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Low Bone Mineral Density in the Early Menopausal Transition: Role for Ovulatory Function

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Objective and Context: The objective of the study was to determine whether luteal abnormalities or measures of sex steroid hormones collected across a menstrual cycle were associated with bone mineral density (BMD) at the total hip or lumbar spine.

Design and Setting: The Study of Women's Health Across the Nation is a longitudinal, community-based study conducted at seven clinical sites. Study of Women's Health Across the Nation includes a daily hormone study substudy in which daily urine samples are collected for one menstrual cycle (up to a maximum of 50 d) each year.

Participants: Participants included 643 pre- and perimenopausal women, aged 43–53 yr.

Main Outcome Measures: BMD of the lumbar spine and total hip was measured by dual-energy x-ray densitometry. Daily urine samples were assayed for estrone conjugates, pregnanediol glucuronide, LH, and FSH, and the information from across the menstrual cycle was expressed as area under the curve (AUC). BMD levels were evaluated in relation to three menstrual cycle attributes: 1) absence or presence of ovulation; 2) luteal phase length to menstrual cycle length ratio; and 3) ovulatory disturbances, defined as anovulatory cycles or cycles with short luteal phases (<10 d).

Results: Lower urine estrone conjugate AUC and higher urine FSH AUC were significantly associated with lower BMD. However, luteal abnormalities based on menstrual cycle attributes were not significantly associated with BMD at the total hip or lumbar spine after adjustment for age, body mass index, urinary hormone concentrations, menopausal status, and race/ethnicity.

Conclusions: Direct measures of urinary hormones, not menstrual cycle luteal abnormalities, were associated with lower levels of BMD. (***J Clin Endocrinol Metab* 91: 3780–3785, 2006**)

DURING THE MENOPAUSAL transition, menstrual cycles are increasingly likely to be anovulatory, with changes in menstrual cycle lengths and bleeding patterns (1). An increasing proportion of menstrual cycles include a prolonged follicular phase or shortened luteal phase, reflecting delayed ovulation or anovulatory cycles (2). However, relatively few studies have assessed the association between bone mineral density (BMD) and these changes in menstrual cycle characteristics during the transition to menopause.

Studies of atypical luteal phase attributes (*i.e.* short luteal phases or evidence of anovulation) and BMD have focused on healthy premenopausal women (3, 4), select samples such as infertile women (5), women with marked undernutrition (6, 7), or women engaged in intense work- or sport-related physical activity (8–12). Frequent anovulatory cycles can have a marked impact on bone. A study of healthy premenopausal women aged 21–42 yr found that those with one or more anovulatory cycles had a bone mineral decline of 6.4 ± 3.8 mg/cm^3 per year (10). Spine trabecular bone mass loss, measured by quantitative computed tomography, was attributed to endogenous progesterone concentrations, rather than low estradiol concentrations. Moreover, female athletes with alterations in ovarian function (*e.g.* short, inadequate luteal phases), but without amenorrhea, also exhibited compromised BMD (4).

This study examines the associations between BMD and hallmarks of menopausal transition, *i.e.* changes in the urinary hormone excretion and in menstrual cycle attributes in a general population of pre- and perimenopausal women, without selection for clinical syndromes or pathology. Three menstrual cycle attributes were considered: 1) the absence or presence of ovulation; 2) the ratio of the luteal phase to menstrual cycle length; and 3) ovulatory disturbances, as indicated by anovulatory cycles or menstrual cycles with short luteal phases (<10 d). Additionally, we related the urinary products of estrogens, progesterone, FSH, and LH from an entire menstrual cycle to BMD level.

