOvul; n = 9), exercising ovulatory (ExOvul; n = 14), and exercising luteal phase defects (ExLPD; n = 10). The three groups were matched for age (27.6 ± 1.0 yr), weight (60.6 ± 1.9 cm), and reproductive maturity (14.5 ± 1.0 yr). PdG production during the luteal phase was lower ($P = 0.004$) in the ExLPD women compared to that in the SedOvul group (2.4 ± 0.4 *vs.* 5.1 ± 0.6 ng/mL creatinine, respectively). The ExOvul group also had less ($P < 0.01$) PdG production during the luteal phase (3.5 ± 0.3 ng/mL creatinine) compared to the SedOvul group. The total production of PdG, as assessed by area under the curve analysis, was also lower ($P < 0.001$) in the ExOvul and ExLPD groups compared to that in the SedOvul group. E1C production, however, was not different ($P > 0.05$) among the groups, except for E1C during the early follicular phase, which was lower ($P = 0.043$) in the ExLPD group than that in the SedOvul group. BMD and biochemical markers of bone metabolism were unaffected by and not associated with the compromised progesterone environment, but BMD values at the proximal femur (r = 0.354; $P = 0.061$) and total body (r = 0.359; $P = 0.056$) were associated with decreased early follicular E1C production. We conclude the following. 1) Luteal phase disturbances occur independent of training volume, and volume of training does not have to be severe to result in menstrual disturbances. 2) As a result of exercise, disturbance in progesterone production is not associated with decreased bone mass. 3) Long follicular phases are associated with reduced estrogen production during the early follicular phase, which are both associated with decreased bone mass. 4) Provided the estradiol status is adequately maintained, BMD is unaffected by decreased progesterone production associated with short and inadequate luteal phases in exercising women. (*J Clin Endocrinol Metab* **82:** 2867–2876, 1997)

MENSTRUAL history and menstrual status are important factors that influence trabecular bone mass. In female athletes, a significant reduction in bone mass in amenorrheic athletes has been reported by a number of investigators that is attributable to the chronic hypoestrogenemia characteristic of amenorrhea (1–5). It is well established that bone mineral density (BMD) in female athletes with amenorrhea is decreased not only in the vertebral bodies of the lumbar spine, but also at appendicular sites throughout the skeleton, in a manner similar to the decreased bone mass observed in postmenopausal women (1, 3, 5, 6). The common denominators in these two clinical situations are reduced levels of estradiol and irregular or absent ovulation.

Estrogen status and menstrual cyclicity, therefore, play an important modulatory role in the positive effect of exercise training on BMD. The beneficial effects of the mechanical loading of exercise training on bone mass is demonstrated by the higher BMD observed in female athletes with ovulatory menstrual cycles compared to that in sedentary ovulatory women (7, 8). The favorable action of physical activity, however, on bone mass is attenuated in the face of amenorrhea and decreased levels of estrogen (1–5). For example, the BMD of athletes with exercise-induced amenorrhea, although much lower than that observed in ovulatory athletes and nonathletes, is somewhat higher than the BMD observed in untrained sedentary women with amenorrhea (4). These findings suggests that exercise reduces, but does not prevent, decreased BMD in women with amenorrhea or estrogen deficiency; exercise alone cannot substitute for an optimal estrogen status (4). Consequently, female athletes with suspected ovarian hormone alterations and menstrual disturbances may also be at risk for decreased bone mass.

Subtle menstrual alterations, such as short and inadequate luteal phases, do not present obvious clinical symptoms, such as amenorrhea, but may also negatively affect bone

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health via the altered ovarian hormone environment. Prior *et al.* (9) reported decreased BMD in female athletes with subtle ovulatory disorders, suggesting that the decreased bone mass was due to decreased progesterone levels associated with ovulatory disorders. Recent investigations in female athletes and sedentary women with subtle menstrual cycle alterations do not support the conclusion that luteal phase disturbances and decreased luteal phase progesterone are associated with decreased bone mass (10, 11). These conflicting results have given rise to intense debate, as evidenced by several letters to the editor recently published (12, 13). It was our intention to examine this topic in detail and provide insight into what influence, if any, subtle menstrual disturbances specifically associated with decreased progesterone production have on BMD.

The primary purpose of this study was to determine whether decreased ovarian progesterone production (associated with short and inadequate luteal phases in exercising women) was associated with decreased BMD of the total body, lumbar spine, and proximal femur by comparing exercising women with luteal phase abnormalities to ovulatory sedentary and ovulatory exercising women. The secondary purpose of this study was to compare the biochemical markers of bone formation and bone resorption in these same groups.

Subjects and Methods

Subjects

Forty-six women met the following general eligibility criteria. They were 1) between the ages of 18–35 yr; 2) in good health, as determined by a medical examination, including a normal Papanicolaou smear within the past year; and 3) free of any chronic disease, including hyperprolactinemia and thyroid disease. They also had 4) not experienced any recent (within 12 months) change in menstrual status, 5) an appropriate activity history, 6) not taken any form of hormonal therapy for at least 12 months, 7) no history of an eating disorder or depressive illness within the past 3 yr, 8) no contraindications that might preclude participation in the study, and 9) not taken any medication that would interfere with calcium metabolism within the past 3 yr. The specific eligibility criteria outlined below were met by 33 of the 46 subjects screened, and these women were included in this study.

Subject categorization

The sample population consisted of a cohort of women that were classified as either 1) sedentary eumenorrheic females (SedOvul) performing no more than 1 h of aerobic activity/week for the past 12 months, with a peak oxygen uptake (VO_2 peak) less than 35 mL/kg·min; or 2) runners running at least 2 h or 16 km/week for the past 12 months and a VO_2 peak greater than 35 mL/kg·min (ExOvul, exercising ovulatory; ExLPD, exercising with luteal phase defects).

Menstrual categorization

Women were eligible for this study if they had apparently eumenorrheic cycles of 24–32 days. 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 with oligomenorrhea (defined as irregular menstrual cycles occurring every 39–90 days) and primary or secondary amenorrhea were excluded from the analyses for this study because we were interested in determining the impact of subtle menstrual disturbances on bone health.

