Unchanged biochemical indices of bone turnover despite fluctuations in 1,25-dihydroxyvitamin D during the menstrual cycle

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Abstract. To examine the effect of endogenous oestrogens on calcium metabolism during the menstrual cycle, fasting blood and urinary samples were obtained every day throughout the menstrual cycle in 5 young women. Bone turnover was estimated by serum alkaline phosphatase and fasting urinary excretions of hydroxyproline and calcium. Serum levels of oestradiol (E2), oestrone (E1), and androstenedione (A) showed the well known cyclic fluctuations, the serum 1,25-dihydroxyvitamin D (1,25(OH)2D) nearly doubled from the early follicular phase to the time of ovulation, although 25-hydroxyvitamin D (25OHD) and 24,25-dihydroxyvitamin D (24,25(OH)2D) were almost unchanged. No correlation between the rise in the serum 1,25(OH)2D level and the measured parameters of calcium metabolism was observed.

In view of these findings, the 1,25(OH)2D3 serum concentration measured in women with functioning ovaries can only be interpreted in the context of the menstrual cycle. The published normal range in women for the metabolite may also require reinterpretation.

Calcium metabolism reflects a dynamic equilibrium between two pools: the skeletal pool consisting of 99% of total body calcium and the extracellular fluid pool consisting of 1%. Many endocrine mechanisms regulate this equilibrium with the main purpose of keeping the calcium in the extracellular fluid pool within narrow physiological limits. Parathyroid hormone (PTH), calcitonin (CT), and 1,25-dihydroxyvitamin D (1,25(OH)2D) are believed to be the three most important hormones in this regulatory mechanism (Rasmussen et al. 1974).

In the last years it has convincingly been demonstrated that oestrogens influence the synthesis of 1,25(OH)2D (Tanaka et al. 1977). In normal women serum 1,25(OH)2D levels are elevated during pregnancy (Kumar et al. 1979; Whitehead et al. 1981) and lactation (Kumar et al. 1979; Hillman et al. 1981), although, controversially, some groups have suggested that the serum 1,25(OH)2D levels are low in postmenopausal women and increase by intake of oestrogen (Gallagher et al. 1980). It is therefore not surprising that since the development of sensitive assays for calcium regulatory hormones some groups (Pitkin et al. 1978; Baran et al. 1980; Gray et al. 1982) have examined the effect of the 10-fold changes of oestrogens on these hormones and calcium metabolism during the normal menstrual cycle.

Unfortunately, the data are inconclusive since both normal (Baran et al. 1980) and increased (Pitkin et al. 1978; Gray et al. 1982) levels of calcium regulatory hormones have been postulated during the cycle. Inaccurate definition of the time of ovulation (Baran et al. 1980) could partially explain this discrepancy. Examination of serum vitamin D metabolites and other indices of calcium metabolism during the menstrual cycle has therefore been the aim of the present study.
Materials and Methods

Participants

Five normal menstruating young women from the laboratory staff, aged 23-29 years, were each studied throughout one menstrual cycle (range 26-35 days). All were nulligravida, healthy and none took any medication during the period of the study. Each participant gave her informed consent. Fasting blood and urinary samples were obtained every day during one menstrual cycle. All samples were stored at -20°C until assayed. To obviate inter-assay variability, assays of the individual biochemical parameters from each participant were performed in a single assay run and all determinations were made in duplicate or triplicate (vitamin D metabolites).

Methods

Vitamin D measurements. Serum concentrations of 25OHD, 1,25(OH)2D and 24,25(OH)2D were determined by a modification of the methods of Shepard et al. (1979). The methods involve specific extraction procedures, followed by chromatography on Sephadex LH 20, Lipidex 5000, and high pressure liquid chromatography including known internal standards to determine recovery of each metabolite. The metabolites were finally measured by competitive protein binding assays (1,25(OH)2D and 24,25(OH)2D) or UV detection (25OHD). The intra-assay variations were: 25OHD 10%, 1,25(OH)2D 13%, and 24,25(OH)2D 14%, respectively.

The serum levels of oestradiol (E2), oestrone (E1), and androstenedione (A) were determined by radioimmunoassay techniques.

E2 assay. Antiserum, 125I-labelled and E2 standards were supplied by Nordiclab, Finland. 0.2 ml serum was extracted by diethylether, and polyethylene glycol was used for separation of the antibody-bound fraction. The intra- and inter-assay variations were 11% and 14%, respectively, and the sensitivity was 11 pmol/l.

E1 assay. Antiserum, 3H-labelled E1 and standards were supplied by Wien Laboratories Inc., N.J., USA. One ml serum was extracted by methylenechloride, and dextran coated charcoal was used for separation of the antibody-bound fraction. The intra- and inter-assay variations were 5% and 10%, respectively, and the sensitivity was 55 pmol/l.

A assay. 3H-labelled A was supplied by New England Nuclear, Boston, M.A., USA, antiserum against A-11-protein conjugate from Wien Laboratories Inc., N.J., USA; and standard A from Sigma, Miss., USA. 0.2 ml serum was extracted by iso-octane and the antibody-bound fraction was separated by a gelcentrifugation procedure. The intra- and inter-assay variations were 7% and 12%, respectively, and the sensitivity was 0.9 nmol/l.

