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Serum Soluble Interleukin-6 Receptor and Biochemical Markers of Bone Metabolism Show Significant Variations During the Menstrual Cycle

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ABSTRACT

We examined sequential changes of bone-resorbing cytokines and bone metabolic markers and the effect of ovarian hormones on bone metabolism during the menstrual cycle in 10 healthy Japanese women, aged 22–43 yr, with normal ovarian function. Serum soluble interleukin-6 receptor (sIL-6R) showed a significant variation; a rise during the early and late follicular periods followed by a fall during the early luteal period ($P = 0.0423$, $P = 0.0334$) and an increase during the mid and late luteal periods. There were significant changes in the levels of markers of bone formation: a rise in serum bone-specific alkaline phosphatase (ALP) during the mid and late follicular ($P = 0.0265$) periods and a fall in serum carboxyl-terminal propeptide of type I procollagen (PICP) during the midluteal period ($P = 0.0161$). As for the levels of bone resorption markers, urinary type I collagen C-telopeptide breakdown products (CTx) and free deoxypyridinoline (D-Pyr) decreased significantly during the early and midfollicular periods, urinary free D-Pyr and serum pyridinoline cross-linked carboxyl-terminal telopeptide of type I collagen (ICTP) ($P = 0.0440$) increased significantly during the early luteal period, and urinary CTx, free D-Pyr, and serum ICTP decreased significantly during the late luteal period ($P = 0.0170–0.0008$). The serum PTH level was significantly higher during the follicular than the luteal period ($P = 0.0132$). Serum sIL-6R significantly correlated with urinary CTx ($r = 0.190$, $P < 0.05$) and serum ALP ($r = 0.209$, $P < 0.05$) and serum estradiol with intact osteocalcin ($r = 0.309$, $P < 0.0005$) and serum ALP ($r = 0.181$, $P < 0.05$). These observations strongly suggest that cyclic variations in the levels of bone formation and resorption markers and of a bone-resorbing cytokine may be modulated by cyclic changes in serum steroid hormones during the menstrual period. In addition, the specific days of biochemical events in the menstrual cycle are crucial for evaluating osteoclastic and osteoblastic activities in pre- and perimenopausal women or in women starting GnRH agonist therapy. (*J Clin Endocrinol Metab* **83:** 326–332, 1998)

ESTROGEN withdrawal, whether caused by natural or by surgical menopause, results in bone loss by stimulating bone resorption and elevates the levels of biochemical markers for bone resorption and formation. Administration of estrogen to postmenopausal women prevents bone loss or increases bone mass, reducing the levels of biochemical indices for bone turnover. Several biochemical markers for bone metabolism, which are now considered to be more specific than those used previously, have been newly developed. Some of these markers showed reduced levels 2 weeks after the start of hormone replacement therapy in older women, indicating that alterations in estrogen levels cause variations in the concentrations of biochemical markers of bone metabolism relatively shortly after the start of treatment (1).

Recent studies on the role of estrogen in bone metabolism have shown the involvement of cytokines, such as interleukin (IL)-1 and IL-6. Pacifici *et al.* (2, 3) reported that estrogen withdrawal allows peripheral blood monocytes to secrete more IL-1 in postmenopausal women, and that estrogen replacement inhibits IL-1 secretion by monocytes in those women. Manolagas and his co-workers reported (4) that IL-6 is involved in the stimulation of bone resorption induced by estrogen deficiency. Tamura *et al.* (5) showed that IL-1 greatly stimulates the differentiation of osteoclast precursors into mature osteoclasts in the presence of soluble IL-6 receptors (sIL-6R) in cocultures of mouse bone marrow and primary osteoblastic cells. Because estrogen shows significant cyclic variation during the menstrual period, these findings have prompted us to investigate variations in the levels of cytokines during the menstrual period.

