UC Davis
UC Davis Previously Published Works

Title
Bone resorption is affected by follicular phase length in female rotating shift workers.

Permalink
https://escholarship.org/uc/item/0n55491w

Journal
Environmental health perspectives, 111(4)

ISSN
0091-6765

Authors
Lohstroh, Pete N
Chen, Jiangang
Ba, Jianming
et al.

Publication Date
2003-04-01

DOI
10.1289/ehp.5878

Peer reviewed

eScholarship.org Powered by the California Digital Library
University of California

Bone Resorption Is Affected by Follicular Phase Length in Female Rotating Shift Workers

Pete N. Lohstroh,[1] Jiangang Chen,[1] Jianming Ba,[2] Louise M. Ryan,[3] Xiping Xu,[4] James W. Overstreet,[1] and Bill L. Lasley[1]

[1]Center for Health and the Environment, University of California, Davis, California, USA; [2]Department of Pharmacology, School of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania, USA; [3]Department of Biostatistics, and [4]Department of Environmental Health, Harvard School of Public Health, Boston, Massachusetts, USA

Stressors as subtle as night work or shift work can lead to irregular menstrual cycles, and changes in reproductive hormone profiles can adversely affect bone health. This study was conducted to determine if stresses associated with the disruption of regular work schedule can induce alterations in ovarian function which, in turn, are associated with transient bone resorption. Urine samples from 12 rotating shift workers from a textile mill in Anqing, China, were collected in 1996–1998 during pairs of sequential menstrual cycles, of which one was longer than the other (28.4 vs. 37.4 days). Longer cycles were characterized by a prolonged follicular phase. Work schedules during the luteal-follicular phase transition (LFPT) preceding each of the two cycles were evaluated. All but one of the shorter cycles were associated with regular, forward phase work shift progression during the preceding LFPT. In contrast, five longer cycles were preceded by a work shift interrupted either by an irregular shift or a number of "off days." Urinary follicle-stimulating hormone levels were reduced in the LFPT preceding longer cycles compared with those in the LFPT preceding shorter cycles. There was greater bone resorption in the follicular phase of longer cycles than in that of shorter cycles, as measured by urinary deoxypyridinoline. These data confirm reports that changes in work shift can lead to irregularity in menstrual cycle length. In addition, these data indicate that there may be an association between accelerated bone resorption in menstrual cycles and changes of regularity in work schedule during the preceding LFPT. ***Key words:*** **bone resorption, deoxypyridinoline (DPD), follicular phase length, luteal-follicular phase transition (LFPT), shift work.** ***Environ Health Perspect*** **111:618–622 (2003). doi:10.1289/ehp.5878 available via** ***http://dx.doi.org/*** **[Online 9 December 2002]**

Although the association between ovarian function and bone loss is well recognized, the minimal hormonal requirements for maintaining healthy bones are poorly defined. Exercise-induced amenorrhea (Beitins et al. 1991; Broocks et al. 1990; Bullen et al. 1985), prolactin-secreting tumors (Klibanski et al. 1980; Schlechte et al. 1983), and gonadotropin-releasing hormone (GnRH)-induced hypogonadism (Scharla et al. 1990; Surrey and Judd 1992) all result in bone loss in women. Female athletes have been shown to lose bone despite increased skeletal loading, a situation that places these women at increased risk for bone injuries (Cann et al. 1984). Older studies suggested that bone loss in healthy young women is related to abnormalities of progesterone production (Bullen et al. 1985; Prior 1990). However, recent reports have indicated that even modest exercise can result in alterations of ovarian function characterized by perturbations of the follicular phase but not the luteal phase of the menstrual cycle (De Souza et al. 1997; Waller et al. 1996; Winters et al. 1996). These subtle alterations of the follicular phase were associated with decreased bone integrity and an increased incidence of bone fractures (De Souza et al. 1997). Other data suggest that stress-related effects on bone health are associated with alterations in the rise of follicle-stimulating hormone (FSH) during the late luteal phase of the menstrual cycle, which in turn alter the follicular phase of the next cycle (De Souza et al. 1998).