Subjects and Methods

Study population

The data came from pre- and perimenopausal women in the Daily Hormone Study (DHS), a substudy of the Study of Women's Health Across the Nation (SWAN). SWAN is a multisite, longitudinal, population-based study examining the natural history of the menopausal transition in 3302 women whose baseline age was 42–52 yr at study initiation (1996). The DHS began in 1997 with the first follow-up evaluation of women enrolled in SWAN. The four sites (Detroit, MI, area; Chicago, IL; Boston, MA; and Pittsburgh, PA) with African-American enrollees recruited approximately 90 participants each, with half being African-American and the remaining Caucasian. The three sites with Chinese, Japanese, and Hispanic (Oakland, CA; Los Angeles, CA; and Newark, NJ) enrollees targeted 238, 180, and 90 participants, respectively. This sampling and recruitment strategy ensured an equal rep-

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Abbreviations: AUC, Area under the curve; BMD, bone mineral density; BMI, body mass index; CV, coefficient of variation; DHS, Daily Hormone Study; SWAN, Study of Women's Health Across the Nation; uE1c, urine samples for estrone conjugates; uFSH, urine samples for FSH; uLH, urine samples for LH; uPdG, urine samples for pregnanediol glucuronide.

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resentation of the different minority groups among the study participants. A total of 867 women completed a daily collection of specimens across one menstrual cycle or 50 d. Of these, 840 had sufficient data to determine whether there was evidence of luteal activity (see subsequent definition). Whereas urine and data were collected from enrollees at the seven research sites (Boston, MA; Chicago, IL; Detroit, MI; Los Angeles, CA; Newark, NJ; Oakland, CA; and Pittsburgh, PA), bone was not measured at the Chicago and Newark sites; therefore, the sample for these analyses included Caucasian (n = 208), African-American (n = 121), Chinese (n = 144), and Japanese (n = 167) women. An institutional review board approved the study protocol at each site, and written informed consent was obtained from each participant.

Measurement of variables

Bone mineral densitometry. Bone measurements of the lumbar spine and total hip (grams per square centimeter) were made with the Hologic 2000 densitometers (Hologic, Bedford, MA) (Pittsburgh and Oakland) and 4500A densitometers (Boston, Detroit area, and Los Angeles). The protocol included the use of a positioning device (Osteodyne, Research Triangle Park, NC) to facilitate the reproducible measurement of the proximal femur. The study-wide quality control program included the daily measurement of an anthropomorphic spine phantom at each site, calibration with a spine phantom to provide cross-site and cross-time calibrations, and a site-level review of all participant scans for specified criteria. Synarc, Inc. (Waltham, MA) reviewed 5% of all scans as well as those scans with potential problems based on *a priori* criteria. They defined the ultimate status of these particular scans as acceptable, requiring reanalysis, or rejected (13).

Demographic and physical measurements. Each study participant provided information about her reproductive history, sociodemographic characteristics, medical history, and lifestyle behaviors by completing questionnaires. Information included menstrual status, parity, oral contraceptive use, smoking practices, and alcohol history. Participants were measured for weight (kilograms) and height (centimeters) using a calibrated balance-beam scales and a stadiometers, respectively. During these measurements, women wore a single layer of light clothing and no shoes. Body mass index (BMI) was calculated as a participant's weight (kilograms) divided by the square of her height (meters).

Hormone assays. For the DHS, daily first-voided urine samples were collected for one complete menstrual cycle or up to a maximum of 50 d. These urine samples were assayed for LH (uLH) and FSH (uFSH) as well as estrone conjugates (uE1c) and pregnanediol glucuronide (uPdG) using assays adapted to chemiluminescent technology on the ACS-180 automated chemiluminescence analyzer (Bayer Diagnostic Corp., Tarrytown, NY). The reporting range for the uPdG assay was 0.005–25.5 μg/ml, the minimum detectable concentration was 0.0001 μg/ml, and the inter- and intraassay coefficients of variation (CVs) were 17.8 and 7.7%, respectively. The reporting range for the uE1c assay was 5.10–408.0 ng/ml, the minimum detectable concentration was 0.10 ng/ml, and the inter- and intraassay CVs were 11.5 and 8.1%, respectively. The reporting range for the uLH assay was 0.1–55.2 mIU/ml, the minimum detectable concentration was 0.1 mIU/ml, and the inter- and intraassay CVs were 10.9 and 4.6%, respectively. The reporting range for the uFSH assay was 0.3–136 mIU/ml, the minimum detectable concentration was 0.3 mIU/ml, and the inter- and intraassay CVs were 11.4 and 3.8%, respectively. The daily urinary hormone values were normalized for creatinine concentrations and expressed as a ratio of the hormone to creatinine concentrations.