Bone loss because of suppression of ovarian estrogen production may occur in women administered GnRH agonists (6, 7). A simple means of identifying women with increased bone resorption associated with accelerated bone loss is clinically important in the management of those requiring GnRH agonists. Markers of bone turnover are now being studied to identify women who will lose bone before bone density measurements can detect such losses. The percent change in bone mineral density at L1-L4 after 6 months of GnRH agonist treatment correlated inversely with the percent change of cross-linked N-telopeptides of type I collagen from baseline to month 4 (8). Evaluating the baseline values of biochemical markers is crucial for detecting a percent change from baseline in those women who start GnRH agonists therapy.

When bone turnover in pre- and perimenopausal women

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is examined, the specific days of the menstrual cycle on which blood and/or urine specimens are collected are of clinical importance. The determinations made of sex steroids in the early follicular phase may not adequately represent sex steroid concentrations for premenopausal women throughout the menstrual cycle. Multiple blood or urine samples (or both) may be necessary to adequately characterize the associations between estrogens and skeletal health in premenopausal women (9). It is therefore worthwhile to assess alterations in metabolic markers of bone turnover during the menstrual cycle in healthy women with normal cycles.

Subjects and Methods

Subjects and preparation of samples

Ten healthy Japanese women ranging in age from 22–43 yr (mean, 33 ± 7 yr) with no history of menstrual cycle irregularity were investigated. None of the volunteers had had any chronic diseases or were taking any medication known to affect bone metabolism (*e.g.* renal disease; hyperparathyroidism; hyperthyroidism; diabetes mellitus; or corticosteroid, anticonvulsant, and oral contraceptive therapy). All subjects were ambulatory and engaged in their usual activities. All subjects gave informed consent before entering the study.

Blood samples were drawn on the first day of menstruation, followed by sampling three times a week (Monday, Wednesday, and Friday) until 4 days after the subsequent menstruation. All blood samples were collected in the fasting state between 0800–0900 h and were allowed to clot at room temperature for approximately 1 h. Thereafter, the serum was separated off by centrifugation and stored at −20 C. Urine samples were collected as second void at the same time and were also stored at −20 C.

Analytical methods for serum cytokines

Serum IL-6 levels (Amersham International, Buckinghamshire, UK) and serum sIL-6R levels (Quantikine, human sIL-6R immunoassay; R & D Systems, Minneapolis, MN) were measured using a sandwich enzyme-linked immunosorbent assay (ELISA).

Serum IL-1β values were determined using a solid-phase ELISA, which uses an antibody for IL-1β bound to a microtiter plate together with a biotinylated antibody to IL-1β and Amdex amplification reagent (Amdex N.S., Buckinghamshire, UK).

The minimum detectable doses of serum IL-6, sIL-6R, and IL-1β are 0.70 pg/mL, 140 pg/mL, and 0.1 pg/mL, respectively. The intra- and interassay coefficients of variation (CV) were 2.1–4.3% and 3.8–6.3% for serum IL-6, 2.3–8.6% and 4.2–6.4% for serum sIL-6R, and <10% and <10% for serum IL-1β, respectively.

Makers of bone turnover

Bone formation. A serum bone-specific alkaline phosphatase (ALP) assay (Alkphase-B; Metra Biosystems, Mountain View, CA) was performed using a microplate coated with an anti-bone-specific ALP monoclonal antibody. The bound ALP activity was measured by using *p*-nitrophenyl phosphate as a substrate (10). The minimum detectable level of this assay is 2 U/L, and the intra- and interassay CVs were 2.2–3.8% and 2.1–3.8%, respectively.

Serum concentrations of carboxy-terminal propeptide of type I procollagen (PICP) were measured using an equilibrium RIA (11) whose lowest detectable concentration is 25 ng/mL. The intra- and interassay CVs were 1.7–6.7% and 1.3–5.1%, respectively.

Serum intact osteocalcin (OC) was measured using a sandwich enzyme immunoassay (EIA) that employs polyclonal antibodies against N-terminal 20 residues (amino acids 1–20) and against C-terminal 7 residues (amino acids 43–49) (12). The sensitivity of the EIA is 1.0 ng/mL and the intra- and interassay CVs were 2.5–5.4% and 4.7–5.7%, respectively.