Experimental protocol

Volunteers were initially interviewed by phone and, if eligible, 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 log and collect timed 8-h urine samples beginning on day 2, 3, or 4 of the menstrual cycle (day 1 defined as the first day of menstrual bleeding) until the onset of the next menses every month for two or three consecutive study cycles (minimum of two study cycles). Timed 8-h urine samples were defined as overnight urine sample collections that were initiated each night upon retiring, continued throughout the night, and terminated each morning upon arising after the first morning void. The date and time of sample collection were recorded appropriately. 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 the prelabeled tubes and store in the refrigerator. The samples for each week of a given study cycle were delivered to the laboratory on a weekly basis. During each study cycle, a weekly blood sample was drawn, and a second morning urine sample was collected. At this visit, urine collection tubes were given to the subject for the next week. Weekly blood samples were analyzed for estradiol, progesterone, FSH, and LH. Daily urine samples were analyzed for creatinine (Cr), urinary free LH, pregnanediol-3-glucuronide (PdG), and estrone conjugates (E1C). Weekly urine samples were analyzed for the bone markers.

Subjects were weighed (kilograms) weekly, and menstrual bleeding patterns and training reports were monitored daily via diary and training cards that were reviewed weekly with the investigators. Any significant change in dietary habits was documented. During the 3 months of enrollment, each subject completed a maximal exercise test to exhaustion to document training status and peak aerobic power (VO_{2peak}), and a body fat analysis and a BMD assessment were performed.

Determination of menstrual phase dynamics

Ovulatory status was determined for all subjects. All subjects with anovulatory cycles were deleted from these analyses because luteal phase abnormalities and decreased progesterone production were the outcome of interest with respect to bone mass. Progesterone production, not estradiol, was the unique descriptor that distinguished the ovulatory groups (sedentary and exercise) from the ExLPD group. A compromised estrogen status has previously been shown to affect bone health (1–5) and would probably have confounded the interpretation of the effects of decreased progesterone production on bone health. Only 1 (of 9) of the sedentary women and 6 (of 24) of the exercising women completed 2 cycles; all other subjects completed 3 cycles.

The day of the LH surge (day 0) was identified by the urinary LH peak and by the concurrence of the day of or the day after the midcycle E1C peak. As there is a delay between the plasma and urinary peaks of LH, 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 or ovulation (14). The total days of the follicular and luteal phase were calculated, using the day on which the onset of menses was noted and the day of the LH surge. The follicular phase 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 was defined as the difference between cycle length and follicular phase length. A luteal phase abnormality was defined as either short, when a luteal phase length was less than 10 days, or inadequate, when peak PdG was less than 3 μg/mg Cr for 3 or more midluteal phase days. For the exercising women, a luteal phase abnormality had to be displayed in at least two cycles for a given subject to be categorized in the luteal phase abnormality group. For the sedentary group, all cycles monitored had to meet ovulatory criteria for them to be included in the analyses. Data from all completed cycles for each subject were averaged and were determined to be the most representative of the overall ovarian steroid status of the subjects. Two sedentary women were excluded for luteal phase abnormalities. Four exercising women were excluded for anovulatory cycles, and two were excluded for amenorrhea.

An integrated value for the urinary excretion of E1C and PdG was calculated for the cycle by integrating the area under the curve (AUC) for the urinary metabolite. For the total cycle, the AUC was calculated from day 1 to the onset of the next menses. Menstrual phase dynamics were also compared among the groups via the method of mean steroid

levels recently described by Winters *et al.* (15), where alignment of the cycle is based on the day of menstruation. E1C excretion was divided into three periods: 1) period 1 reflects ovarian activity during follicular recruitment and was defined as days 2–5; 2) period 2 reflects the growth and development of steroidogenically active follicles and was defined as day 6 to the day of the LH surge; and 3) period 3 reflects E1C production by the corpus luteum and was defined as the day following the LH surge to the day before the onset of the next menses. PdG excretion was divided into two periods: 1) period 1 reflects excretion of PdG by the adrenal glands and was defined as days 6–10; and 2) period 2 reflects PdG excretion by the corpus luteum and was defined as the day after the LH surge to the day before the onset of the next menses.

Daily urine collections

Subjects were required to collect 8-h timed daily urine samples beginning on day 2 of the menstrual cycle (day 1 defined as the first day of menstrual bleeding) until the onset of the next menses for three consecutive menstrual cycles. Timed 8-h urine samples were overnight urine collections that were initiated each night upon retiring, continued throughout the night, and terminated each morning upon arising after the first morning voiding. Refrigerated 10-mL aliquots of urine delivered to the laboratory were checked, aliquoted into polyethylene tubes, and frozen at −80 C. Samples for urinary free 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 interassay variance was less than 3.4%. RIA determination of urinary LH was performed at the Reproductive Endocrinology Laboratory at New Britain General Hospital (New Britain, CT).

Urine samples were analyzed for E1C and PdG by the methods of Munro *et al.* (16). E1C and PdG were both indexed to the Cr level of the same sample to adjust for variations in urine volume. E1C and PdG are expressed as nanograms or milligrams 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 assay is 7.8 ng/mL, and that of the PdG assay is 0.15 μg/mL. Values below the low end sensitivity were reported at the minimum detection limits. The intraassay coefficients of variations 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).

The validity of these techniques, as representative of the 24-h pattern of excretion of LH, E1C, and PdG, has been previously calculated by other investigators (14). Beitins *et al.* (14) collected urine samples from three untrained women with ovulatory menstrual cycles for a period of 14 days during the early follicular phase, the late follicular phase, and the luteal phase, as three separate 8-h aliquots. The excretion of LH, E1C, and PdG was measured in each aliquot and as a total of the 24 h, and the data were expressed as nanomoles of Cr. The correlations between the overnight 8-h aliquots and 24-h excretion were 0.80, 0.63, and 0.60 for LH, E1C, and PdG, respectively.

Serum hormone measurements

Blood samples were collected between 0700–1000 h into standard 9.5-mL serum tubes after a 12-h fast. Blood samples were allowed to clot for 20 min before centrifugation. Aliquots of serum were placed into polyethylene tubes and stored at −80 C until analysis. Serum estradiol, progesterone, FSH, and LH were evaluated using commercially available kits (Diagnostic Products) in single assay sets. All serum steroid and gonadotropin assays were performed in duplicate at the Institute for Toxicology and Environmental Health, University of California (Davis, CA). All samples were thawed only once for each assay procedure.