For each study participant, two summary measures of urinary hormone concentrations were computed: the mean urinary hormone level from her entire menstrual cycle and the cumulative hormone concentration estimated by integrating the area under a participant's hormone curve for her entire menstrual cycle. Before integration, all cycle lengths were standardized to 28 d to control for differences in hormone concentrations derived from the number of collection days.

Menstrual cycle characteristics. Menstrual cycle attributes were based on participants' self-reported menstrual bleeding patterns. Menstrual cycle status was classified as either premenopausal (*i.e.* at least one menstrual cycle in the 3 months before study entry, with no changes in cycle regularity) or early perimenopausal (*i.e.* menses in the 3 months before recruitment, with changes in regularity).

The first day of the data collection window for the daily urine collection was the menstrual period start date, whereas the final day of the collection period coincided with the day before the next menstrual period. Seven women did not experience a subsequent menstrual period and stopped collection on d 50; their information was excluded from data analyses because exact menstrual cycle lengths were unknown. For each menstrual cycle, a validated algorithm, which uses moving averages across the cycle to locate the 5 d during the follicular phase when uPdG concentrations are the lowest, was employed to detect a significant increase in uPdG concentrations as evidence of luteal activity and presumed ovulation (14). The criterion for luteal activity was a 3-fold increase in uPdG concentrations above this lowest level for at least 3 consecutive days. Menstrual cycles not meeting these criteria were classified as anovulatory based on no evidence of luteal activity.

Follicular phase length was defined as the number of days from the first cycle day to and including the day of ovulation. Luteal phase length was calculated as the difference between the cycle length and the follicular phase length.

For each menstrual cycle, three measures of menstrual cycle attributes were defined. The first attribute was the presence or absence of luteal activity, a marker for ovulation (15). The second attribute was the luteal phase index, a ratio of the luteal phase length to the total cycle length for ovulatory cycles only, a measure of progesterone exposure (10). Finally, the third attribute was the presence or absence of an ovulatory disturbance, defined as a cycle that was either anovulatory or had a luteal phase of less than 10 d (10).

Statistical analysis

The data management and analysis was conducted using SAS (version 8.0; SAS Institute, Cary, NC). Univariate statistics were calculated for all variables and the distributions were evaluated for normality. To address skewness and satisfy the normality assumption for parametric tests, BMD, BMI, and the urinary hormone measures were log transformed. Associations among variables were examined using a Student's *t* test (for continuous normally distributed variables), a Wilcoxon test for nonparametric data or a χ^2 test of homogeneity for categorical variables. All *P* values calculated to evaluate statistical significance were based on two-sided tests at a value of $P < 0.05$.

Preliminary analyses identified age, BMI, cumulative urinary hormone concentrations [as measured by the integrated area under the curve (AUC)], menopausal status, menstrual cycle length, and race as important covariates. Age was centered at 43 yr, the minimum age for the study participants to reduce the possibility of collinearity between age and other variables and their interaction terms. Moreover, this approach permits a more straightforward application of the results to a clinical setting: a centered age set to 0 corresponds to a 43-yr-old woman, and relevant effects can be computed based on differences in ages relative to this minimum. Multiple linear regression analysis was used to evaluate the associations between menstrual cycle characteristics and BMD. Statistical models related each menstrual cycle attribute to BMD with and without adjustment for the cumulative hormone concentrations. Then, to determine whether such an adjustment altered the association between BMD and menstrual cycle characteristics (*i.e.* ovulatory/anovulatory cycles, luteal-phase index, ovulatory disturbances), these analyses were rerun including the AUC variables corresponding to the four urinary hormones (uE1c, uFSH, uPdG, and uLH). The models also included interactions among the three menstrual cycle attributes and race/ethnicity to assess disparities in BMD across ethnic groups (16). Because the associations between BMD and menstrual cycle attributes did not vary significantly by race/ethnicity, these interaction terms were not included in the final models.

