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Changes in Biochemical Bone Markers during Pregnancy and Puerperium

To elucidate the changes in bone turnover during pregnancy and puerperium, we measured serially the levels of serum osteocalcin and urine deoxypyridinoline (Dpy) as markers of bone formation and bone resorption, respectively, in 22 healthy women with normal pregnancy. Nineteen non-pregnant women served as control. The Dpy levels increased significantly at 16 weeks of pregnancy and remained elevated thereafter. The levels of osteocalcin, however, were significantly decreased at 16 weeks of pregnancy and elevated later at 6 weeks postpartum. Bone turnover ratio (Dpy/osteocalcin) continued to rise during pregnancy, but returned to control levels 6 weeks after delivery. Dpy levels and bone turnover ratio during puerperium tended to be higher in 17 breast-feeding women than those of 5 exclusive bottle-feeders. In conclusion, bone resorption begins to increase from the second trimester of pregnancy and calcium release from bone tissue might play a major role in calcium homeostasis during the whole period of pregnancy as well as during lactation.

Key Words: *Biochemical Markers; Pregnancy; Lactation; Disorder, Calcium Metabolism; Osteoporosis*

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INTRODUCTION

Calcium requirements are substantially increased during pregnancy and lactation. Total calcium accumulation in term fetuses is known to be average 21 g (range 13-33 g) (1). Of this, 80% is developed at the third trimester of pregnancy, when there is rapid mineralization of the fetal skeleton (2). During lactation, maternal calcium loss is 280-400 mg/day (up to 1,000 mg/day), i.e., approximately four times higher than during pregnancy (3).

Calcium regulatory mechanisms are altered during pregnancy and lactation to meet the increased demands for fetal skeleton formation and breast milk production. Calcium homeostasis is maintained mainly by controlling intestinal calcium absorption, renal calcium excretion and skeletal calcium release. Intestinal calcium absorption and renal excretion rates are known to be increased during pregnancy, although the importance of bone resorption is still debatable (4). Enhanced maternal bone resorption, however, is reported to play a major role in compensating for calcium loss during lactation (4-7).

Under conditions of normal homeostasis, bone resorption and formation are tightly coupled in the process of bone remodeling and the former precedes the latter. In contrast to bone mineral density, biochemical bone markers represent short-term changes in bone turnover (8). Thus, to assess the changes in bone turnover during pregnancy and puerperium, we measured the levels of serum osteocalcin and urine deoxypyridinoline (Dpy) as markers of bone formation and bone resorption, respectively.

MATERIALS AND METHODS

Thirty-three healthy and well-nourished women in early pregnancy, who visited the Samsung Seoul Hospital for prenatal care, volunteered for a follow-up study throughout pregnancy and puerperium. Only normal singleton pregnancies with term deliveries and an uneventful puerperium were included in the study. Any pregnancies accompanied by pre-existing medical conditions that affected calcium metabolism, such as thyroid disease, renal disease and fracture, were excluded. Based on these criteria, 22 women were selected for this study from among the initial 33. For the control group, 19 healthy non-pregnant and well-nourished female volunteers with normal regular menstrual cycles were recruited.

To control for diurnal variations in biochemical markers of bone turnover, samples of fasting venous blood between 8-10 a.m. and first-voided morning urine were taken to assay osteocalcin and Dpy, respectively. The

concentrations of intact osteocalcin (NovoCalcin® kit: Metra Biosystems, CA, U.S.A.) and total Dpy (PYRILINKS-D® kit: Metra Biosystems, CA, U.S.A.) were measured by competitive immunoassay. The Dpy level was corrected for the urine creatinine (Cr) value. The intra-assay and inter-assay coefficients of variation for osteocalcin were below 9% and below 14%, respectively, and those for Dpy were below 10% and below 13%, respectively.

Gestational ages in the pregnant group and ovarian follicular growth in controls were determined using transvaginal ultrasonograpy (7 MHz probe, 128 XP, Acuson, CA, U.S.A.). Biochemical studies were performed five times in the pregnant group, at 8, 16, 25 and 36 weeks of pregnancy and at 6 weeks postpartum. The markers were also measured three times in the control group, in the early follicular phase (menstrual cycle day 3), during the pre-ovulatory phase (diameter of dominant follicle >18 mm) and in the mid-luteal phase (7 days after ovulation). Mean values of three measurements were employed, to minimize variation of the biochemical bone markers during the menstrual cycle (9, 10).

For statistical analysis, two-sample t-test, Wilcoxon rank sum test and repeated-measures analysis of variance (ANOVA) were used. All data are expressed as means± standard error of mean (SEM) and $p<0.05$ was considered statistically significant.