Serum N-mid OC levels were measured based on a two-site immunoradiometric assay (IRMA) for human OC using human OC as a standard and two monoclonal antibodies raised against human OC, a solid-phase anti-25–37 region, and a tracer anti-5–13 sequence of the molecule (13). The minimum detectable concentration of IRMA is 0.4 ng/mL, and the intra- and interassay CVs were 1.7–5.2% and 2.3–6.8%, respectively.

Bone resorption. Urinary type I collagen C-telopeptide breakdown products (CTx) were measured by an ELISA (Cross Laps ELISA, Osteometer Biotech A/S, Herlev, Denmark) based on an immobilized synthetic peptide with an amino acid sequence specific for a part of the C-telopeptide of the α1-chain of type I collagen (14, 15). The sensitivity of the method is 50 μg/L, and the intra- and interassay CVs were 1.0–3.7% and 2.6–4.5%, respectively.

Urinary free deoxypyridinoline (D-Pyr) was measured by an ELISA that uses a monoclonal antibody having less than 1% cross-reactivity with free Pyr (Pyrilinks-D, Metra Biosystems) and no significant interaction with cross-linked peptides (16). The urinary excretion of bone resorption markers was corrected using the urinary creatinine concentration measured by a standard colorimetric method. This technique's lowest detectable concentration is 3 nM, and the intra- and interassay CVs were 3.6–9.5% and 6.3–10.3%, respectively.

Serum concentrations of pyridinoline cross-linked carboxy-terminal telopeptide of type I collagen (ICTP) were measured with an equilibrium RIA (17). The sensitivity is 1.0 ng/mL, and the intra- and interassay CVs were 4.8–6.9% and 3.9–7.7%, respectively.

Other biochemical indices

Serum ALP (U/L) was measured spectrophotometrically using *p*-nitrophenyl phosphate as the substrate, according to the method recommended by the German Society of Clinical Chemistry. Serum PTH (ng/mL) was determined by RIA using intact PTH as the standard (18). Serum PTH-related protein (PTHrP) was measured using a two-site IRMA (Nichols Institute Diagnostics, San Juan Capistrano, CA) (19). The minimum detectable levels of serum PTH and PTHrP were 100 ng/mL and 0.2 pmol/L, respectively. The intra- and interassay CVs were 3.7–7.2% and 2.6–9.3% for PTH and 3.5–9.5% and 2.7–4.5% for PTHrP, respectively. Serum FSH, LH, estradiol (E_2), and progesterone (P_4), and the serum levels of calcium and inorganic phosphorus were analyzed according to standard laboratory methods. Serum calcium value was corrected for serum albumin using the following equation: corrected calcium = calcium + [40-albumin (g/L)] 0.02 mmol/L.

All samples from an individual woman were analyzed in the same assay.

Statistical analysis

Each cycle was divided into a follicular period (FP) and a luteal period (LP) by the serum LH peak. FP and LP were normalized by lengths to eliminate interindividual variations in cycle and phase lengths as described in the literature (20). FP was further divided into early (days −14 to −12), mid (days −10 to −6), and late (days −4 to −2), and LP into early (days 2–4), mid (days 6–10), and late (days 12–14), respectively. Values from each subject were expressed as the percent changes from values at the LH peak. The significance of the differences among cytokines and indices of bone metabolism for different menstrual periods (early FP *vs.* early LP, etc.) was assessed using Student's *t* test for paired data by means of Statistical Package (SAS Institute, Cary, NC). Regression analysis was used in determining the relationship of ovarian steroids, sIL-6R, and bone metabolic markers during the menstrual cycle. Except where explicitly indicated, mean values are expressed as mean ± SD.