Bone turnover markers

Urinary assays. Urine samples were collected as the second morning sample after an overnight 12-h fast. Five-milliliter aliquots were frozen at −80 C. Cross-linked N-terminal telopeptides of type I collagen were assayed by an enzyme-linked immunosorbent assay (Osteomark, Ostex, Seattle, WA). Intraassay variability was 5.2%. Assay sensitivity was 20 nmol/bone collagen equivalents. Values were normalized to Cr concentrations determined in each sample. Deoxypyridinoline (DPD) was assayed by an enzyme-linked immunosorbent assay (Metra Biosystems, Palo Alto, CA). Intraassay variability was less than 2.0%. Assay sensitivity was 1.1 nmol/L DPD. Values were normalized to Cr determined in each sample.

Serum assays. Blood samples were collected between 0700–1000 h after a 12-h fast. Samples were collected into serum separator tubes, allowed to clot at room temperature for 20 min, and centrifuged. Two-milliliter aliquots were frozen at −80 C. Serum osteocalcin was analyzed via RIA (Nichols Institute Diagnostics, San Juan Capistrano, CA). Intraassay variability was 4.0%. Assay sensitivity was 0.05 ng/mL. Serum bone-specific alkaline phosphatase was measured via an immunoradiometric assay (Tandem, Hybritech, San Diego, CA). Intraassay variability was 3.0%. Assay sensitivity was 0.7 U/L. Serum type I procollagen peptide was measured by a enzyme-linked immunoassay (Prolagen-C, Metra Biosystems, Palo Alto, CA). Intraassay variability was 2.4%. Assay sensitivity was 1 ng/mL. All bone markers were analyzed in the Core Laboratory at the University of Connecticut Health Center (Farmington, CT).

BMD assessment

Areal BMD (grams per cm^2) of the total body, lumbar spine (L2–L4), and right proximal femur was measured via dual energy x-ray absorptiometry (DXA) using a Hologic QDR2000 (Waltham, MA). The total body, lumbar spine, and proximal femur scans were performed at the standard scan speed in the array mode requiring 30 min to complete the entire procedure with only minimal radiation (<5–7 mrem). The short term precision of this instrument is 1% *in vivo*. In our laboratory, the short term precision determined in 10 young adult females scanned 7–10 days apart by the same technician is 0.9% for the lumbar spine, 1.2% for the proximal femur, and 0.9% for the total body. Scans for each subject were completed during the 3-month study period by the same experienced and certified technician.

Training volume

Training activities were recorded daily. Factors recorded on the training cards included number of kilometers run and time of each run. Training cards were monitored weekly and collected monthly. Training volume was defined as the actual number of kilometers run per week as recorded in the training logs. Training hours were defined as the number of hours run per week plus the hours per week of other physical activity.

Peak exercise testing

The VO_2 peak was determined by metabolic measurement of expired gases during a progressive treadmill test to volitional exhaustion. The VO_2 peak was assumed to have been attained if three of the four following criteria were met: 1) no increase in oxygen uptake greater than 150 mL with increasing exercise intensity (plateau criterion), 2) respiratory exchange ratio greater than 1.1, 3) a rating of perceived exertion of 18 or greater, and 4) a heart rate within 5–10 beats of the predicted maximal heart rate. The treadmill test was a continuous graded test to exhaustion that was modified appropriate to each subjects' 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). Inspired flow oxygen concentration and carbon dioxide concentration were continuously sampled during the test. The analyzers were calibrated before and after each test with standardization grade gas mixtures.

Body composition testing

Body fat was determined via skinfold testing (17). Body composition was estimated via skinfold measurements at various sites, including the tricep, subscapula, illiac crest, abdomen, and thigh, with a constant pressure skinfold caliper (Holtain, UK). All measurements were made

in triplicate on the right side of the body by one investigator (test-retest correlation, r = 0.96).

Statistical methods

The number of observations in this study provided more than a 99.5% probability of detecting differences larger than 2 SD (*i.e.* outside the approximate normal range) and an 80% probability of detecting differences larger than 1 SD (*i.e.* outside the central 68% of the normal range) to be significant at the 5% level. (A statistical power of 80% is commonly regarded as the minimal ethical power in studies involving human subjects.) We regarded differences of smaller than 1 SD as not being of practical physiological and clinical importance, and therefore, we did not design the experiment to detect them. All demographic data among the three groups were analyzed via a one-way ANOVA. All training logs, menstrual phase dynamics, hormonal parameters, and bone parameters were analyzed via a one-way ANOVA. Urinary hormonal data were analyzed by assessing the AUC as calculated by the trapezoidal method after the baseline has been subtracted. Analysis of variance was then performed on the AUC. Pearson product-moment correlation coefficients were calculated to measure significant relationships between the independent and dependent variables. A significance level of 0.05 was used to detect significant differences. *Post-hoc* analyses (least significant squares) were performed when significant F ratios were found.

Results

Demographic characteristics

The demographics of the study participants are presented in Table 1. The groups were matched for age, height, and weight. As expected, the sedentary women had a greater ($P < 0.02$) percent body fat than the two exercising groups. The groups were also matched for age of menarche and reproductive maturity (Table 1), factors important for studies of menstrual cycle dynamics. The ExOvul and ExLPD groups were matched for training characteristics (Table 1), including number of kilometers run per week and number of hours spent in physical activity per week; these parameters were significantly different from those in the SedOvul group. As expected, the ExOvul and ExLPD groups both had a higher peak VO_2 than the SedOvul group. The total PdG AUC was inversely correlated with kilometers run per week (r = −0.467; $P = 0.008$) and hours spent in physical activity per week (r = −0.518; $P = 0.003$). The mean PdG during the luteal phase was also inversely correlated with kilometers run per week (r = −0.461; $P = 0.009$) and hours spent in physical activity per week (r = −0.533; $P = 0.002$).