The number of women in the workforce has grown over the past 20 years and continues to expand. Because a majority of working women are in their reproductive years, there is public concern about exposures in the workplace that could adversely affect menstrual function, fertility, or pregnancy. As a consequence of this concern, several studies have been designed to identify such hazards (Eskenazi et al. 1995; Gold et al. 1995a, 1995b; Lasley et al. 1995; Schenker et al. 1995). Despite clear evidence that subtle stressors such as changes in work schedule also may have adverse effects on reproductive health, there have been few studies on such nonchemical hazards, and there is little understanding of their mechanism(s) of action. Such stressors not only may have intrinsic adverse effects, but also they may exacerbate the effects of other workplace hazards. The presence of these stressors also can confound interpretation of the results of studies designed to evaluate other putative hazards.

Previous studies have indicated that stressors as subtle as night work or shift work can lead to irregular menstrual cycles (Miyauchi et al. 1992) through perturbations of the diurnal rhythms of reproductive hormones. The present study was conducted to test the hypothesis that stresses associated with the disruption of the regular work schedule induce alterations in ovarian function which, in turn, are associated with transient bone resorption. Specifically, we assessed work shift status, ovarian hormone profiles, and bone metabolites during two consecutive menstrual cycles of female workers to determine if a delay of ovulation and lengthening of the follicular phase are induced by the changing of work-shift regularity, and if these alterations of the menstrual cycle are associated with increased bone resorption.

Materials and Methods

Subjects. Twenty-one healthy Chinese female workers on rotating work shifts in a textile mill in Anqing, China, were recruited into the study during 1996–1998. The Human Subjects Committees at the Harvard School of Public Health and the China Medical Institutes approved all study procedures, and informed consent was obtained from each participant (Cho et al. 2002; Ronnenberg et al. 2000). Daily early morning urine samples were collected by the subjects during consecutive menstrual cycles. Paired sequential cycles were identified in which one of the pair was longer than the other. Of the 21 subjects enrolled, 9 subjects were excluded from the study because of noncompliance with the study protocol. Five subjects did not collect daily urine samples during the luteal-follicular phase transition (LFPT). Samples collected from the other 4 excluded subjects were too dilute to provide reliable information for follicular phase determination, as shown by low levels of creatinine in the sample (< 0.2 mg/mL). There was no association between the noncompliance of the subjects and their work shift schedules, and no other criteria were used to exclude subjects from the study.

Address correspondence to B.L. Lasley, Center for Health and the Environment, University of California, One Shields Avenue, Davis, CA 95616 USA. Telephone: (530) 752-8506. Fax: (530) 752-5300. E-mail: bllasley@ucdavis.edu

P. Lohstroh and J. Chen contributed equally to this paper.

The research described in this paper was funded by National Institutes of Health grants ES06198, ES05707, and P42 ES04699.

Received 9 July 2002; accepted 9 December 2002.

Of the 12 women (23–31 years of age) in the study, 11 were cotton-weaving workers and one was a laboratory technician. All 12 women worked rotating shifts during the study period, but the types of work shift were not known to the investigators until after the laboratory analyses were complete.

Menstrual cycle definitions. The menstrual cycle length was defined as the number of days from the onset of one period of menstrual bleeding to the day before the onset of the next period of menstrual bleeding. All cycles were determined to be ovulatory, as evidenced by a sustained rise of urinary pregnanediol-3-glucuronide (PdG) for 10 or more days immediately preceding the onset of menstruation. The midcycle urinary FSH peak was used as a biomarker to indicate the day of ovulation (Li et al. 2002) and for follicular phase length determination. The follicular phase length was defined as the number of days from the first day of menstruation up to and including the day of the FSH peak. The remaining days in the cycle were defined as the luteal phase. The LFPT was defined as the interval including day –8 to –1 of the preceding cycle, when day 1 is the first day of the menstrual bleeding in the study cycle. In other words, the LFPT was the last 8 days of the preceding cycle. Work schedules were evaluated during the LFPT preceding each of the two study cycles. FSH profiles were determined during the LFPT preceding the second of the two study cycles.

Sample collection and storage. The urine samples (3–5 mL) were self-collected, stored frozen without preservatives in the subjects' home refrigerator freezers at –10°C, then transferred to the laboratory at the end of each menstrual cycle for storage at –35°C. All samples collected during the entire cycle were thawed and analyzed for estrone conjugates (E1C), PdG, FSH, and creatinine, and then were refrozen. When cycles were shown to be ovulatory and the follicular phase had been defined, the samples collected in the follicular phase were thawed for a second time and analyzed for deoxypyridinoline (DPD).

