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Concentrations of Follicle-Stimulating Hormone Correlate with Alkaline Phosphatase and a Marker for Vitamin K Status in the Perimenopause

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ABSTRACT

Serum alkaline phosphatase (ALP), a gross marker of bone turnover, has been reported to be elevated after menopause, a period characterized by hallmark increases in follicle-stimulating hormone (FSH). Whether the ALP rise coincides with the perimenopausal transition when changes in FSH, estrogen levels, and menstrual cycles are first apparent is not known. The purpose of this cross-sectional study was twofold: (1) to characterize the influence of the perimenopausal transition on ALP activity and (2) to correlate ALP activity with more precise markers for bone, osteocalcin (OC), and vitamin K status assessed with undercarboxylated osteocalcin (ucOC). Thirty-eight studies of hourly FSH were conducted on cycle day 6 of the follicular phase in perimenopausal women volunteers, aged 40–54 years (mean body mass index [BMI] = 24.2 ± 0.5). Mean FSH was used to define the perimenopausal stage (early perimenopausal, mean FSH ≤ 15 IU/L, n = 27; late perimenopausal, mean FSH > 15 IU/L, n = 11). As expected, late perimenopausal women had irregular and longer menstrual cycles, lower estradiol (E_2) and estrone (E_1) levels, and a lower frequency of ovulations vs. the early group. ALP was higher (76.5 ± 8.3 vs. 58.3 ± 2.7 IU/L, p = 0.045) compared with the early perimenopausal group. In a subsample (n = 10), OC was associated with ALP (r = 0.69, $p < 0.03$), FSH was positively related to ucOC concentrations (r = 0.7, $p < 0.03$), and women with E_1 concentrations <40 pg/ml had double the percentage of ucOC compared with those where E_1 was >40 pg/ml (46.3% ± 6.6% vs. 22.0% ± 3.1%, $p < 0.006$). Clinical markers of the perimenopause are associated with a nonspecific but inexpensive marker of enhanced bone turnover (i.e., higher ALP) and correlate well with more precise markers of bone activity. These findings suggest that health-promotion strategies for preserving bone should be instituted well before the last menstrual period.

INTRODUCTION

THE RAPID BONE LOSS after menopause brought on by the dramatic decline in estradiol (E_2) has become one of the major reasons for treatment of postmenopausal women with hormone replacement therapy (HRT).[1,2] Little is known, however, about the nature and time course of the menopause-mediated bone loss or whether preemptive signs may exist in women approaching their last menstrual cycles.

The years before menopause (perimenopause transition) have only recently been studied but are now considered to be a time of dramatic fluc-

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tuations in both gonadotropins and ovarian steroids because of the diminished influence of the ovarian follicular reserve and, thus, loss of tight negative feedback, which is responsible for the regular cyclicity of the menstrual cycle.[3,4] In response to rising follicle-stimulating hormone (FSH) levels, the ovary intensifies its production of estrogen despite a decline in the number of follicles, and episodes of hyperestrogenism have been reported in the perimenopause.[4]

A number of bone biomarkers in both blood and urine have been used to assess bone turnover in the menopause, with varying levels of precision.[5–7] Although state-of-the-art assays have been developed that identify the isoenzyme bone-specific (ALP), these tests are expensive and seldom are included in a routine medical examination. Healthy women are more likely to have a measure of serum ALP (a less specific marker of osteoblast activity). A rise in serum ALP has been associated with decreased bone mineral density in postmenopausal women (mean age 59.1 $\pm$ 0.3 years).[8]

Only a few studies to date have examined the relationship between markers of early reproductive aging (i.e., prior to menopause) and bone function. Slemenda et al.[9] reported significant bone loss in irregularly cycling perimenopausal women in a longitudinal, 3-year study. However, the significance of these findings is not clear because data from the perimenopausal subjects were combined with data from postmenopausal women to predict rate of bone loss in order to limit the impact of occasional high estrogen measurements and low bone loss rates.

In a cross-sectional study of bone-related variables in women at different stages of the perimenopausal transition, a marked elevation of ALP was present only in subjects with FSH values >40 IU/L.[10] Perimenopausal women with FSH values between 20 and 40 IU/L showed essentially similar concentrations to premenopausal subjects. In this study, single measures of FSH were obtained at random on the day of the clinic visit and not drawn at an equivalent phase in the menstrual cycle, thus raising questions about the reliability of the gonadotropin characterization. Moreover, retrospective recall in this and other studies has been the only method used to assess cycle length. Thus, the current understanding of how the dynamic hormone changes during the perimenopause relate to bone turnover remains obscure.