Menstrual cycle dynamics

Menstrual cycle dynamics (Table 2) were different among the groups; however, menstrual cycle length was similar among the sedentary and two exercising groups, ranging from 23–32 days (Table 1). By design, all of the sedentary women included in this evaluation were ovulatory. Two sedentary women tested were either anovulatory or had a luteal phase abnormality and were not included in this study. These results in sedentary women are to be expected, as menstrual phase abnormalities occur in the general population at a prevalence rate of approximately 2–5% (18, 19). Also by design, the three groups were very similar for all methods of comparing their overall estrogen status (Table 2) independent of exercise status and menstrual categorization. Figures 1 (E1C and PdG) and 2 (serum estradiol and progesterone) display the estrogen status of the groups.

The only measure of estrogen status that differentiated the SedOvul group from the ExLPD group was mean E1C on days 2–5 (Table 2). The ExLPD groups had delayed follicular recruitment, as evidenced by the lower EIC levels on days 2–5. The ExOvul group also trended ($P = 0.09$) toward lower E1C levels on these same days.

TABLE 1. Subject characteristics

	SedOvul	ExOvul	ExLPD	Probability
	(n = 9)	(n = 14)	(n = 10)	
Demographics				
Age (yr)	26.4 ± 1.3	30.4 ± 1.4	25.2 ± 2.1	0.065
Height (cm)	164.0 ± 1.6	167.0 ± 2.2	161.8 ± 1.9	0.195
Weight (kg)	63.6 ± 5.5	60.4 ± 2.2	58.3 ± 2.4	0.577
Body fat (%)	26.5 ± 2.7	21.1 ± 1.5[a]	19.0 ± 1.3[b]	0.022
Reproductive characteristics				
Age of menarche (yr)	13.8 ± 0.5	13.0 ± 0.4	12.9 ± 0.3	0.340
Reproductive age (yr)	12.7 ± 1.2	16.9 ± 1.4	12.9 ± 2.2	0.179
Cycle parameters				
Menstrual cycle length (days)	28.6 ± 0.7	27.6 ± 0.7	26.9 ± 0.8	0.394
Day of LH surge	15.8 ± 0.7	14.7 ± 0.7	17.9 ± 0.7[c]	0.018
Number of bleeding days	5.3 ± 0.2	5.0 ± 0.3	5.5 ± 0.4	0.383
Follicular phase length (days)	15.8 ± 0.7	14.7 ± 0.8	20.5 ± 1.7[d]	0.001
Luteal phase length (day)	12.8 ± 0.5	12.8 ± 0.4	6.4 ± 1.5[d]	0.0002
Training characteristics				
VO_2 peak (mL/kg/min)	30.2 ± 1.6	40.9 ± 1.5[a]	42.9 ± 1.5[b]	
Running/week (km)	2.1 ± 1.8	32.3 ± 3.7[a]	25.6 ± 5.6[b]	0.00006
Training/week (h)	0.5 ± 0.3	4.7 ± 0.7[a]	4.5 ± 0.6[b]	0.00007
Resting heart rate (bpm)	74.3 ± 2.3	60.7 ± 2.4[a]	61.8 ± 1.2[b]	0.0003
Resting systolic blood pressure (mmHg)	109.8 ± 3.1	115.9 ± 2.9	111.4 ± 2.3	0.502
Resting diastolic blood pressure (mmHg)	65.5 ± 2.5	77.5 ± 2.2	67.2 ± 2.8	0.018

Values are mean ± SEM.
[a] SedOvul *vs.* ExOvul.
[b] SedOvul *vs.* ExLPD.
[c] ExOvul *vs.* ExLPD.
[d] SedOvul and ExOvul *vs.* ExLPD.

TABLE 2. Menstrual cycle dynamics

	SedOvul	ExOvul	ExLPD	Probability
	(n = 9)	(n = 14)	(n = 10)	
E1C parameters				
Peak E1C day	14.6 ± 0.7	13.8 ± 0.7	16.0 ± 0.6	0.088
E1C (μg/mL/Cr)	27.1 ± 2.0	22.2 ± 2.1	19.3 ± 1.7[a]	0.043
E1C/Cr day 6-ovulation (μg/mL/Cr)	47.2 ± 4.4	51.9 ± 2.9	40.9 ± 4.9	0.411
Peak E1C/Cr (μg/mL/Cr)	77.1 ± 6.7	88.3 ± 5.4	83.4 ± 7.6	0.476
E1C/Cr luteal phase (μg/mL/Cr)	48.3 ± 3.5	46.8 ± 4.0	46.6 ± 4.7	0.937
PdG parameters				
Peak PdG day	22.2 ± 0.8	22.0 ± 0.6	22.4 ± 0.6	0.956
PdG/Cr day 6–10 (ng/mL/Cr)	0.8 ± 0.1	0.6 ± 0.1	0.7 ± 0.9	0.448
Peak PdG/Cr (ng/mL/Cr)	8.3 ± 0.7	6.1 ± 0.6	5.7 ± 2.0	0.412
PdG/Cr luteal phase (ng/mL/Cr)	5.1 ± 0.6	3.5 ± 0.3[b]	2.4 ± 0.4[a]	0.004
Area under the curve analysis				
E1C/Cr (μg/mL/Cr)	416.9 ± 39.3	543.1 ± 47.4	457.8 ± 49.1	0.167
PdG/Cr (ng/mL/Cr)	55.6 ± 5.7	34.9 ± 3.3[b]	15.4 ± 3.1[c]	0.00001
Luteinizing hormone				
Peak LH Day	15.8 ± 0.7	14.7 ± 0.7	17.9 ± 0.7[d]	0.018
Peak LH (mIU/L)	102.5 ± 13.4	85.1 ± 7.4	85.8 ± 10.2	0.414

Values are mean ± SEM.
[a] SedOvul *vs.* ExLPD.
[b] SedOvul *vs.* ExOvul.
[c] SedOvul and ExOvul *vs.* ExLPD.
[d] ExOvul *vs.* ExLPD.

The most significant characteristics that differentiated the ExLPD group from the SedOvul and ExOvul groups were luteal phase dynamics and progesterone production. The ExLPD group produced significantly less progesterone than either the SedOvul or ExOvul group, as assessed by comparing the mean PdG levels of the luteal phase and by the AUC for PdG. The ExLPD group had a significantly shorter luteal phase and a later LH surge day than either the SedOvul or ExOvul group (Table 1). Figures 1 (E1C and PdG) and 2 (serum estradiol and progesterone) also clearly display the decreased progesterone status observed in the ExLPD group compared to the SedOvul and ExOvul groups. The ExLPD group also had a significantly longer follicular phase than either the SedOvul or ExOvul group.