Results

Values at LH peak

The mean cycle length was 28.5 ± 1.9 days (range, 26–31 days). The FP consisted of 15.4 ± 1.9 days (range, 13–18 days), and the LP of 13.1 ± 0.6 days (range, 12–14 days). All investigated cycles were ovulatory, as judged from the rise in serum P_4 values during LP. Values at the LH peak are given in Table 1.

TABLE 1. Concentrations of serum sIL-6R and bone metabolic markers at LH peak

	sIL-6R (ng/mL)	B-ALP (U/L)	PICP (ng/mL)	Intact OC (ng/mL)	N-mid OC (ng/mL)	CTx (μg/mMCr)	D-Pyr (nM/mMCr)	ICTP (ng/mL)	ALP (U/L)	PTH (ng/mL)	PTHrP (pM/L)	Ca (mEq/L)	P (mg/dL)	E_2 (pg/mL)	P_4 (ng/mL)	FSH (U/L)	LH (U/L)
Mean	22822	10.2	113.3	4.8	10.1	246.5	5.54	5.2	56.0	474.5	32.0	7.6	107.2	261	0.9	13.4	32.9
SD	5840	2.4	30.8	3.0	2.3	68.0	1.47	6.0	18.0	153.9	5.0	2.0	32.5	82	0.4	4.5	13.4

Analysis of variables

Gonadotropins and ovarian sex steroids. Serum LH showed its well-known midcyclic peak ($P < 0.0001$), and serum FSH varied significantly ($P = 0.0329 \sim P < 0.0001$) showing a midcyclic rise ($P < 0.0001$). Serum E_2 showed an increase during the mid FP to peak values at ovulation ($P = 0.0053 \sim P < 0.0001$), a rapid decrease after ovulation ($P = 0.0002$), and a rise during the mid LP. Serum P_4 showed an increase after ovulation ($P < 0.0001$) with a midluteal maximum ($P < 0.0001$).

Serum cytokines. Serum sIL-6R showed a rise in the early FP, followed by a fall in the mid FP and an increase in the late FP. Following a fall in the early LP, serum sIL-6R rose, showing an overall increase during the LP (Fig. 1A). There were significant differences in serum sIL-6R between both the early FP ($P = 0.0423$) and the mid FP ($P = 0.0334$) and the early LP. Serum concentrations of IL-6 and IL-1β were below detectable levels.

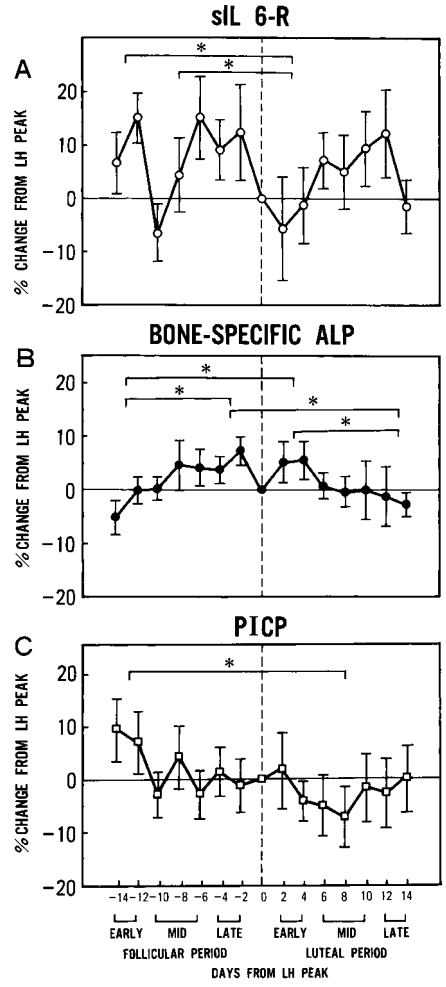

FIG. 1. Mean curves (±SE) for serum levels of sIL-6R (A, ○-○), bone-specific ALP (B, ●-●), and carboxy-terminal propeptide of type I procollagen (C, □-□) during menstrual cycle. Each cycle was divided into FP and LP by LH peak. Values are expressed as percent changes from values at LH peak. *, $P < 0.05$.