Laboratory analyses. Assays for urinary E1C, PdG, and the beta subunit of FSH were performed as previously described (Munro et al. 1991; Qiu et al. 1998). Urinary DPD was measured using the Pyrilinks-D kit (Metra Biosystems, Inc., Mountain View, CA). All assay results were indexed by the concentration of creatinine in the same urine sample. In the present data set, the interassay coefficients of variation for E1C, PdG, FSH, and DPD were 10.4, 10.4, 12.9, and 11.7%, respectively.

Statistical analysis. The hormone values of urinary E1C, FSH, and PdG, as well as urinary DPD values, were compared between longer cycles and shorter cycles by using 2-way analysis of variance with repeated measures. The area under the curve (AUC) of DPD was calculated by trapezoidal rule (Holder et al. 1999) and compared between longer cycles and shorter cycles by paired *t*-test. All data are presented as mean ± SEM. We considered $p < 0.05$ significant.

Results

Seven subjects had a cycle pair in which a longer length cycle was followed by a shorter cycle, and five subjects had a shorter length cycle followed by a longer cycle (Table 1). The mean length (± SEM) of the longer cycles was 37.4 ± 1.3 days, and the mean length of the shorter cycles was 28.8 ± 0.74 days. The follicular phase length was significantly longer in the longer cycles compared with the shorter length cycles (21.9 ± 1.2 vs. 14.2 ± 0.56 days; $p < 0.001$), but there was no difference in the lengths of the luteal

Table 1. Characteristics of menstrual cycles.

	First cycle		Second cycle	
Subject	Cycle length (days)	Follicular length (days)	Cycle length (days)	Follicular length (days)
1	31	17	34[a]	18
2	33[a]	18	22	14
3	31	17	39[a]	25
4	33[a]	18	31	15
5	38[a]	22	31	15
6	37[a]	20	30	14
7	37[a]	21	28	12
8	46[a]	29	29	10
9	29	15	46[a]	31
10	28	13	35[a]	20
11	33[a]	20	27	14
12	28	14	38[a]	21

[a]Longer length cycle.