The ExOvul group demonstrated minor suppression of corpus luteum progesterone production compared to that in the SedOvul group, as evidenced by significantly lower AUC PdG values for the entire cycle and lower mean PdG levels during the luteal phase than those in the SedOvul group. However, the length of the luteal phase in this group was similar to that in the SedOvul group.

Bone parameters

There were no significant differences among the three groups when comparing BMD at the total body, lumbar spine (L2–L4), and right proximal femur (Table 3). Bone formation markers, including bone-specific alkaline phosphatase, type I procollagen peptide, and osteocalcin, were similar among the three groups (Table 3). Markers of bone resorption, including N-terminal telopeptides of type I collagen/Cr and DPD/Cr were comparable among the three groups, although the DPD/Cr approached significance ($P < 0.07$). No significant relationships were observed between total E1C AUC and total PdG AUC and BMD at any of the sites assessed. BMD at the proximal femur ($r = 0.353$; $P = 0.061$) and total body ($r = 0.359$; $P = 0.056$) trended toward significant correlations with EIC on days 2–5 (Fig. 3).

Discussion

Prior (20) proposed that progesterone has a trophic effect on bone, and that exercising women with subtle menstrual disturbances associated with decreased luteal phase progesterone production have compromised bone mass. To test this theory, we evaluated bone health in three groups of women with comparable estrogen status but varying progesterone status: 1) consistently ovulatory sedentary women, 2) consistently ovulatory exercising women, and 3) exercising women consistently experiencing short or inadequate luteal phases. Our study clearly demonstrates that despite significant differences in all parameters of ovarian progesterone production in ExLPD women, the BMD at all sites is the same as that observed in women with normal progesterone status. Moreover, BMD at all sites was not correlated with progesterone production. It is likely that estradiol is the primary ovarian steroid maintaining bone health in these exercising women with luteal phase abnormalities, as the overall estradiol environment is similar among the three groups. These data do not support the theory that progesterone has an independent effect on bone or that subtle menstrual disturbances, such as luteal phase inadequacy and short luteal phases, adversely affect bone health (9). It seems, instead, that provided a normal estradiol status is maintained, BMD is unaffected by decreased progesterone production associated with short and inadequate luteal phases in exercising women.

Despite a lack of correlation between reduced progesterone production and BMD, exercise was associated with a decrease in PdG in the luteal phase and a trend for decreased E1C in the early follicular phase, days 2–5. This window of hormone measurement (days 2–5) has been defined as that period reflective of follicular recruitment, increased estrogen, and increased inhibin production (21). It should be noted that although this window (days 2–5) of measurement of estrogen status differs among the groups, it fails to be reflective of the overall estrogen environment experienced