To clarify the relationship between reproductive aging prior to menopause and bone loss, we undertook a cross-sectional study of selected bone biomarkers in perimenopausal women using an intensive blood-sampling protocol to more precisely define reproductive hormones during the early follicular phase and prospective charting to monitor menstrual cycle length. We hypothesized that the measurement of serum ALP, an inexpensive and commonly obtained laboratory test in clinical practice, may have some utility for monitoring perimenopausal bone activity.

Osteocalcin (OC), a bone protein secreted by osteoblasts, is a new specific biomarker of bone turnover.[11] The level of OC that would indicate bone turnover in the postmenopause is not well defined, as osteoporotic women have values that extend throughout and just beyond the normal range.[12] Moreover, lack of a standardized commercial assay complicates comparison of concentrations among studies.[13]

Undercarboxylated osteocalcin (ucOC) is a precise indicator of vitamin K status. It reflects vitamin K's role in carboxylating glutamic acid residues on the protein that subsequently facilitates the binding of OC to hydroxyapatite in bone.[14–16] Both OC and ucOC can be measured in the same assay.

Until now, OC assays were designed to monitor supraphysiological concentrations in disease states. In addition, older assays were limited by their inability to distinguish the intact molecule from fragments.[17] Recently, a new assay specific for the intact protein has been developed. As a secondary aim of this study, we set out to establish the feasibility of using a modified assay specific for the intact OC protein to measure physiological concentrations present in healthy women. The undercarboxylated form was also examined for changes during the climacteric years.

MATERIALS AND METHODS

Before initiation of the study, approval of the clinical protocol was obtained from the University of Michigan Hospitals Institutional Review Board (IRB) for use with human subjects. All volunteers provided written informed consent. This study was undertaken as part of a larger study of the effects of HRT on the neuroreproductive axis of perimenopausal women (NIH grant NUR01 01373, N. Reame, P.I.).

Subjects

At the time of recruitment, 37 subjects met the following eligibility criteria: aged 40 or older, intact uterus and ovaries, a screening body mass index (BMI) of 20–30 kg/m^2, a spontaneous menstrual cycle within the preceding 6 months, a history of normal menstrual cycles and at least one pregnancy carried to term, normal endocrine screen, no current medical or psychiatric illness, no current use of oral contraceptives, no pregnancy or breastfeeding in the past 6 months, and no current history of dieting, excessive exercise, or alcohol consumption. One normal cycling subject was studied again 38 months later when she was experiencing irregular cycles, yielding a total of 38 studies. For the substudy of OC and ucOC, values from 1 subject (postmenopausal) whose last menstrual cycle was 18 months prior to the study was included for comparison with 9 perimenopausal subjects.

Study protocol

Subjects were admitted between cycle days 4 and 8 to the outpatient division of the General Clinical Research Center of the University of Michigan Hospitals for an 8-hour intensive blood-sampling protocol (0900–1700 hours). To assess general health status, subjects underwent a psychosocial and medical health interview. They also kept a daily diary of menstrual symptoms/bleeding and medication usage for up to 45 days. In all subjects, the results of screening studies for hemoglobin, hematocrit, liver function, insulin, glucose, and serum cholesterol were in the normal range for healthy volunteers after an overnight fast.

During the 8-hour study, blood samples were obtained through an indwelling intravenous (i.v.) forearm catheter every 10 minutes to assess pulsatile luteinizing hormone (LH) secretion, hourly for FSH measures, and every 4 hours for E_2 and progesterone (P) determinations. A fasting sample of blood was drawn at 0900 hours (just prior to the 8-hour sampling regimen) for determination of serum ALP, OC, ucOC, prolactin (PRL), total testosterone (T), free T, estrone (E_1), dehydroepiandrosterone sulfate (DHEAS), serum calcium, phosphate, albumin, thyroid-stimulating hormone (TSH), thyroxine (T_4), total triiodothyronine (T_3) and liver enzymes, alanine aminotransferase (ALT), and aspartate aminotransferase (AST). Three single blood draws were collected at 1-week intervals following the follicular phase study day for measurement of E_2 and P to determine the ovulatory status of the study cycle.