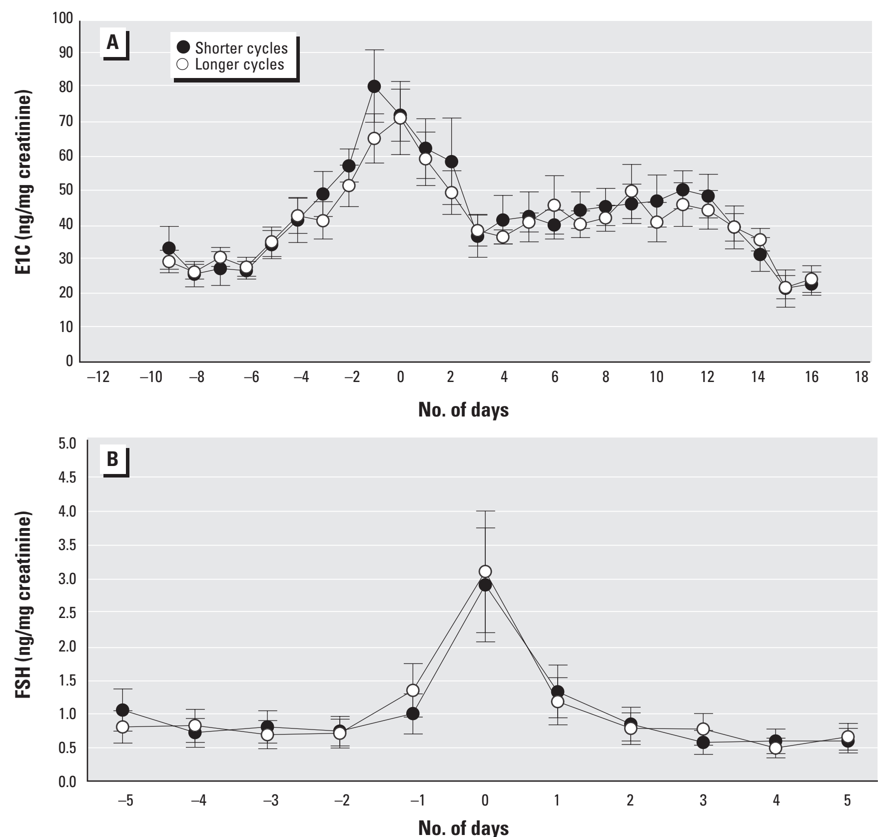

Figure 1. Daily urinary E1C concentrations (*A*) and FSH concentrations (*B*) indexed by creatinine concentrations of the same sample for 12 women that had pairs of sequential menstrual cycles in which one was of shorter length and the other was of longer length; both groups of cycles were aligned to the day of the midcycle FSH peak (day 0). (*A*) There was no statistical difference between E1C values on any day when the shorter and longer cycles were aligned in this manner ($p > 0.05$). (*B*) There was no statistical difference between FSH values on any day when the shorter length and longer cycles were aligned in this manner ($p > 0.05$).

phases between the two cycle types (14.6 ± 0.75 and 15.5 ± 0.36 days; $p > 0.05$).

Urinary hormone metabolite profiles were characterized by a gradual rise and abrupt fall of E1C (Figure 1A), with a single, prominent periovulatory FSH peak on approximately day 14 (shorter length cycles) or day 22 (longer cycles) after the onset of menstruation. When the midcycle FSH peak was defined as day 0, the E1C profiles from day –5 to day 0 were not different between the two groups of cycles ($p > 0.05$) (Figure 1A). Neither the FSH peak concentrations (2.95 ± 0.85 vs. 3.14 ± 0.91 ng/mg Cr, $p > 0.05$) (Figure 1B) nor the PdG profiles (data not shown) were statistically different between the two cycle groups. The only difference between the hormone profiles in the shorter length cycles and longer cycles was that the urinary E1C and FSH peaks were delayed in the longer cycles (Figure 1A). In addition, FSH levels were significantly lower during the interval from day –7 to day –3 of the LFPT preceding longer cycles compared with FSH levels in the same interval prior to shorter length cycles (from 0.35 ± 0.04 ng/mg Cr vs. 0.69 ± 0.12 on day –7 to 0.56 ± 0.10 ng/mg Cr vs. 0.74 ± 0.11 on day –3; $p = 0.045$) (Figure 2).

DPD concentrations during the follicular phase were compared between the longer cycles and the shorter cycles. In general, DPD concentrations gradually increased during the late follicular phases of longer cycles. Trend analysis showed that the slope of the urinary DPD trend line for longer cycles was statistically different from zero, whereas there was no statistically significant trend for shorter length cycles ($p = 0.021$ for long cycles) (Figure 3). Mean concentrations of DPD were significantly higher in longer cycles compared with shorter length cycles on day –1 before the FSH peak (66.9 ± 6.62 vs. 54.2 ± 4.42 nmol/L; $p = 0.043$) and on day –2 (62.3 ± 5.57 vs. 48.4 ± 5.68 nmol/L; $p = 0.026$), whereas DPD levels were not significantly different between the two cycles in the early and midfollicular phases (from 52.2 ± 4.28 vs. 57.3 ± 5.56 on day –9 to 70.9 ± 11.4 vs. 53.1 ± 3.5 on day –4, longer cycles vs. shorter cycles; $p > 0.05$). To address the overall effects of irregular work schedule changes on bone resorption, the AUC for DPD was analyzed to evaluate the excretion of DPD over time during the follicular phase of menstrual cycles. The area under the DPD concentration curve from day –8 to day –1 before the FSH peak was significantly greater (429.2 ± 29.2 vs. 389.9 ± 24.8; $p = 0.042$) in longer cycles compared with shorter cycles, and this difference in bone resorption was greater (189.8 ± 15.5 vs. 163.8 ± 13.3; $p < 0.004$) when only the late follicular phase (day –4 to –1) was considered.