Results

The demographic and health characteristics of the 643 DHS participants with known cycle lengths are presented in Table 1. Their mean age was almost 47 yr with a range of 43–53 yr. Approximately 80% of menstrual cycles had evidence of luteal activity (*i.e.* an ovulatory cycle). Women with

TABLE 1. Selected demographic and health characteristics of DHS participants

	Ovulatory cycles (n = 513)	Anovulatory cycles (n = 116)
Age (yr), mean ± SD	46.2 ± 2.4[a]	48.0 ± 2.5[a]
BMI (kg/m^2), average ± SD	26.4 ± 6.6[b]	28.0 ± 7.5[b]
Current smoker	47 (9.2)	12 (10.4)
Race		
African-American (%)	92 (18.0)	25 (21.5)
Caucasian (%)	168 (32.9)	38 (32.8)
Chinese (%)	119 (23.3)	24 (20.7)
Japanese (%)	131 (25.7)	29 (25.0)

[a] $P < 0.0001$ for intergroup differences.
[b] $P < 0.05$ for intergroup differences.

ovulatory cycles were significantly younger (46 *vs.* 48 yr) and had a lower BMI (24 *vs.* 28 kg/m^2) than women with anovulatory cycles.

Women with ovulatory cycles had higher uE1c concentrations and lower uFSH and uLH concentrations than women with anovulatory cycles (Table 2). Menstrual cycle length was significantly different between the two groups: women classified as anovulatory had longer cycle lengths, on average, than those classified as ovulatory (38.7 *vs.* 29 d). Only 2% (n = 10) of women with ovulatory cycles had a luteal phase length less than 10 d. Of note, there were no significant differences between the two groups with respect to spine or hip BMD (Table 2). More than 80% of the women with anovulatory cycles were classified as early perimenopausal, whereas only 15% were premenopausal (Table 2). By contrast, among women with ovulatory cycles, 66% were perimenopausal and 33% were premenopausal. More than 60% of the ovulatory group had menstrual cycle lengths between 26 and 31 d, compared with 13% for the anovulatory group. In the latter group, approximately two thirds were more than 31 d in length.

Menstrual cycle luteal function attributes

There were no significant associations among the three measures of luteal function (ovulatory/anovulatory, luteal phase index, and ovulatory disturbance) and BMD at either bone site. There was, as expected, a significant positive association ($P < 0.0001$) between log-transformed BMI and BMD at the total hip and the lumbar spine, whereby women with higher BMI levels also had higher BMD (data not shown).

Urinary hormone characteristics

Table 3 summarizes the results from the multiple variable regression models of the association between BMD and cu-

TABLE 2. BMD, menstrual cycle, and urinary hormone characteristics for DHS participants by luteal activity status

	Ovulatory cycles (n = 513)	Anovulatory cycles (n = 116)	*P* value[a]
BMD (g/cm^2), mean ± SD			
Total hip BMD	0.935 ± 0.14	0.941 ± 0.15	0.68
Lumbar spine BMD	1.062 ± 0.13	1.059 ± 0.14	0.86
Menstrual cycle attributes			
Luteal-phase index[b], mean ± SD	0.50 ± 0.1	N/A	
Ovulatory disturbance (%)[c]	10 (1.9)	116 (100)	
Hormone concentrations, mean ± SD			
uPdG (pg/ml)[d]	2.4 ± 3.1	1.1 ± 6.8	N/A
uE1c (ng/ml)	53.8 ± 45.3	43.1 ± 36.2	<0.0001
uFSH (mIU/ml)	21.4 ± 25.3	79.3 ± 80.4	<0.0001
uLH (IU/ml)	3.3 ± 5.7	7.2 ± 8.7	<0.0001
Cumulative hormone concentrations (AUC), mean ± SD			
uPdG (pg/ml)[d]	67.7 ± 36.7	34.7 ± 189.6	N/A
uE1c (ng/ml)	1495.1 ± 653.6	1272.9 ± 624.3	0.001
uFSH (mIU/ml)	579.7 ± 389.8	1966.3 ± 1662.5	<0.0001
uLH (IU/ml)	90.3 ± 57.5	178.8 ± 165.9	<0.0001
Other menstrual cycle characteristics			
Menopausal status			
Late perimenopausal (%)	0 (0.00)	1 (0.9)	
Early perimenopausal (%)	334 (65.6)	95 (81.9)	
Premenopausal (%)	166 (32.6)	17 (14.7)	
Undetermined (%)	9 (1.8)	3 (2.6)	
Average cycle length (d) ± SD	29.0 ± 4.3	38.7 ± 12.2	
Less than 26 d (%)	122 (23.8)	24 (20.7)	
26–28 d (%)	218 (41.5)	8 (6.9)	
29–31 d (%)	106 (20.7)	7 (6.0)	
31 d or more (%)	67 (13.1)	77 (66.4)	
Average follicular phase (d) ± SD[e]	14.3 ± 4.4	N/A	
Average luteal phase (d) ± SD[e]	13.7 ± 2.1	N/A	