Assays

Reproductive Hormone Assays. Plasma E_2 (assay sensitivity 5 pg/ml) and P (assay sensitivity 0.2 ng/ml) were measured using radioimmunoassay (RIA) kits (Diagnostic Products Corp., Los Angeles, CA, and Radioassay Systems Laboratories, Carson, CA). All samples for a subject were measured in the same assay. Intraassay and interassay coefficient of variation (CV) for E_2 was 5.1% and 9%, respectively. Intraassay and interassay CV for P was 2.3% and 9.2%, respectively. Plasma LH and FSH were determined by Delfia® fluorometric immunoassay (IFMA) (Wallac Inc., Gaithersburg, MD). All samples were measured in the same assay. Gonadotropin concentrations are reported as international units per liter (IU/L). The LH assay standard is calibrated against the World Health Organization (WHO) Second International Standard for pituitary LH for immunoassay 80/552. FSH is calibrated against the WHO Second International Reference Preparation of human pituitary FSH/LH for bioassay 78/549. For LH and FSH assays, the limit of detection was 0.05 IU/L, and the interassay CV was 6.8% and 4.8%, respectively. For analysis, values below assay sensitivity were assigned the value of assay sensitivity.

ALP Assay. Fasting serum ALP was analyzed by the clinical ligand laboratory of the University of Michigan Hospitals, using the dry slide method, and read by a Vitros 900 Auto Analyzer (model 950)(Johnson & Johnson, Rochester, NY). The assay sensitivity is 20 IU/L, and the CV is 4.5%.

OC and ucOC Assay. OC was measured using a modification of a previously validated immunoradiometric assay (N-tact® Osteo SP) (Incstar, Stillwater, MN) that measures the intact protein.[13] Plasma OC and ucOC were measured in a subset of subjects ($n = 10$, the last 10 subjects studied). OC was measured in untreated plasma. For measurement of ucOC, 400 μl plasma sample was mixed with 8 mg calcium phosphate tribasic (Mallinkrodt, Paris, KY) in micro-Eppendorf tubes, mixing end-over-end for 30 minutes at 4°C and centrifuging at 700*g* for 20 minutes at 4°C

(protocol modified from J. Suttie, University of Wisconsin-Madison). The ucOC was measured in the supernatant. This treatment allows separation of carboxylated and undercarboxylated species.

The assay modification involved extension of the standard curve from 1.5 ng/ml down to 0.20 ng/ml. All samples were run in two assays, with OC and ucOC for a given individual run in the same assay in duplicate. Kits shipped from the company within 5 days of iodination were used for the two assays. Sample volume size used in the assay was 100 μl per tube. Quality control (QC) checks included a perimenopausal plasma sample and a low control (human osteocalcin) provided with the kit, both treated with calcium phosphate tribasic (as described). The intraassay CV for the perimenopausal QC (average 0.72 ng/ml, $n = 2$) was 2.7%. The interassay CV for the low control QC (average 0.775 ng/ml, $n = 2$) was 1.3%.

Assay sensitivity (2 standard deviations [SD] of buffer control) for the two assays analyzed was 0.09 ng/ml. Plasma samples from a perimenopausal and a postmenopausal volunteer were diluted parallel to the standard curve in this modified version (slopes: standard 1.09 $\pm$ 0.018; perimenopausal 1.11 $\pm$ 0.03; postmenopausal 1.11 $\pm$ 0.017). Recovery of serial dilutions of kit standard and perimenopausal and postmeno-pausal samples averaged 74%–119%. Intraassay and interassay CV averaged 6.8% and 9.1%, respectively.

Data analysis

Definition of Study Groups. To examine the effects of reproductive aging, subjects were initially grouped as premenopausal (regular menstrual cycles, no menopausal complaints, FSH $\leq$ 15 IU/L, $n = 14$), or perimenopausal (symptomatic women with irregular cycles). This latter group was further divided into an early ($n = 13$) and late ($n = 11$) perimenopausal group according to an FSH value of 15 IU/L. This value has been used to define study groups in other studies of perimenopausal bone loss and is associated with low pregnancy rates in infertile patients.[18] Because the normally cycling and early perimenopausal groups did not differ in BMI (23.4 $\pm$ 0.5 vs. 24.5 $\pm$ 0.7 kg/m^2, respectively, p = NS) or FSH (10.1 $\pm$ 0.8 vs. 8.5 $\pm$ 1.2 IU/L, respectively, p = NS), E_2 (47.9 $\pm$ 7.3 vs. 95.8 $\pm$ 20.5 pg/ml, respectively, p = NS), and ALP (59.7 $\pm$ 2.9 vs. 56.8 $\pm$ 4.8 IU/L, respectively, p = NS) concentrations, they were combined for all further analyses ($n = 27$) and are referred to as the early perimenopausal group.