Fasting urinary hydroxyproline was determined by a spectrophotometric method (Larsen et al. 1982, subm. for publ.). The method involves oxidation of hydroxyproline to pyrrole followed by a reaction with Ehrlich's reagent. The method includes a toluene extraction of the pyrrole derivative from alkaline aqueous solution, which gives a specific determination of hydroxyproline. Within the normal concentration range the intra- and inter-assay variation were 5% and 9%, respectively. The detection limit was 10 µmol/l.

Serum and urinary calcium were measured by atomic absorption spectrophotometry using a Perkin-Elmer 403 and serum levels were corrected for individual variations in serum protein (Christiansen et al. 1975). Total serum protein was measured refractometrically and serum alkaline phosphatase enzymatically, according to the Scandinavian recommendations (The Committee of Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology 1974).

After a 12-h overnight fast the participants emptied their bladder between 8 a.m. and 9 a.m. Sixty min later a urine sample was obtained for determination of calcium, hydroxyproline, and creatinine. Calcium and hydroxyproline excretions relative to creatinine were obtained from the concentration ratio expressed in mmol/mmol.

Calculations

In each participant the time of ovulation was estimated by subtracting 14 days from the first day of the second menstruation during the study.

All values given as a function of the menstrual cycle are mean values of 2 succeeding days.

Results

As shown in Fig. 1 there was a 10-fold rise in circulating E2 level from day -12 to day 0, and intermediate levels in the luteal phase.

Serum levels of the other two steroid hormones, E1 and A, increased significantly during the menstrual cycle and reached maximum levels on about day 0.

The biochemical indices of bone turnover are illustrated as a function of the menstrual cycle in Fig. 2. The mean values (and SEM) were virtually unchanged for all three parameters and the slope of the regression lines connecting the mean concentrations are not statistically different from zero. Serum calcium either expressed in raw data or corrected to serum protein, showed no significant changes during the cycle. The total protein-corrected serum calcium in the 5 patients between day -14 and -8, and between -2 and +3 was 2.46 mmol/l and 2.44 mmol/l, respectively (not shown).
Fig. 1.
Serum levels of oestradiol (E$_2$), oestrone (E$_1$) and androstenedione (A) in five normal young women during the menstrual cycle. Values are given as mean ± SEM.

Fig. 2.
Fasting urinary hydroxyproline/creatinine, fasting urinary calcium/creatinine, and serum alkaline phosphatase in 5 normal young women during the menstrual cycle. Values are given as mean ± SEM.

Fig. 3
Individual changes in serum concentrations of vitamin D metabolites. The serum samples were analysed corresponding to the minimum and maximum serum concentrations of oestradiol.
The individual values of the vitamin D metabolites at day –12 and day 0 are given in Fig. 3.

All 5 participants showed an increase in serum 1,25(OH)2D, $P < 0.05$ (Student's t-test for paired data). On the other hand, serum 24,25(OH)2D and 25OHD concentrations were unchanged (24,25(OH)2D: +16%; 25OHD: −2%).

Discussion

Although the number of participants in the present study was small, the increase in serum 1,25(OH)2D seems convincing, since the intra-assay variation of our 1,25(OH)2D assay was 13%, and the mean change was 56%. The samples selected for determining vitamin D metabolites were chosen after the serum steroid hormone had been determined. Thus, the samples were taken in the early follicular phase and at the days in the cycle with the highest serum 1,25(OH)2D levels.

Our data support the findings of Gray et al. (1982), but differ from a previous study which reported no change in the serum concentrations of PTH, CT, and 1,25(OH)2D on days 3 and 13 of the menstrual cycle in normal women (Baran et al. 1980). In their study, the serum concentration of oestradiol on day 13 was two to three times higher than the value on day 3, which indicates that the samples were drawn before ovulation, as the authors also admitted. Fluctuations in the vitamin D binding protein (DBP) during the menstrual cycle might explain the mid-cycle peak in the serum level of 1,25(OH)2D. Our protein binding assay for 1,25(OH)2D measures the total concentration of 1,25(OH)2D, both bound and free fractions. The total value could increase if the bound fraction is increased. However, the circulating levels of DBP are known not to change during a normal menstrual cycle (Boullon et al. 1977).

The found alterations in serum 1,25(OH)2D levels cannot be explained with certainty. It is well known that oestrogens influence calcium metabolism. Serum calcium levels and urinary calcium and hydroxyproline excretion rise after the menopause, and treatment with natural or synthetic oestrogens lower both the serum calcium and the urinary calcium and hydroxyproline excretion rate in post-menopausal women, because of inhibition of bone resorption (Gallagher & Nordin 1975; Nordin et al. 1975; Frumar et al. 1980). In the present study, however, there was no change in serum calcium and the two indices of bone resorption were unchanged during the cycle. It therefore seems possible that the tenfold increase in serum E2 directly, or indirectly by an increased PTH secretion rate or a hitherto unknown mechanism, increases the synthesis of 1,25(OH)2D. Surprisingly, this ‘primary’ increase of 1,25(OH)2D does not affect the measured parameters of calcium metabolism.

In view of these findings, the 1,25(OH)2D serum concentration measured in women with functioning ovaries can only be interpreted in the context of the menstrual cycle. The normal range in women for the metabolite may also require reinterpretation.

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