[a] The *P* values for intergroup (*i.e.* ovulatory *vs.* anovulatory) differences were derived using Student's *t* tests. These values were not adjusted for multiple comparisons.
[b] The ratio of luteal phase length to cycle length (for ovulatory cycles only).
[c] An ovulatory disturbance is defined as either a short luteal phase (<10 d) or an anovulatory cycle
[d] The intergroup differences in uPdG values were not tested for statistical significance because the classification of menstrual cycles as ovulatory or anovulatory is based on uPdG levels.
[e] Follicular phase length is defined as the number of days from the first cycle day up to and including the day of ovulation. Luteal phase length is the difference between the cycle length and the follicular phase length.

TABLE 3. Associations between BMD and urinary sex steroid hormone excretion across a menstrual cycle (AUC), in models adjusted for age and BMI

Effect	Total hip BMD			Lumbar spine BMD		
	Beta[a]	SE (beta)	*P* value	Beta	SE (beta)	*P* value
Log(uE1c)[b]	**0.0282**	**0.0119**	**0.02**	**0.0303**	**0.0126**	**0.02**
Age[c]	0.0010	0.0018	0.59	0.0009	0.0019	0.62
Log(BMI)	0.3761	0.0231	<0.0001	0.2005	0.0248	<0.0001
Log(uFSH)[b]	**−0.0133**	**0.0072**	**0.06**	**−0.0154**	**0.0075**	**0.04**
Age[c]	0.0019	0.0019	0.31	0.0020	0.0020	0.32
Log(BMI)	0.3618	0.0236	<0.0001	0.1835	0.0252	<0.0001
Log(uPdG)[b]	0.0012	0.0071	0.86	0.0064	0.0075	0.31
Age[c]	0.0012	0.0019	0.53	0.0014	0.0020	0.48
Log(BMI)	0.3714	0.0239	<0.0001	0.1988	0.0255	<0.0001
Log(uLH)[b]	−0.0099	0.0071	0.16	−0.0109	0.0075	0.15
Age[c]	0.0013	0.0019	0.48	0.0013	0.0020	0.51
Log(BMI)	0.3605	0.0242	<0.0001	0.1827	0.0259	<0.0001

Bold numbers are statistically significant.
[a] The regression coefficients have been adjusted for age, BMI, menstrual-cycle length, menopausal status, and race.
[b] Cumulative hormone concentration, calculated using the AUC measure.
[c] Age is defined as a respondent's actual age minus the minimum age (43 yr) for all respondents.

mulative sex steroid hormone measures. Log-transformed uE1c AUC was positively associated with both total hip and lumbar spine BMD after adjustment for relevant covariates (age, BMI, menstrual cycle length, menopausal status, race/ethnicity): women with higher uE1c concentrations (positive beta coefficient) had significantly higher BMD level at both bone sites.

Log-transformed uFSH AUC was negatively associated with BMD at a $P < 0.05$ for the lumbar spine indicating that pre- and perimenopausal women with lower uFSH concentrations exhibited higher BMD at the lumbar spine. There were no statistically significant associations between BMD at both bone sites and uPdG AUC and uLH AUC, after adjustment for the relevant covariates.