Statistical Analysis. Physiological data are presented as mean $\pm$ SE. Mean values of FSH, LH, E_2, and P were determined from the serial values obtained across the 8-hour daytime sampling interval during the follicular phase study. To adjust for differential binding of calcium by varying albumin levels, each subject's serum total calcium/albumin ratio was proportionally corrected to a standard albumin level of 4.2 g/dl, using the method of Garton et al.[19] The percent of undercarboxylated osteocalcin (% ucOC) is the calculated value:

$$\%\text{ucOC} = \frac{\text{ucOC}}{\text{Total OC}} \times 100$$

The distribution of physiological values was explored for normality. Where appropriate data transformation was used for skewed distributions (e.g., logarithmic transformation for sex steroid data, reciprocal transformation for ALP), transformation did not change the outcome of the results, and the transformation data are not reported. Correlation analysis was performed using Pearson r test, and comparison between groups was made using Fisher's exact test (for frequencies), independent sample t test, or Mann Whitney U test. In two separate subanalyses on % ucOC and estrogen status, subject data were grouped by a cutoff of 20 pg/ml for E_2 and 40 pg/ml for E_1, based on reports of bone loss at these concentrations.[9] p values were derived using the two-tailed tests conducted at the 0.05 level.

RESULTS

Clinical characteristics

At the time of the study, 31 of the 37 subjects (84%) had experienced their most recent menstrual period within the past 3 months. In 5 others (13%), the last menstrual period was within the past 5 months, and in 1 subject (3%) the last menses was 9 months earlier. Of the 38 menstrual cycles assessed, 25 (66%) were ovulatory, as determined by a midluteal P level $\geq$4 ng/ml.

Table 1 lists the clinical characteristics of the two groups of women, early perimenopausal (FSH $\leq$ 15 IU/L) and late perimenopausal (FSH $>$ 15 IU/L) during the follicular phase study. There were no significant differences between groups for age or body size characteristics. As expected, the late perimenopausal group had higher mean gonadotropin concentrations ($p < 0.001$), lower plasma E_2 levels ($p = 0.006$), and lower E_1 levels ($p < 0.05$).

FSH concentration had a significant effect on mean cycle length ($p < 0.01$); the positive correlation was significant ($r = 0.66$, $p < 0.001$). Early perimenopausal women (FSH range 4–15 IU/L) had cycle lengths ranging from 15 to 63 days. In contrast, menstrual bleeding intervals ranged from 27 to 161 days in the late perimenopausal group (FSH range 20–98 IU/L). Anovulatory cycles were present in 73% of the late perimenopausal women, a higher rate than observed in the early perimenopausal group (28%, $p < 0.003$).

No group differences were observed for PRL or DHEAS concentrations or in thyroid function or liver enzyme activity (ALT, AST). The expected effects of the perimenopause on T concentrations were observed. Serum phosphate did not differ between groups, but serum calcium, when corrected for a serum albumin of 4.2 g/dl, was significantly lower in the late perimenopausal group (9.5 $\pm$ 0.1 vs. 9.8 $\pm$ 0.1 mg/dl, $p = 0.03$).

Reproductive aging and serum markers of bone health

All subjects had ALP concentrations in the normal range for this enzyme, 30–130 IU/L. In the late perimenopausal group, the concentration of ALP was higher than in the early perimenopausal group (76.5 $\pm$ 8.3 vs. 58.3 $\pm$ 2.7 IU/L, $p < 0.05$) (Fig. 1). Correlations between the measures of reproductive aging and bone metabolism are shown in Table 2. ALP concentrations were correlated with higher mean FSH ($p = 0.01$) and higher mean LH ($p < 0.001$) on day 6 $\pm$ 2 of the menstrual cycle. Additionally, there was a trend for higher ALP levels with longer cycle lengths ($p = 0.06$). An association between ALP and E_2 concentrations was not observed ($p = 0.40$). Serum calcium was positively associated with age ($p < 0.04$). The negative association between serum calcium and FSH was not significant ($p = 0.09$).