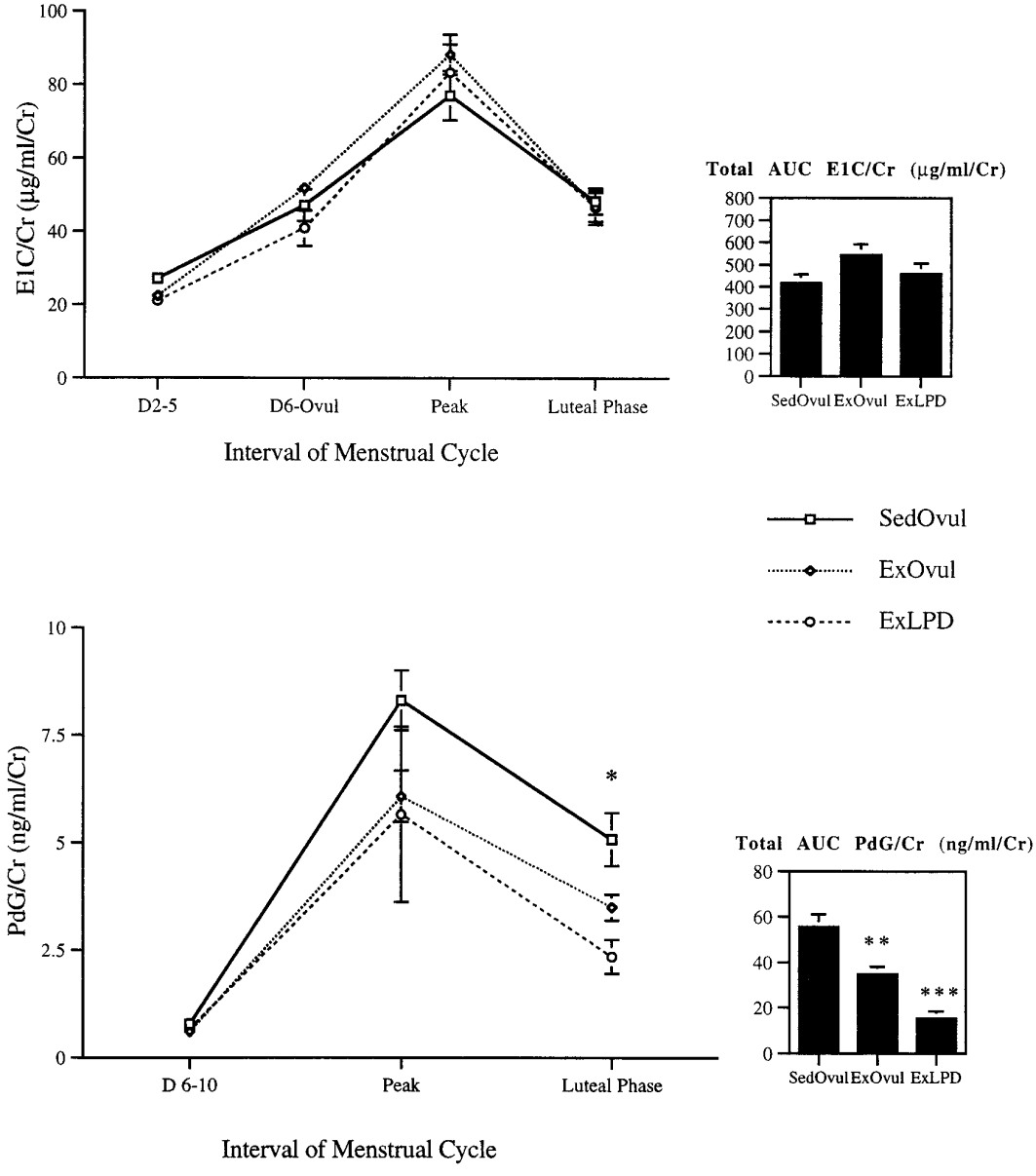

FIG. 1. Urinary excretion of E1C (micrograms per mL Cr) and PdG (nanograms per mL Cr) during different periods of the menstrual cycle in SedOvul, ExOvul, and ExLPD groups. *Inset graphs* show total E1C (micrograms per mL Cr) and PdG (nanograms per mL Cr) over the entire menstrual cycle for each group. Significant differences are shown: *, SedOvul *vs.* ExOvul and ExLPD and ExOvul *vs.* ExLPD; **, SedOvul *vs.* ExOvul; ***, SedOvul and ExOvul *vs.* ExLPD.

by a given individual. Rather, this window is indicative of a clear delay in follicle recruitment that results in a delay in estradiol production associated with long follicular phases (and short luteal phases).

In exercising subjects with luteal phase defects in whom the decrease in PdG was most severe, there was a lengthening of the follicular phase compared to that in the exercising women with normal luteal phase progesterone production, a lowered production of estrogen in the early follicular phase, and a shortening of the luteal phase compared to those in both groups. Taken together, these data support the concept that exercise concomitantly decreases both luteal phase progesterone production and early follicular phase estrogen production and that the severity of these defects moves in parallel with the more severe decreases in progesterone production associated with the most severe deficit in early follicular phase estrogen production. Although there was no discernible relationship between BMD and progesterone indexes in the present study, the relationship between BMD and lowered estrogen production in the early follicular phase approached significance ($P = 0.06$). Therefore, reductions in both luteal phase progesterone and early follicular phase estrogen production may be related to exercise training, and although the change in the measured progesterone production appears to be relatively independent of BMD, the estimation of lowered estrogen production in the early follicular phase appears to have an association with BMD. In addition to exercise training *per se*, nutritional

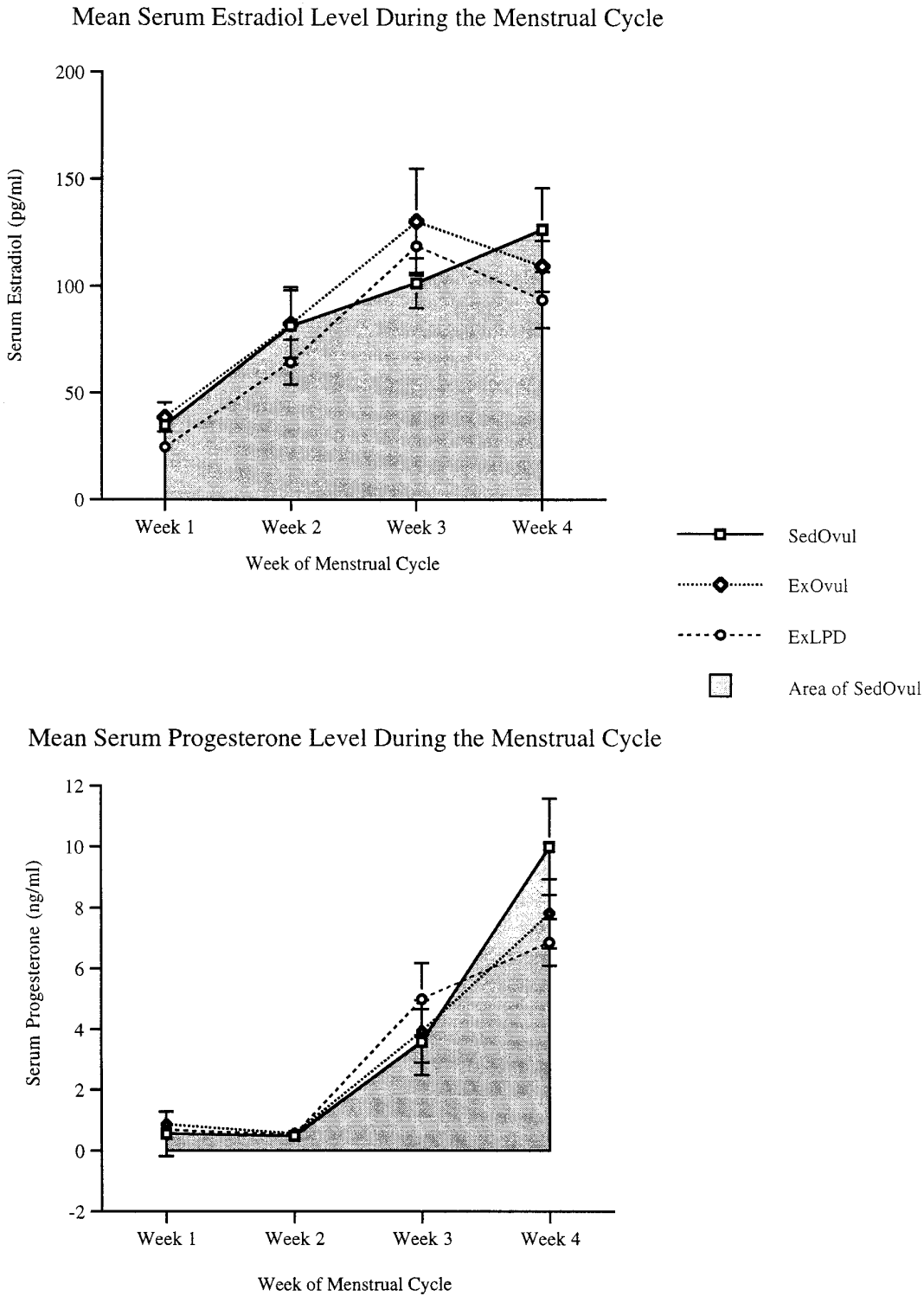

FIG. 2. Mean serum estradiol and progesterone levels during the menstrual cycle in SedOvul, ExOvul, and ExLPD groups.

factors, including caloric intake and diet composition, may also impact bone health and menstrual phase dynamics and should be evaluated concurrently in future investigations of this issue.