Work schedules were available for all 12 of the cycles preceding the shorter length study cycles and for 10 of the cycles preceding the 12 longer study cycles. A regular, forward progression of work shifts (day–day–swing–swing–graveyard–graveyard–off day–off day) with no more than two consecutive shifts was found preceding all but one of the 12 shorter length cycles (first cycle of subject 1). In contrast, only 5 of the 10 longer cycles were associated with this normal progression, and 4 of the 5 remaining longer cycles (second cycle of subjects 3, 9, 10, and 12) were preceded by extra off days, which broke the regularity of the work schedules. The LFPT preceding the remaining longer menstrual cycle (first cycle of subject 8) was characterized by an extended period of work (swing–swing–swing–graveyard-day) (Table 2).

Discussion

Previous reports indicate that even subtle stressors, such as night work or shift work, can increase the incidence of irregular menstrual cycles in healthy young women (Miyauchi et al. 1992). In the present study, rotating shift workers had some shorter length menstrual cycles (27–31 days) as well as some longer cycles (33–46 days). The hormone profiles of these cycles show that the variations in cycle length resulted primarily from differences in the length of the follicular phase of the cycle rather than the length of the luteal phase. Our findings suggest that the disruption of the regularity of rotating shift work is associated with longer menstrual cycles characterized by a delay in the day of ovulation and a lengthening of the follicular phase. We also found evidence of increased bone resorption in the follicular phases of these longer cycles. In this study, we were able to identify pairs of sequential menstrual cycles that included cycles of different lengths. Our ability to study consecutive cycles of the same woman decreases the possibility that changes in nutrition, general health, or lifestyle would contribute to changes in hormone secretion or bone accretion. In fact, all of the endocrine parameters that we measured were similar in the longer and shorter length cycles except for those associated with the delay of ovulation.

Our previous study of young women who exercised regularly demonstrated that perturbations of FSH secretion during the LFPT are associated with a delay of ovulation in the subsequent cycle and a prolongation of its follicular phase (De Souza et al. 1998). Thus, it appears that this transition period from the luteal phase of one cycle to the follicular phase of the next cycle is particularly sensitive to effects of environmental stressors. The present

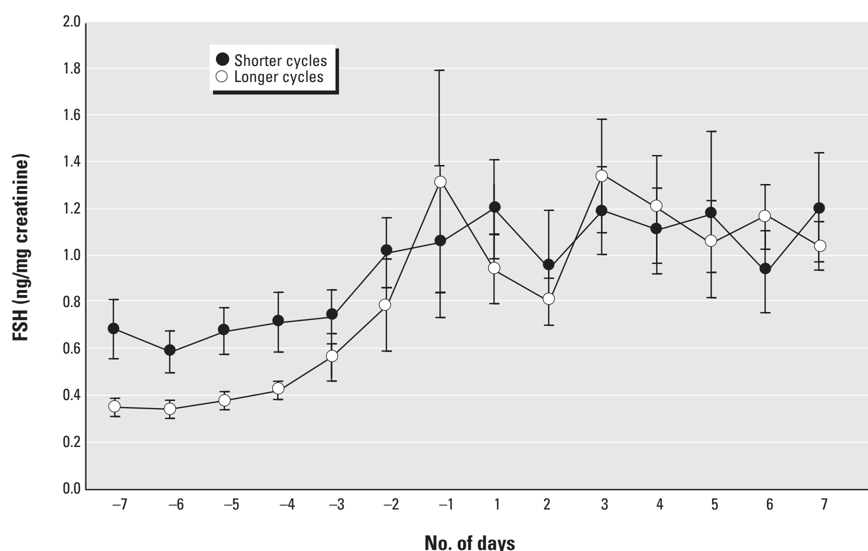

Figure 2. Daily urinary FSH profiles during the LFPT preceding shorter cycles and longer cycles. Day 1 is the first day of the next menstrual bleeding. FSH levels were significantly lower during the interval from day –7 to day –3 of the LFPT preceding longer cycles compared with those in the same interval prior to shorter length cycles ($p < 0.05$).