Table 1. Clinical Characteristics of Perimenopausal Women by Screening FSH

	Early perimenopause FSH $\leq$ 15 IU/L (n = 27)	*Late perimenopause FSH $\leq$ 15 IU/L (n = 11)*	p *value*
Age (years)	46.3 $\pm$ 0.8[a]	48.6 $\pm$ 0.9	0.1[b]
BMI (kg/m2)[c]	23.9 $\pm$ 0.4	24.7 $\pm$ 1.3	0.5
Menstrual cycle length (days)	29.6 $\pm$ 2.3	76.0 $\pm$ 15.3	0.0001[b]
FSH (IU/L)	9.3 $\pm$ 0.7	39.4 $\pm$ 6.6	<0.001
LH (IU/L)	5.8 $\pm$ 0.7	23.9 $\pm$ 4.7	<0.001
E_2 (pg/ml)	71.0 $\pm$ 11.4	27.0 $\pm$ 7.1	0.006
E_1 (pg/ml)	73.4 $\pm$ 9.7	48.4 $\pm$ 9.9	<0.05
P (ng/ml)	0.3 $\pm$ 0.08	0.2 $\pm$ 0.006	0.4
OV+	22/27 (82%)	3/11 (27%)	<0.003[d]
Prolactin (ng/ml)	8.3 $\pm$ 0.7	6.1 $\pm$ 0.8	0.06
DHEAS (ng/ml)	971.6 $\pm$ 94.1	977.0 $\pm$ 214.5	<1.0
Total T (ng/ml)	0.3 $\pm$ 0.02	0.2 $\pm$ 0.04	<0.02
Free T (pg/ml)	0.4 $\pm$ 0.08	0.9 $\pm$ 0.2	0.03
TSH (μU/ml)	2.1 $\pm$ 0.3	2.8 $\pm$ 0.4	<0.3
T_4 (μg/dl)	7.3 $\pm$ 0.3	6.5 $\pm$ 0.4	0.1
T_3 (ng/dl)	134.0 $\pm$ 4.6	121.0 $\pm$ 5.2	0.1
ALT (U/L)	26.0 $\pm$ 2.2	25.3 $\pm$ 3.0	<0.9
AST (U/L)	25.4 $\pm$ 1.6	24.5 $\pm$ 2.1	0.7

[a]Values are mean $\pm$ SE. *t* tests reported unless otherwise indicated.

[b]Mann Whitney U test.

[c]BMI, body mass index; FSH, follicle-stimulating hormone; LH, luteinizing hormone; E_2, estradiol; E_1, estrone; P, progesterone; OV+, midluteal progesterone rise of $\geq$4 ng/ml; DHEAS, dehydroepiandrosterone sulfate; T, testosterone; TSH, thyroid-stimulating hormone; T_4, thyroxine; T_3, triiodothyronine; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

[d]Fischers Exact Test

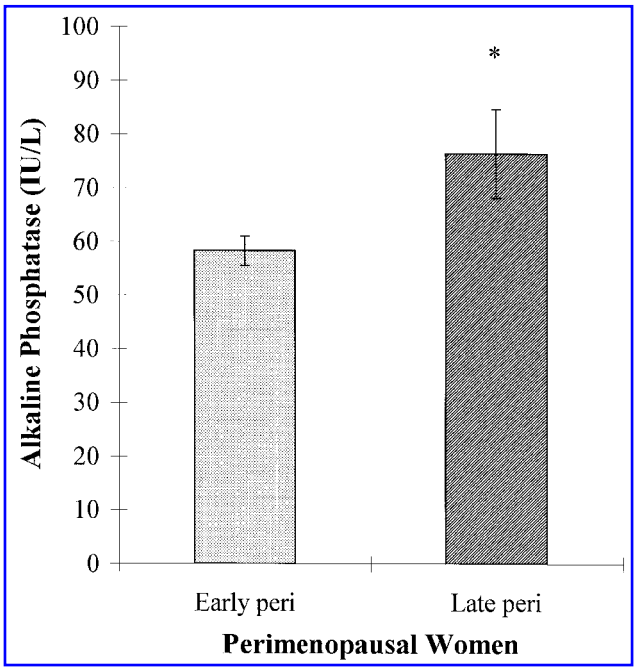

FIG. 1. Comparison of fasting serum ALP for early perimenopausal (peri) women with FSH ≤ 15 IU/L ($n = 27$) vs. late perimenopausal women with FSH > 15 IU/L ($n = 11$) in the early follicular phase (menstrual cycle days 4–8). Late perimenopausal women with FSH > 15 IU/L had significantly higher concentrations of serum ALP. *$t = 2.08$; $p < 0.05$.