Individual regression models including the three menstrual cycle attributes as well as the ovarian and gonadotropin hormones are presented in Table 4. Higher levels of $_{\log}$uE1c AUC was positively associated with both total hip and lumbar spine BMD, independent of whether measures of menstrual cycle characteristics were included in the

TABLE 4. Associations between BMD and menstrual cycle characteristics in models including uE1c or uFSH AUC and adjusted for BMI

Effect	Total hip BMD			Lumbar spine BMD		
	Beta[a]	SE (beta)	*P* value	Beta[a]	SE (beta)	*P* value
Ovulatory/anovulatory						
Ovulatory/anovulatory	0.0073	0.0139	0.60	0.0121	0.0147	0.40
Log(BMI)	0.3804	0.0233	<0.0001	0.2046	0.0248	<0.0001
Log(uE1c)[b]	**0.0285**	**0.0123**	**0.02**	**0.0308**	**0.0129**	**0.02**
Ovulatory/anovulatory	0.0018	0.0148	0.90	0.0054	0.0156	0.73
Log(BMI)	0.3653	0.0239	<0.0001	0.1863	0.0255	<0.0001
Log(uFSH)[b]	**−0.0141**	**0.0078**	**0.07**	**−0.0167**	**0.0081**	**0.04**
Luteal-phase index (only ovulatory cycles)[c]						
Luteal-phase index	−0.0331	0.0636	0.61	−0.0157	0.0670	0.81
Log(BMI)	0.3905	0.0268	<0.0001	0.2097	0.0283	<0.0001
Log(uE1c)[b]	**0.0326**	**0.0148**	**0.03**	**0.0327**	**0.0154**	**0.04**
Luteal-phase index	−0.0336	0.0641	0.60	−0.0084	0.0673	0.90
Log(BMI)	0.3788	0.0268	<0.0001	0.1926	0.0283	<0.0001
Log(uFSH)[b]	−0.0040	0.0101	0.69	−0.0176	0.0104	0.09
Ovulatory disturbance[d]						
Ovulatory disturbance	−0.0069	0.0129	0.59	−0.0064	0.0136	0.63
Log(BMI)	0.3804	0.0233	<0.0001	0.2044	0.0248	<0.0001
Log(uE1c)[b]	**0.0289**	**0.0122**	**0.02**	**0.0318**	**0.0128**	**0.01**
Ovulatory disturbance	−0.0009	0.0136	0.94	0.0009	0.0143	0.94
Log(BMI)	0.3651	0.0238	<0.0001	0.1851	0.0254	<0.0001
Log(uFSH)[b]	**−0.0143**	**0.0077**	**0.06**	**−0.0178**	**0.0080**	**0.03**

Age is defined as a respondent's actual age minus the minimum age (43 yr) for all respondents. *Bold numbers* are statistically significant.
[a] The regression coefficient has been adjusted for age, BMI, urinary hormone concentrations, menstrual-cycle length, menopausal status, and race.
[b] Cumulative hormone concentration, calculated using the AUC measure.
[c] The ratio of luteal phase length to cycle length (for ovulatory cycles only).
[d] An ovulatory disturbance is defined as either a short luteal phase (<10 d) or an anovulatory cycle.

model. Lower $_{log}$uFSH AUC was associated with higher lumbar spine BMD, even when either ovulatory/anovulatory status or ovulatory disturbances were used as the measure of menstrual cycle attribute. There were no statistically significant associations between BMD levels and $_{log}$uLH and $_{log}$uPdG AUC, after adjustment for age, BMI, race, menstrual-cycle length, and menopausal status.

Discussion

This study evaluated the relationship between BMD and the markers of menopausal transition, *i.e.* changes in the endogenous hormone environment and menstrual cycle characteristics, in a multiethnic, community-based study of pre- and perimenopausal women aged 43 and 53 yr. Our study demonstrated that before the final menstrual period, hip and spine BMD levels were significantly associated with the direct measures of endogenous hormones. Lower cumulative uE1c concentrations, as represented by the area under the hormone curve for an entire menstrual cycle, were consistently associated with lower BMD at both bone sites, whereas higher cumulative uFSH concentrations were associated with lower BMD at the lumbar spine. There was, however, no statistically significant association between total hip and lumbar spine BMD levels and cumulative uPdG exposure. Furthermore, these data showed no evidence of an association between presence/absence of ovulation, the luteal-phase index, or ovulatory disturbances and BMD at either bone site.