Our data are consistent with the reports of Waller *et al.* (11) and Hetlund *et al.* (10) and corroborate the conclusions of Winters *et al.* (15). These reports support the hypothesis that decreases in luteal phase progesterone and early follicular phase estrogen in response to exercise training are physiologically related, and the reduced estrogen, but not the reduced progesterone, results in decreased bone mass. It is only in women with estrogen deficiency and amenorrhea that an

TABLE 3. Bone parameters

	SedOvul	ExOvul	ExLPD	Probability
	(n = 9)	(n = 14)	(n = 10)	
Bone mineral density				
Whole body (g/cm^2)	1.144 ± 0.323	1.194 ± 0.039	1.146 ± 0.022	0.472
Lumbar spine L2-4 (g/cm^2)	1.026 ± 0.041	1.093 ± 0.037	1.034 ± 0.029	0.345
Femoral neck (g/cm^2)	0.875 ± 0.053	0.910 ± 0.059	0.883 ± 0.019	0.673
Biochemical bone markers				
BSAP (U/L)	15.1 ± 1.9	15.9 ± 1.3	17.6 ± 1.2	0.521
Osteocalcin (ng/mL)	7.0 ± 0.5	6.6 ± 0.3	7.2 ± 0.5	0.567
CICP (ng/mL)	107.5 ± 11.5	124.8 ± 12.1	114.7 ± 6.6	0.598
DPD (nM/mM Cr)	7.4 ± 0.3	6.0 ± 0.4	6.2 ± 0.5	0.070
NTx (nM BCE/mmol Cr)	55.7 ± 9.3	49.6 ± 7.1	54.6 ± 5.5	0.814

Values are mean ± SEM.

increased risk of bone loss exists. Several investigators have demonstrated significantly lower BMD at the hip and spine in female athletes with amenorrhea and coincident estrogen deficiency compared to ovulatory women (1–5).

The results of this investigation also demonstrate that subtle menstrual cycle disturbances, such as inadequate luteal phase and short luteal phase, are a common occurrence in female runners independent of training volume and that the volume of training does not have to be severe to result in menstrual disturbances. Other investigators also documented the occurrence of this phenomena in exercising women, particularly runners (14, 22), reporting similar menstrual disorders in recreational level runners (23, 24).

It has been suggested that compromised spinal BMD in exercising women with subtle menstrual disturbances, such as luteal phase abnormalities, may be attributed to changes in luteal phase progesterone production (9), findings in direct contrast to our investigation results and those reported by others (10, 11). Prior *et al.* (9) reported that decreased spinal BMD correlated with subtle disturbances of ovulation among women with differing exercise habits. Prior *et al.* (9) also reported a correlation between decreased spinal trabecular bone mass in exercising women and the number of occurrences of luteal phase abnormalities.

In that investigation (9), however, the authors' method of quantifying progesterone levels during the menstrual cycles may not have been optimal. Blood sampling occurred during the early follicular and midluteal phases of only the first and last cycles of the study year. The early follicular and midluteal samples were then pooled before hormonal evaluation, and this single pooled sample was used as representative of each subject's cycle. Adjusting for the dilution and subtracting the mean follicular phase progesterone level, as performed by Prior *et al.* (9), is not a reliable or accurate means of quantifying progesterone. Furthermore, assessment of ovulatory status was limited to basal body temperature changes, a method not preferred in research as a tool for accurate determination of ovulation (25–29). It may be that Prior *et al.* (9) simply miscategorized their subjects because they did not collect daily blood or urine samples and thus did not accurately document ovulatory status and probable deficient estrogen status.

On the other hand, we obtained daily urine samples for three menstrual cycles to measure PdG and E1C concentrations. This method allowed for a quantitative and qualitative assessment of daily estradiol and progesterone production and allowed us to evaluate the production of these ovarian steroids over an entire menstrual cycle by area under the curve analysis. Moreover, each subject's data are an average of three (in some cases, two) menstrual cycles and, therefore, are more representative of the overall true steroid environment impacting bone health in these women. These methods of ovarian hormone assessments have been shown to provide reliable and accurate results in the assessment of luteal phase abnormalities in women that are difficult to identify (30, 31). Additionally, all anovulatory women were excluded from our investigation due to the likelihood of compromised estrogen status confounding our primary outcome parameter, BMD. Our conclusions, therefore, that decreased progesterone production did not adversely affect BMD in our group of exercising women with short and inadequate luteal phases are based on reliable methodologies.

Another issue of controversy is the evaluation of BMD. Two specific aspects of this issue warrant discussion: the method of assessment and the timing of the measurements. Firstly, much controversy has surrounded the techniques of BMD assessment, specifically quantitative computed tomography (QCT) *vs.* DXA. Unlike our use of DXA bone mass assessments, Prior *et al.* (9) used QCT measurements to assess spinal trabecular BMD. Although it is acknowledged that the precision associated with the two techniques varies, both techniques have been proven reliable in the cross-sectional assessment of bone mass (32), which makes this aspect of the controversy misdirected. The focus should be on the likely miscategorization of subjects in the study by Prior *et al.* (9), as previously discussed. In fact, it is probable that the QCT measurements of spinal BMD were accurate assessments of bone mass, and the subjects in the study were simply miscategorized (due to inaccurate hormonal evaluations). Any differences noted in spinal BMD in their groups were probably not attributable to decreased progesterone, but were more likely attributable to compromised estrogen status.