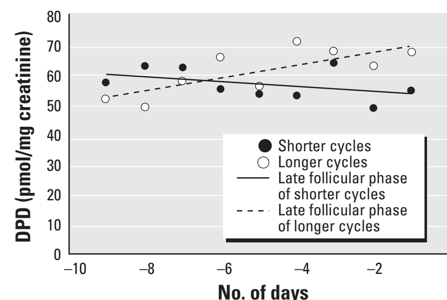

Figure 3. Urinary DPD concentrations indexed by creatinine during the follicular phase of the shorter cycles and longer cycles aligned as shown in Figure 1A. Trend lines show that DPD concentrations gradually increased during the late follicular phases of longer cycles, whereas DPD concentrations gradually decreased during the same interval of shorter cycles.

Table 2. Work schedule during the LFPT prior to each cycle.

	Days prior to first cycle[a]												Days prior to second cycle											
Subject	–12	–11	–10	–9	–8	–7	–6	–5	–4	–3	–2	–1	–12	–11	–10	–9	–8	–7	–6	–5	–4	–3	–2	–1
1	OD	OD	D	D	S	S	S	G	OD	OD	G	G[b]	S	S	G	G	OD	OD	D	D	OD	S	G	G
2	OD	OD	D	D	S	S	G	G	OD	OD	D	D	OD	OD	D	D	S	S	G	G	OD	OD	D	G
3	OD	OD	D	D	S	S	G	G	OD	OD	D	D	OD	OD	OD	OD	OD	OD	OD	OD	OD	OD	OD	OD[b]
4	D	D	S	S	G	G	OD	OD	D	D	NA	NA	D	D	S	S	G	G	OD	OD	D	D	S	G
5	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	D	D	S	S	G	G	OD	OD	D	D	S	S
6	D	D	S	S	G	G	OD	OD	D	D	S	S	OD	D	D	S	S	G	G	OD	OD	S	G	G
7	D	D	S	S	G	G	OD	OD	D	D	S	G	D	D	S	S	G	G	OD	OD	D	D	D	D
8	OD	OD	D	S	S	S	G	D	OD	OD	D	D[b]	OD	OD	D	D	S	S	G	G	OD	S	G	G
9	OD	OD	D	D	S	S	G	G	OD	S	NA	NA	D	S	D	G	OD	OD	OD	OD	OD	S	S	G[b]
10	D	D	S	S	G	G	OD	OD	D	D	S	S	OD	OD	OD	OD	D	D	NA	OD	D	S	S	G[b]
11	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	S	G	G	OD	OD	D	D	S	S	G	G	OD
12	OD	OD	D	D	S	S	G	G	OD	OD	D	D	G	G	OD	OD	D	D	S	OD	OD	OD	OD	OD[b]

Abbreviations: D, day (0600 hr–1400 hr); OD, off day; S, swing (1400 hr–2200 hr); G, graveyard (2200 hr–0600 hr); NA, information not available.
[a]Day 1 is the first day of the next menstrual cycle. [b]Irregular work schedule.

report suggests that changes in the regularity of work shift can have adverse effects on menstrual function when they take place at this time. The frequency of off days during the LFPT may have similar effects, but further investigation of confounding factors is needed before any conclusions can be drawn. The observed differences in FSH profiles during the transition periods preceding shorter and longer cycles may provide an important clue to the underlying mechanism. The apparent inhibition of FSH secretion during this time is consistent with a previous study of women who exercised regularly in which a decreased FSH secretion during the LFPT was 90% predictive of a prolonged follicular phase and delay of ovulation in the following menstrual cycle (De Souza et al. 1998).

This study not only confirms previous reports that shift work and/or night work perturb ovarian function but also it demonstrates an adverse effect of such stressors on bone health. There was significantly greater bone resorption in the follicular phase of longer cycles than in that of shorter cycles, as measured by levels of urinary DPD. DPD is a crosslink of bone type-1 collagen released during the bone resorption process and excreted unmetabolized in the urine. Elevated levels of urinary DPD indicate increased bone resorption. DPD measurements are used to identify and evaluate individuals at risk for accelerated bone loss (Robins et al. 1994). Elevated levels of DPD can be measured before changes in total bone mineral density are observed by densitometry (Fujimura et al. 1997). In addition, DPD measurements can detect whether supplementation and lifestyle interventions are affecting the rate of bone loss. Although DPD concentrations were not measured during the luteal phases of the menstrual cycles in this study, DPD concentrations were not different in shorter cycles and longer cycles during the early follicular phase and midfollicular phase. These similarities, along with the normal hormone profiles observed during the luteal phases of both cycle types, support the concept that differences in bone resorption in this study were limited to the follicular phase. The only difference in the endocrine profiles of the two cycle types was in the early follicular phase and appeared to reflect a delay in follicle recruitment. However, this delay in follicle recruitment did not appear to have an immediate effect on bone mobilization. The differences observed in DPD were not apparent until the late follicular phase, when estrogen levels were essentially the same in the two groups. Thus, the apparent endocrine cause for the loss of bone preceded the detection of bone resorption by several days.