Prior postulated that progesterone has a tropic effect on bone (17). In particular, it was hypothesized that healthy, normal-weight, premenopausal women with decreased ovarian progesterone production associated with anovulatory cycles or short luteal phases would have significantly lower lumbar spine BMD, relative to women without these characteristics. De Souza *et al.* (12) tested this hypothesis among 33 eumenorrheic menstruating women with comparable estrogen status but varying progesterone status and determined that BMD levels of the total body, lumbar spine (L2-L4), and right proximal femur were not correlated with progesterone production. Likewise, our results do not demonstrate that progesterone has an independent trophic effect on bone mass: variation in BMD was not associated with markers of progesterone exposure (*i.e.* the luteal phase index and uPdG AUC). Instead, we found that cumulative uE1c and uFSH levels were positively associated with BMD levels, even after controlling for various measures of menstrual cycle attributes.

Prevalence of ovulatory disturbances, characterized by the presence of anovulatory cycles or a shortened luteal phase (<10 d), increased during perimenopause (17). These patterns were originally described more than 50 yr ago by Collett *et al.* (18) using nonquantitative basal temperature methods to document the day of ovulation in 302 cycles from 146 women aged 17–50 yr. They found that 15.1% of cycles were anovulatory in women 40–50 yr of age, compared with 2% among women 24–35 yr of age. A more recent prospective study of 160 Swedish women who were 61–72 months before the onset of menopause reported that 62% of cycles were ovulatory, whereas only 4.8% cycles were ovulatory among women who were 0–6 months before their last menstrual period (19). Our data were consistent with these findings: among women classified as perimenopausal, 22% of menstrual cycles were anovulatory, a significantly higher ($P = 0.0003$) fraction than among premenopausal women (9%). Yet there was no significant difference between these two groups in the prevalence of shortened luteal phase.

Published reports relating BMD to changes in menstrual cycle characteristics have frequently been inconsistent; they have used samples that are too small to detect important differences and/or too select to gauge accurately the general effects of changes in ovarian function on BMD. For example, a 1-yr prospective study of 66 healthy, premenopausal women reported that women who experienced one or more anovulatory cycles, or more than one short luteal phase per year, exhibited significant decreases in spinal bone density, as measured by quantitative computed tomography (10). Yet several subsequent studies found no significant association between bone mass at the lumbar spine and luteal phase abnormalities in small (less than 54 women) samples of premenopausal women (4, 11, 12).

The current study had the advantage of data from a multiethnic sample of 643 pre- and perimenopausal women without selection for clinical syndromes or pathology. As a result, the relationship between measures of menstrual cycle characteristics and BMD could be characterized more precisely than was the case with earlier studies, many of which lacked the sampling power or diversity required to gauge accurately the effects of changes in menstrual cycle attributes on BMD. Furthermore, the current study addressed the existence of disparities in BMD across race/ethnic groups (16), which the earlier studies did not evaluate. We found no statistically significant difference in the associations between BMD and menstrual cycle attributes by race/ethnicity.

A limitation of the current study is that the data were available for only a single menstrual cycle, and the implementation of enrollment criteria may have disproportionately selected women with the most normal cycles. In addition, this study assumes that the relationships between two BMD measures that comprise a small portion of the skeleton (hip and spine) and urinary hormone metabolites measured across a single menstrual period afford a set of meaningful proxies for the cumulative cellular osteoclast and osteoblast responses to hormone concentrations and receptor activity on those cells. This assumption enables the reader to appreciate the importance of type I error, *i.e.* statistically significant associations that could be observed by chance alone. Another relevant consideration is that the cross-sectional analyses presented in this study cannot conclusively establish the causal association between age-related changes in endogenous hormone levels and changes in BMD. Nonetheless, these analyses provide important baseline results for the further research, using longitudinal data, that would be necessary to evaluate whether or not changes in ovulatory function result in declining BMD.

In conclusion, the largest cross-sectional study to date of the relationship between changes in menstrual cycle attributes and BMD in the early menopausal transition afforded no evidence that menstrual cycle characteristics were associated with BMD levels, independent of measures of

hormone status. Instead, changes in the underlying hormone environment before the final menstrual period, reflected by lower urinary uE1c and higher uFSH concentrations, were significantly associated with lower levels of BMD.

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