In a subsample ($n = 10$), serum ALP was positively associated with OC ($r = 0.69$, $p < 0.03$) (Fig. 2) and showed a statistical trend with ucOC ($r = 0.59$, $p = 0.07$). FSH was positively related to ALP ($r = 0.78$, $p < 0.007$) and ucOC ($r = 0.7$, $p < 0.03$) and showed a trend with OC ($r = 0.56$, $p = 0.09$).

The % ucOC was higher in the group of women with E_1 concentrations <40 pg/ml compared with those with concentrations >40 pg/ml (46.3% ± 6.6% vs. 22.0% ± 3.1%, low E_1 vs. higher E_1, $p = 0.006$). Women with E_1 concentrations <40 pg/ml also had E_2 concentrations <20 pg/ml, representative of a low estrogen environment overall. A trend for higher % ucOC was found in the group of women with E_2 concentrations <20 pg/ml compared with those with concentrations >20 pg/ml (40.5% ± 7.7% vs. 22.8% ± 3.7%, low E_2 vs. higher E_2, $p = 0.07$).

DISCUSSION

The climacteric encompasses the complete span of reproductive aging, generally considered to begin with the decline in fertility after the age of 35. In a woman's early 40s there is an increase in mean FSH concentrations and LH pulsatility before any reduction in estrogen or P.[20] Episodes of hyperestrogenism may also occur secondary to the elevated FSH and accelerated follicular development.[4] Thereafter, there is a dramatic decline in the production of both E_2 and E_1 and notable declines in T and androstenedione.[21] The stage of the climacteric where bone turnover accelerates has not been conclusively established. Several investigators have suggested that accelerated bone turnover commences prior to the actual menopause, but methodological weaknesses have limited the significance of their findings.[9,10,19,22–24] These limitations include the use of single blood samples to assess sex steroid and gonadotropin concentrations, failure to synchronize the timing of blood draws in relation to the menstrual cycle, and the use of heterogeneous study samples poorly defined for menopausal status.

To better characterize the relationship between a gross marker of bone turnover (ALP) and a more precise indicator of bone activity (OC and ucOC) with reproductive aging, we used serial measures to assess sex steroids and gonadotropins in the early follicular phase of the menstrual cycle. In this way, women were classified into early or late perimenopausal groups based on the mean of 9 hourly FSH concentrations on cycle day 6. The use

TABLE 2. PEARSON CORRELATIONS FOR ALP OR CALCIUM WITH AGE AND MARKERS OF REPRODUCTIVE AGING: FSH, LH, CYCLE LENGTH, AND E_2 ($n = 38$)

	Age	*FSH*	*LH*	*Cycle length*	E_2
ALP	0.15	0.41 ($p = 0.01$)	0.56 ($p < 0.001$)	0.31 ($p = 0.06$)	−0.14
Calcium[a]	0.34 ($p < 0.04$)	−0.28 ($p = 0.09$)	−0.08	−0.14	0.22

[a]Corrected for serum albumin of 4.2 g/dl.

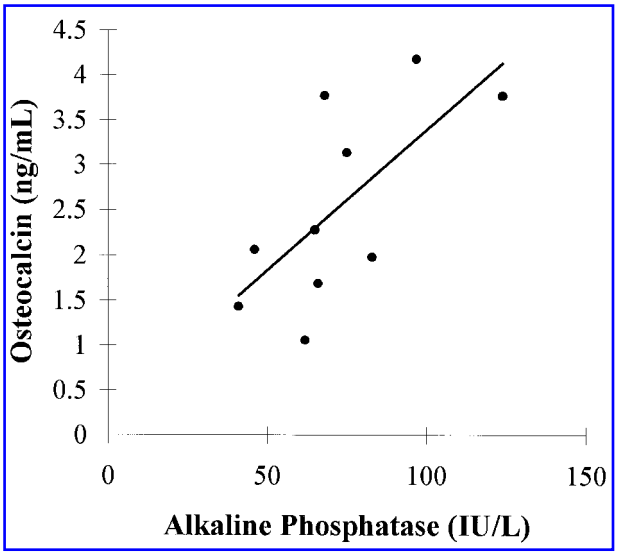

FIG. 2. Relationship between ALP and OC concentrations at 0900 hours ($r = 0.69$, $p < 0.03$, $n = 10$). Menstrual cycle days 4–8 for the perimenopausal women and random day for the postmenopausal woman with 18 months of amenorrhea.