Previous studies have indicated that progesterone is anabolic in women, and reduced production of progesterone is related to bone loss in young women (Bullen et al. 1985; Prior 1990). In contrast to these earlier reports, the present data show no difference in luteal phase progesterone profiles, but rather indicate that subtle changes in the length of the follicular phase and its hormone dynamics are associated with transient bone resorption. This finding is consistent with earlier reports from this laboratory on studies of healthy women (De Souza et al. 1997; Waller et al. 1996). Although it is likely that abnormalities in the follicular phase can result in bone resorption, the underlying mechanism(s) is still not clear. During the long follicular phases observed in this study, estrogen was delayed in reaching normal circulating levels. However, the evidence of bone resorption was not detected in urine until several days later.

In conclusion, the present data indicate that the LFPT is a particularly sensitive period of the menstrual cycle when environmental stressors may be effective in perturbing ovarian function. During this time, stressors such as exercise or changes in work schedule can result in a perturbation of FSH secretion. Diminished FSH secretion during this time appears to delay the recruitment of the next follicle cohort, with a consequent delay of ovulation in the next menstrual cycle and a prolongation of its follicular phase. Although the delay of ovulation does not appear to decrease fecundity as indicated by the periovulatory hormone profiles (Li et al. 2001), one important adverse effect is a transient resorption of bone. Such perturbations, if repetitive, may increase the risk of osteoporosis in later life.

References

Beitins IZ, McArthur JW, Turnbull BA, Skrinar GS, Bullen BA. 1991. Exercise induces two types of human luteal dysfunction: confirmation by urinary free progesterone. J Clin Endocrinol Metab 72:1350–1358.

Broocks A, Pirke KM, Schweiger U, Tuschl RJ, Laessle RG, Strowitzki T, et al. 1990. Cyclic ovarian function in recreational athletes. J Appl Physiol 68:2083–2086.

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

Cann CE, Martin MC, Genant HK, Jaffe RB. 1984. Decreased spinal mineral content in amenorrheic women. JAMA 251:626–629.

Cho SI, Goldman MB, Ryan LM, Chen C, Damokosh AI, Christiani DC, et al. 2002. Reliability of serial urine HCG as a biomarker to detect early pregnancy loss. Hum Reprod 17:1060–1066.

De Souza MJ, Miller BE, Loucks AB, Luciano AA, Pescatello LS, Campbell CG, et al. 1998. High frequency of luteal phase deficiency and anovulation in recreational women runners: blunted elevation in follicle-stimulating hormone observed during luteal-follicular transition. J Clin Endocrinol Metab 83:4220–4232.

De Souza MJ, Miller BE, Sequenzia LC, Luciano AA, Ulreich S, Stier S, et al. 1997. Bone health is not affected by luteal phase abnormalities and decreased ovarian progesterone production in female runners. J Clin Endocrinol Metab 82:2867–2876.

Eskenazi B, Gold EB, Samuels SJ, Wight S, Lasley BL, Hammond SK, et al. 1995. Prospective assessment of fecundity of female semiconductor workers. Am J Ind Med 28:817–831.

Fujimura R, Ashizawa N, Watanabe M, Mukai N, Amagai H, Fukubayashi T, et al. 1997. Effect of resistance exercise training on bone formation and resorption in young male subjects assessed by biomarkers in bone metabolism. J Bone Miner Res 12:656–662.

Gold EB, Eskenazi B, Hammond SK, Lasley BL, Samuels SJ, O'Neill Rasor M, et al. 1995a. Prospectively assessed menstrual cycle characteristics in female wafer-fabrication and nonfabrication semiconductor employees. Am J Ind Med 28:799–815.