Bone formation markers. Serum bone-specific ALP showed a fall in the early FP and rises in the midfollicular, late follicular, and early luteal periods; remained unchanged during the mid LP; and showed a decrease in the late LP (Fig. 1B). The differences in serum bone-specific ALP values were significant between the early and late FPs ($P = 0.0265$), between the early FP and the early LP ($P = 0.0272$), and between both the late FP ($P = 0.0364$) and the early LP ($P = 0.0373$) and the late LP.

Serum PICP increased in the early FP, fluctuated during mid and late FPs, and decreased during the LP (Fig. 1C). There was a significant difference in PICP levels between the early FP and the mid LP ($P = 0.0161$).

Serum intact OC showed a rise during the late FP and a fall during the mid and late LPs. Serum N-mid OC showed a rise in the mid FP and a fall in the late FP and mid LP. The variations in serum intact OC and N-mid OC, however, did not show any significant differences (data not shown).

Bone resorption markers. Urinary CTx showed a fall during the early and mid FPs, a rise in the late FP, and a fall throughout the LP (Fig. 2A). The differences in urinary CTx were significant between the early and late FPs ($P = 0.0086$), and between the late FP and early LP ($P = 0.0170$), mid LP ($P = 0.0008$) or late LP ($P = 0.0143$).

Urinary free D-Pyr showed the same pattern as was observed in urinary CTx, except during the early and mid LPs, in which free D-Pyr showed a fluctuation (Fig. 2B). There was a significant difference between the early FP and the early LP ($P = 0.0347$).

Serum ICTP decreased in the late FP and increased in the early and mid LPs (Fig. 2C). The difference in serum ICTP was significant between the late FP and the early LP ($P = 0.0440$).

Other biochemical indices

Serum PTH showed an increase during the FP and a decrease during the LP (Fig. 3A). There was a significant difference in serum PTH between the late FP and mid LP ($P = 0.0128$). Serum PTHrP showed a fall in the early and mid FPs and in the late LP, fluctuation in the late FP and the mid LP, and a rise in the early LP (Fig. 3B). The difference in serum PTHrP was significant between the mid FP and the early LP ($P = 0.0375$).

Serum ALP levels varied little throughout the menstrual cycle. Serum calcium showed an increase in the early and mid FPs and a fall thereafter, and serum inorganic phosphorus showed a fall during the early FP and the early and mid LPs. We could not find any significant differences in the variations of serum ALP, calcium, and inorganic phosphorus during the menstrual cycles (data not shown).

Relation between variables

The correlations between steroid hormones, sIL-6R, and bone metabolic markers during the menstrual cycle are sum-

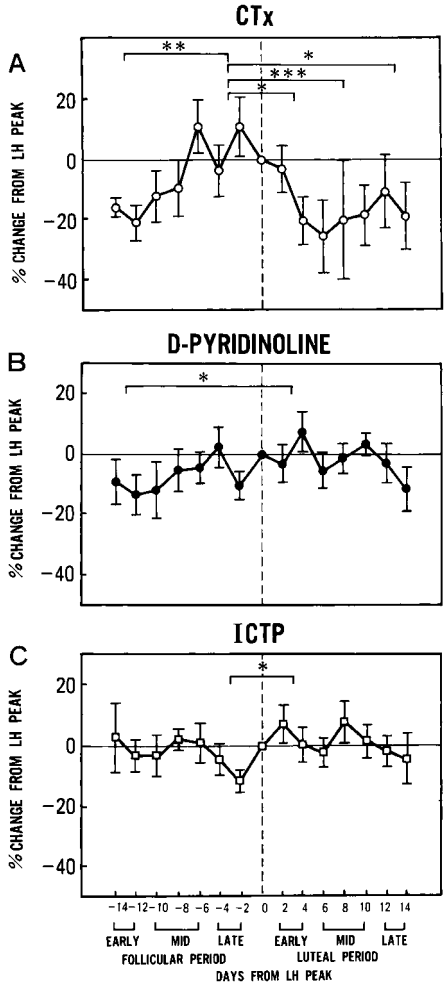

FIG. 2. Mean curves (±SE) for urinary levels of CTx (A, ○-○), D-Pyr (B, ●-●), and serum levels of ICTP (C, □-□) during menstrual cycle (see Fig. 1). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