Secondly, subjects in this investigation had BMD assessments during their menstrual cycle evaluations. An argument can be made against these methods because it is questionable whether the BMD measurements are reflective of menstrual cycle changes occurring during the 2–3 months before BMD assessment. The validity of our BMD data is, therefore, based on the assumption that subtle luteal phase abnormalities occurred consistently in our group of women

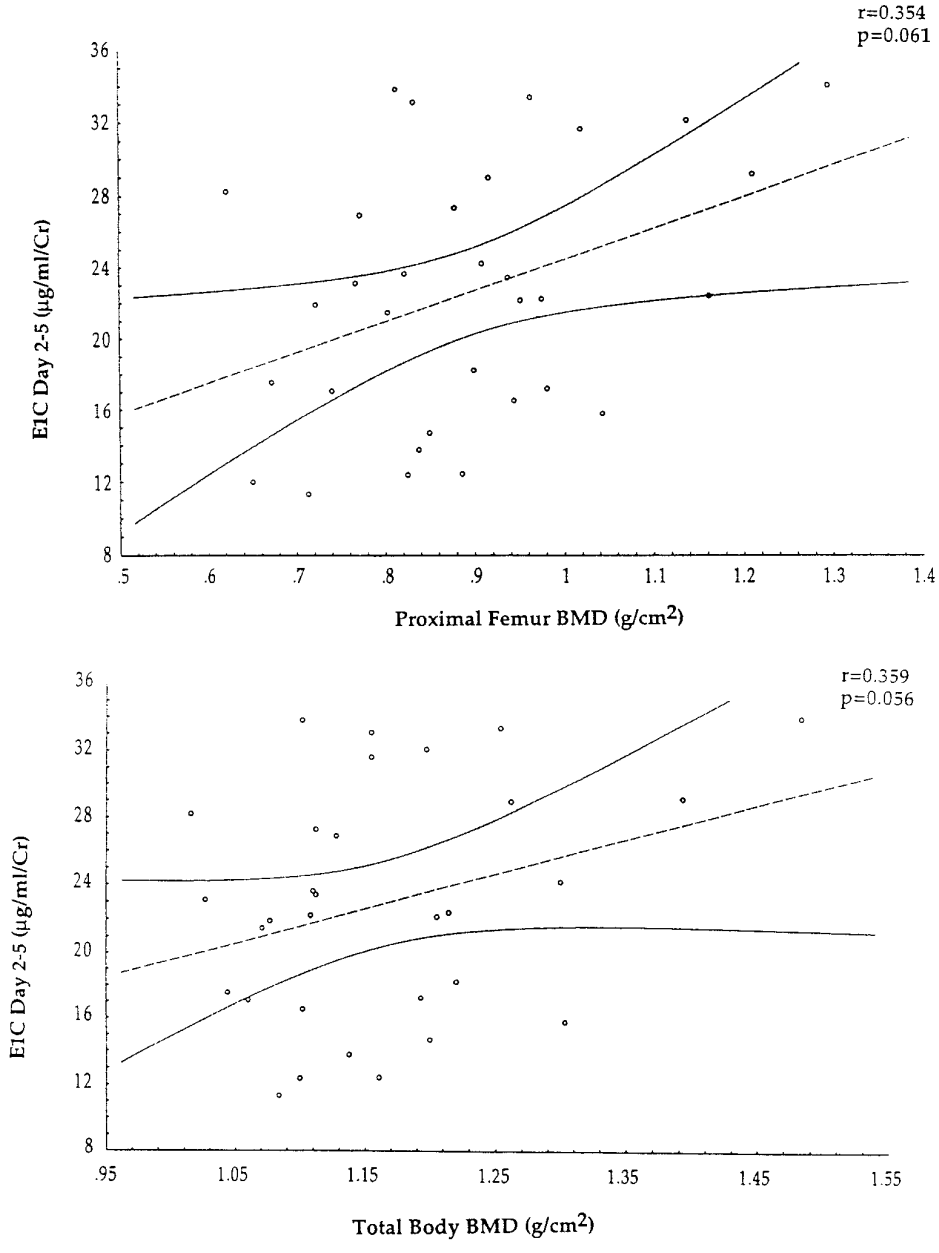

FIG. 3. Significant correlations between E1C (micrograms per mL Cr) on days 2–5 of the menstrual cycle and BMD in the total body and proximal femur.

during the last few years of their training. For this reason, our inclusion criteria were very strict, only including women who had consistently been training at their reported volume for a minimum of 1 yr. Therefore, it is likely that these exercise-related subtle menstrual disturbances were consistent in our subjects, at least throughout the 1 yr before participation in this investigation. Our BMD measurements, then, are very likely to be reflective of the observed menstrual cycle disturbances.

Prior *et al.* (33) has also suggested that bone turnover should be assessed in women with menstrual cycle disturbances. Theoretically, the use of biochemical markers offers a potential advantage over BMD assessment, as short term changes in bone turnover can be reflected by a given bone marker (34, 35). Bone markers are currently used as an adjunctive method for evaluating the response of bone to hormone replacement therapy in postmenopausal women, with changes observed in some markers of resorption within 3 weeks of therapy (34). Our study evaluated bone turnover using biochemical markers of formation and resorption to assess bone metabolism in women with menstrual cycle disturbances. We were unable to find any significant differences in bone turnover among our group of subjects. Specifically, we did not observe any differences in bone formation or resorption based on the markers assessed. Hetland *et al.* (10) also evaluated bone markers in exercising women and did not find significant differences between exercising women with regular menstrual cycles and those with menstrual disturbances. It is noteworthy that the subjects in the Hetland *et al.* (10) study were grouped based on menstrual cycle diaries, and therefore, specific abnormalities, such as anovulation or luteal phase defects, were not identified. Interpre-

tation of that dataset, albeit supportive of our findings, does not provide any insight into the proposed role of progesterone on bone metabolism. The evaluations of bone markers from this study do not support a role for progesterone in increasing bone formation.

In conclusion, it appears that normal endogenous production of estradiol is the most important hormone for maintaining bone mass in women with subtle menstrual cycle disturbances, such as short and inadequate luteal phases. Differences in luteal phase production of progesterone do not appear to influence BMD or biochemical markers of bone turnover. Other consequences of subtle menstrual disturbances associated with running, such as decreased early follicular phase estradiol (days 2–5), may be of greater concern. More importantly, although there was no discernible relationship between bone mass and progesterone indexes in the present study, there was a relationship between bone mass and decreased estrogen production in the early follicular phase. Therefore, reductions of both luteal phase progesterone and early follicular phase estrogen production appear to be related to exercise training *per se*, and although the change in the measured progesterone production appears to be relatively independent of bone mass, the estimation of lowered estrogen production in the early follicular phase appears to be related to bone mass. Well controlled, prospective studies are needed to further evaluate the influence of subtle menstrual cycle alterations on bone health. Future work is also needed to accurately assess ovarian function and hormone production so that the role of early follicular phase estrogen can be elucidated. Provided the overall estradiol status is maintained, bone mass is unaffected by the decreased progesterone production associated with short and inadequate luteal phases in exercising women.

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