Gold EB, Eskenazi B, Lasley BL, Samuels SJ, O'Neill Rasor M, Overstreet JW, et al. 1995b. Epidemiologic methods for prospective assessment of menstrual cycle characteristics in female semiconductor workers. Am J Ind Med 28:783–797.

Holder DJ, Hsuan F, Dixit R, Soper K. 1999. A method for estimating and testing area-under-the-curve in serial sacrifice, batch, and complete data designs. J Biopharm Stat 9:451–464.

Klibanski A, Neer RM, Beitins IZ, Ridgway EC, Zervas NT, McArthur JW. 1980. Decrease bone density in hyperprolactinemic women. N Engl J Med 303:1511–1514.

Lasley BL, Lohstroh P, Kuo A, Gold EB, Eskenazi B, Samuels SJ, et al. 1995. Laboratory methods for evaluating early pregnancy loss in an industry-based population. Am J Ind Med 28:771–781.

Li H, Chen J, Overstreet JW, Nakajima ST, Lasley BL. 2002. Urinary follicle stimulating hormone peak as a biomarker for estimating the day of ovulation. Fertil Steril 77:961–966.

Li H, Nakajima ST, Chen J, Todd HE, Overstreet JW, Lasley BL. 2001. Differences in hormone characteristics of conceptive versus nonconceptive menstrual cycles. Fertil Steril 75:549–553.

Miyauchi F, Nanjo K, Otsuka K. 1992. Effects of night shift on plasma concentrations of melatonin, LH, FSH and prolactin, and menstrual irregularity [in Japanese]. Sangyo Igaku 34:545–550.

Munro CJ, Stabenfeldt GH, Cragun JR, Addiego LA, Overstreet JW, Lasley BL. 1991. Relationship of serum estradiol and progesterone concentrations to the excretion profiles of their major urinary metabolites as measured by enzyme immunoassay and radioimmunoassay. Clin Chem 37:838–844.

Prior JC. 1990. Progesterone as a bone-trophic hormone. Endocr Rev 11:386–389.

Qiu Q, Kuo A, Todd H, Dias JA, Gould JE, Overstreet JW, et al. 1998. Enzyme immunoassay method for total urinary follicle-stimulating hormone (FSH) beta subunit and its application for measurement of total urinary FSH. Fertil Steril 69:278–285.

Robins SP, Woitge H, Hesley R, Ju J, Seyedin S, Seibel MJ. 1994. Direct, enzyme-linked immunoassay for urinary deoxypyridinoline as a specific marker for measuring bone resorption. J. Bone Miner Res 9:1643–1649.

Ronnenberg AG, Goldman MB, Aitken IW, Xu X. 2000. Anemia and deficiencies of folate and vitamin B-6 are common and vary with season in Chinese women of childbearing age. J Nutr 130:2703–2710.

Scharla SH, Minne HW, Waibel-Treber S, Schaible A, Lempert UG, Wuster C, et al. 1990. Bone mass reduction after estrogen deprivation by long-acting gonadotropin-releasing hormone agonists and its relation to pretreatment serum concentrations of 1,25-dihydroxyvitamin D3. J Clin Endocrinol Metab 70:1055–1061.

Schenker MB, Gold EB, Beaumont JJ, Eskenazi B, Hammond SK, Lasley BL, et al. 1995. Association of spontaneous abortion and other reproductive effects with work in the semiconductor industry. Am J Ind Med 28:639–659.

Schlechte JA, Sherman B, Martin R. 1983. Bone density in amenorrheic women with and without hyperprolactinemia. J Clin Endocrinol Metab 56:1120–1123.

Surrey ES, Judd HL. 1992. Reduction of vasomotor symptoms and bone mineral density loss with combined norethindrone and long-acting gonadotropin-releasing hormone agonist therapy of symptomatic endometriosis: a prospective randomized trial. J Clin Endocrinol Metab 75:558–563.

Waller K, Reim J, Fenster L, Swan SH, Brumback B, Windham GC, et al. 1996. Bone mass and subtle abnormalities in ovulatory function in healthy women. J Clin Endocrinol Metab 81:663–668.

Winters KM, Adams WC, Meredith CN, Loan MD, Lasley BL. 1996. Bone density and cyclic ovarian function in trained runners and active controls. Med Sci Sports Exerc 28:776–785.