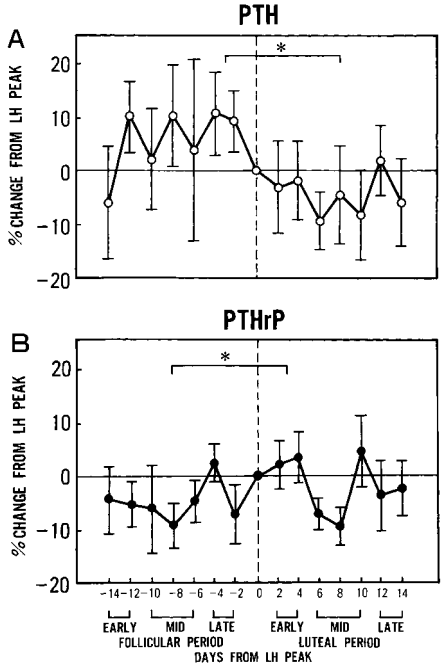

FIG. 3. Mean curves (±SE) for serum levels of PTH (A, ○-○) and PTHrP (B, ●-●) during menstrual cycle (see Fig. 1). *, $P < 0.05$.

marized in Table 2. Peaks of serum sIL-6R and bone metabolic marker levels are summarized in Table 3. We then calculated the correlations in the percent changes from values at the LH peak between E_2 and serum sIL-6R or the other biochemical indices when the intervals between the peaks of each of the markers and that of E_2 were eliminated theoretically. Serum E_2 significantly correlated with serum PTH ($r = 0.252$, $P = 0.0164$) when PTH preceded 8 days before E_2 peak (Table 3).

Discussion

The serum sIL-6R level has been shown to be significantly reduced after estrogen therapy at 6 months, but no significant change in the serum IL-6 level was observed in other studies (21). Moreover, serum sIL-6R levels were correlated with serum intact OC, estrone, E_2, ALP, urinary pyridinoline (Pyr), and D-Pyr, suggesting that the levels of serum sIL-6R, not serum IL-6, reflects the change of bone metabolism caused by estrogen replacement (21). Although it is speculated that changes in circulating sIL-6R may reflect changes in the production of this factor by bone marrow cells, it is entirely possible that other tissues also contribute to variations in serum sIL-6R levels. Conflicting results have been reported concerning the estrogen regulation of cytokine production by human bone marrow cells after menopause or discontinuation of estrogen replacement. There was an association between estrogen withdrawal and an increased release of bone-resorbing cytokines by human bone marrow cells (22), but no association between estrogen deficiency and cytokine production (23). This is the first report showing both a significant variation in serum sIL-6R levels and a correlation of serum sIL-6R levels with serum ALP and with urinary CTx during the menstrual period. Changes in sIL-6R in the serum, however, are unlikely to cause changes in bone metabolism. The sIL-6R in the serum is inert because it is bound in a ternary complex with soluble gp130 and IL-6. However, sIL-6R in the bone microenvironment may well be involved in bone metabolism.

Variations in serum and urinary levels of biochemical indices across the menstrual cycle have been investigated in several studies (20, 24–31). Recently, specific receptors for estrogen have been demonstrated in osteoblasts (32, 33) and osteoclasts (34), and P_4 receptors have been found in bone cells (35), indicating the possibility that sex steroids may act directly on bone cells. In this study, serum E_2 significantly correlated with intact OC and serum ALP, whereas serum P_4 correlated with serum bone-specific ALP and intact OC and inversely with urinary CTx and serum calcium (Table 2).