of frequent blood sampling to detect dynamic FSH secretion permits a more precise determination of the true perimenopausal state. With this intensive sampling technique, we were able to detect differences in ALP concentrations associated with an FSH value >15 IU/L. This level of FSH has been shown to be indicative of early reproductive aging and is much lower than that observed in prior studies of menopausal effects on bone turnover when single estimates of FSH were obtained.[9,10,23]

The higher concentrations of ALP in our late perimenopausal group would seem to be unrelated to chronological age, given the similar ages of the two study groups. Moreover, in the early perimenopausal group, the normally cycling women were significantly younger (mean age 43.2 ± 0.7 vs. 49.5 ± 0.8 years, $p < 0.001$) than those with irregular cycles, but ALP levels were similar. Thus, there appears to be no age-related influence on ALP function during the midlife years.

To what extent the observed elevation in ALP in women with an FSH value >15 IU/L is associated with clinical manifestations of bone loss is not known, as bone density measures were not obtained in this study. Future studies should incorporate a longitudinal design across the perimenopausal transition to assess bone biomarkers and their relation to bone density.

Previous reports have been inconsistent about the relationship between bone turnover markers or bone density and E_2. Some have reported a negative relationship between bone biomarkers (OC) and E_2,[9] whereas others have found no relationship between bone density and E_2.[24] Despite significantly lower levels of E_2 in the late perimenopausal group, we found no relationship between day 6 mean E_2 and ALP ($r = -0.14$, $p = 0.40$) when data from the entire sample were assessed. In this study, as in previous studies, E_2 was determined on a single day, which may have hampered our ability to determine the more chronic estrogen environment and the true relationship to ALP activity. Additionally, a single menstrual cycle differs in length from the average remodeling cycle of bone (approximately 4–8 months)[25,26] and, therefore, may not be capturing the total activity that gave rise to ALP concentrations at one time.

Using a new IRMA assay for intact protein (N-tact), we also demonstrated that OC is detectable, albeit at low concentrations, in healthy perimenopausal women. By this method, we were able to show for the first time that FSH is related to ucOC concentrations. The biochemical influence of the undercarboxylated species of OC is currently not known, but ucOC has been reported to predict the risk of hip fracture among elderly women.[27] Moreover, ucOC concentrations were higher in postmenopausal women than in cycling women in a study where menopause was defined by recall of last menstrual period.[14] Additionally, in this study, ucOC elevations occurred in concert with elevations in ALP, although not at conventional levels of significance. Thus, reproductive aging may be important in the phenomenon of elevated ucOC after menopause.

This study also reports a possible link between estrogen status and ucOC. Of particular interest is that E_1 (the predominant circulating estrogen after menopause) was a better discriminator than E_2 (the dominant estrogen before menopause) in determining the degree to which OC was undercarboxylated. In our very small sample of climacteric women ($n = 10$), those with E_1 concentrations <40 pg/ml had more than a twofold increase in % ucOC. The role that estrogen may have in the carboxylation of the bone protein is unknown and needs elucidation. Given the relatively higher free concentrations of E_1 vs. E_2 during the postmenopausal years,[28] the bioactive influence of E_1 on postmenopausal bone may be underestimated.[29]

CONCLUSIONS

The findings from this cross-sectional study performed in a group of healthy perimenopausal women with well-characterized gonadotropin and sex steroid levels confirm that signs of reproductive aging (elevated gonadotropins and prolonged cycles) are associated with elevated ALP concentrations and, in turn, with OC, a more specific bone marker. To confirm the clinical significance of this finding, future studies should include measures of bone density. Measurement of ALP in healthy perimenopausal women offers a nonspecific but inexpensive marker of bone loss that is commonly determined as part of the physical examination in midlife women. Furthermore, these data suggest that health-promotion strategies for preserving bone should be instituted well before the last menstrual period.

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