To the best of our knowledge, there has been only one report that dealt with OC levels during the menstrual cycle. Nielsen *et al.* (20) showed a variation in serum OC with significantly higher levels during the LP. The authors measured serum OC by RIA modified from that of Price and Nishimoto (36). In the present study, we measured serum

TABLE 2. Correlation between steroid hormones, sIL-6R, and/or bone metabolic markers during menstrual cycle

	sIL-6R	B-ALP	PICP	Intact OC	N-mid OC	CTx	D-Pyr	ICTP	ALP	PTH	PTHrP	Ca	P
sIL-6R	1.000												
B-ALP	−0.064	1.000											
PICP	0.032	0.034	1.000										
Intact OC	−0.024	0.008	0.027	1.000									
N-mid OC	0.103	0.307[c]	0.147	0.168	1.000								
CTx	0.190[f]	0.062	0.009	0.024	−0.179[f]	1.000							
D-Pyr	−0.050	0.286[d]	−0.236[e]	0.089	0.114	−0.123	1.000						
ICTP	0.172	0.124	−0.116	−0.241[e]	−0.136	−0.158	0.280[d]	1.000					
ALP	0.209[f]	0.158	−0.017	0.114	0.054	0.173	−0.040	−0.112	1.000				
PTH	−0.013	0.024	0.057	0.144	−0.021	0.121	−0.069	0.044	0.247[e]	1.000			
PTHrP	0.018	−0.221[f]	0.177	0.086	0.010	−0.137	0.123	0.152	−0.103	−0.087	1.000		
Ca	0.047	−0.108	0.010	−0.096	−0.170	0.159	−0.347[a]	−0.176[f]	0.030	0.143	−0.285[d]	1.000	
P	0.143	−0.063	0.216[f]	0.049	−0.170	0.247[e]	−0.183[f]	−0.089	0.018	0.294[d]	−0.110	0.504[d]	1.000
E_2	0.001	0.134	0.043	0.309[b]	0.018	0.190	0.010	−0.077	0.181[f]	0.005	0.045	0.098	0.108
P_4	−0.088	−0.279[d]	−0.047	0.200[f]	0.107	−0.280[d]	0.176	0.062	−0.029	0.005	0.032	−0.215[f]	−0.161
FSH	0.113	0.076	0.143	−0.084	0.123	0.076	−0.104	0.075	0.088	0.315[c]	−0.207[f]	0.131	0.186[f]

B-ALP, Bone-specific ALP, P, inorganic phosphorus.
[a] Correlation coefficients are significant at <0.0001.
[b] Correlation coefficients are significant at <0.0005.
[c] Correlation coefficients are significant at <0.001.
[d] Correlation coefficients are significant at <0.005.
[e] Correlation coefficients are significant at <0.01.
[f] Correlation coefficients are significant at <0.05.

TABLE 3. Peaks and correlations between serum E_2 and bone metabolic markers such as serum sIL-6R after theoretical elimination of intervals between peaks

	E_2	sIL-6R	BALP	PICP	CTx	D-Pyr	ICTP	PTH	PTHrP
Peak (days from LH peak)	2.4 ± 4.2	−2.0 ± 8.9	−2.0 ± 7.2	−4.2 ± 9.9	0.8 ± 6.5	2.2 ± 9.3	3.6 ± 5.6	−5.4 ± 3.5	2.4 ± 7.7
r value		−0.215	0.063	0.136	−0.151	−0.149	0.103	0.252	−0.167
P value		0.0544	0.5986	0.2238	0.2238	0.1147	0.3342	0.0164	0.0916
Days preceding (minus) or lagging behind (plus) E_2		−4	−4	−6	−2	0	+2	−8	0

intact OC with sandwich EIA (12), and serum N-mid OC with two-site IRMA (13). However, we could not find any significant variations in serum intact OC and N-mid OC levels during the menstrual period. The most likely explanation for these discrepancies is the difference in the epitope specificity of the antibodies between their study and our study. Nielsen *et al.* (20) further showed that serum OC and serum E_2 correlated best when the OC peak occurred approximately 7 days after E_2 ($r = 0.69$; $P < 0.05$), suggesting a stimulatory effect of estrogen on osteoblastic activity with a time lag of 7 days. The authors ascribed the time lag to stimulation of osteoblastic proliferation rather than to stimulation of the activity of the existing osteoblasts. The peak in intact OC preceded the E_2 peak by 2.5 ± 2.1 days in this study. There was no significant correlation between intact OC and E_2 when intact OC preceded before E_2 by 2 days. However, serum intact OC significantly correlated with serum E_2 when there was no time lag (Table 2).

PICP is a globular trimer that is cleaved extracellularly from the carboxy-terminus of procollagen. As PICP, which is not incorporated into bone matrix, is not cleared by the kidney, circulating PICP levels correlate with the bone collagen synthesis rate and osteoblastic activity (37–39). Serum PICP showed a variation dissimilar to that of serum bone-specific ALP, and behaved somewhat differently from serum OC and bone-specific ALP in that it was not significantly higher in women in either early or late postmenopause than in premenopausal women (40, 41).

Based on these results, a divergence among the changes in bone-specific ALP, intact OC, and PICP could reflect a complex process of bone formation, a stimulatory effect of estrogen on osteoblastic activity involving stimulation of osteoblastic differentiation and proliferation and of the activity of existing osteoblasts (42), and differences in the behavior of bone remodeling units at different sites in the skeleton.

In addition to the above direct effects of estrogen on bone cells, an indirect mechanism could explain variations in markers of bone metabolism during the menstrual cycle. Serum PTH was significantly correlated with serum E_2 with a time lag of 8 days. Our results support the findings and the explanations of Pitkin *et al.* (25) and El-Hajj Fuleihan *et al.* (31), but are not in agreement with reports in which no midcyclic elevations were found in serum PTH (26, 29, 30). Recently, Thys-Jacob and Alvir (43) observed a significant decline in total and ionized calcium at midcycle, with an increase of E_2 in both the asymptomatic and the premenstrual syndrome groups. Only in the premenstrual syndrome group was the peak midcycle intact PTH significantly elevated compared with early follicular levels. However, we could not find any explanations for these discrepancies. Concerning this mechanism, Riggs *et al.* (44–46) found a 40–50% increase in serum immunoreactive PTH and a concomitant decrease in serum calcium after 10–26 weeks of conjugated estrogen treatment in postmenopausal osteoporotic women. An *in vitro* study (47) suggested that estrogen might directly enhance PTH secretion.

A variation in urinary CTx during the menstrual cycle confirmed our previous finding of a significant variation in the urinary levels of cross-linked N-telopeptides of type I collagen (48). The same profile was observed for urinary free D-Pyr. The serum ICTP, however, showed a different pattern from urinary CTx or free D-Pyr. This is consistent with another study that showed the nadir of serum ICTP during the FP and its peaks during the LP (49). ICTP is reported to be cleaved during the degradation of type I collagen and to correlate with bone resorption assessed by either histomorphometry or calcium kinetic studies (50) and is mainly cleared by the kidney (17). Twenty four-hour endogenous creatinine clearance has been reported to be higher during the LP than during the FP and to correlate with the production of ovarian hormones (51, 52), indicating that the decrease in urinary CTx and free D-Pyr during the LP was a result of decreased production rather than decreased renal clearance.

In conclusion, our observations of significant cyclic variations in biochemical markers of bone metabolism suggest that cyclic variations in markers of bone formation and resorption and a bone-resorbing cytokine might be modulated by cyclic changes in sex steroids during the menstrual period. In addition, the specific days of biochemical events in the menstrual cycle are crucial for evaluating osteoblastic and osteoclastic activities in pre- and perimenopausal women, or in women who start GnRH agonist therapy. The physiological significance of the variation in osteoclastic and osteoblastic activities during the menstrual cycle remains to be elucidated further in relation to skeletal health in premenopausal women.

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