RESULTS

There were no differences in baseline characteristics between the two groups (Table 1). Fourteen women (63.6%) gave birth by vaginal delivery and eight (36.4%) by cesarean section. After parturition, eight women (36.4%) fed their infant with breast milk only. Five women (22.7%) used bottle-feeding exclusively and the other nine (40.9%) used a mix of bottle-feeding and breast-feeding.

Fig. 1 illustrates the changes in bone turnover during pregnancy and puerperium. When compared with non-pregnant controls, the urinary levels of Dpy (nM/mM Cr) were higher at 16 weeks of pregnancy (6.9±0.4 vs. 5.4 ±0.3, $p<0.05$) and remained elevated thereafter. The serum levels of osteocalcin (ng/mL), however, were decreased at 16 weeks of gestation (3.6±0.3 vs. 4.9±0.5, $p<0.05$) and increased later at six weeks postpartum (11.5±0.8 vs. 4.9±0.5, $p<0.05$). In comparison with the levels seen at 8 weeks of pregnancy, the Dpy levels were significantly increased at 36 weeks of pregnancy and six weeks postpartum. Osteocalcin levels were significantly decreased at both 16 and 25 weeks of pregnancy and increased at six weeks postpartum.

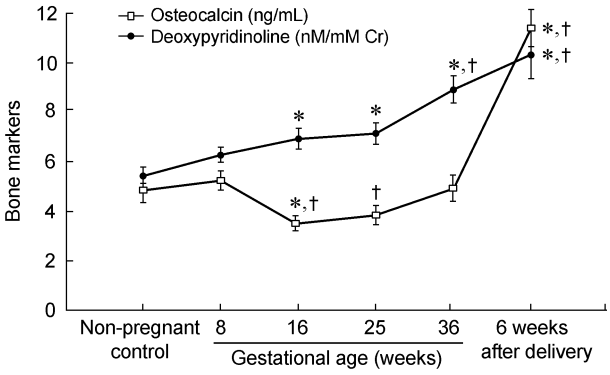

Fig. 1. Changes in bone markers during pregnancy and puerperium. Data are expressed as mean±SEM. *$p<0.05$ vs. non-pregnant control, Wilcoxon rank sum test; †$p<0.05$ vs. 8 weeks of pregnancy, repeated-measures ANOVA.

Fig. 2 shows the changes in the bone turnover ratio expressed as Dpy/osteocalcin ratios during the study. The ratios at 16 weeks (2.1±0.2), 25 weeks (2.1±0.2) and 36 weeks (2.7±0.3) of pregnancy were significantly higher than those seen both in non-pregnant controls (1.2±0.1) and at 8 weeks of pregnancy (1.4±0.2). After delivery, the ratio (1.3±0.2) returned to control levels due to a sharp increase in osteocalcin.

Changes in bone markers following delivery were evaluated by feeding status (Table 2). Breast-feeding women showed lower levels of osteocalcin compared with those

Table 1. Baseline characteristics of study subjects

	Control (n=19)	Case (n=22)
Age (years)	30.1±0.9 (22-36)	29.5±0.7 (24-38)
Body weight (kg)	50.8±1.3 (38.4-63.8)	57.3±1.7 (41.4-68.6)
Height (cm)	158.6±1.2 (151-172)	159.4±1.3 (149-169)
Body mass index (kg/m^2)	20.2±0.5 (16.8-21.5)	22.5±0.7 (17.0-26.7)
Age of menarche (years)	14.7±0.4 (12-19)	13.9±0.6 (13-18)
Length of menstrual cycle (days)	30.4±0.9 (26-40)	29.2±0.7 (25-42)
Parity	0.13±0.2 (0-2)	0.18±0.1 (0-2)

Data are expressed as mean±SEM with the range in parentheses.
Two-sample t-tests revealed no significant differences between groups.

Table 2. Changes in bone markers in relation to mode of feeding

	AMF alone (n=5)		BMF (n=17)	
	PA 36 wks	PP 6 wks	PA 35 wks	PP 6 wks
Osteocalcin (ng/mL)	3.6±1.1	13.2±1.5	4.8±0.7	10.4±1.0
Deoxypyridinoline (nM/mM Cr)	8.3±0.6	8.9±1.2	9.1±0.7	13.2±1.2
Bone turnover ratio*	3.1±0.7	0.7±0.2	2.6±0.4	1.5±0.3

Data are expressed as mean±SEM.
Two-sample t-tests revealed no significant differences at both PA 36 weeks and PP 6 weeks between groups.
AMF, artificial milk feeding; BMF, breast milk feeding; PA, pregnancy at; PP, postpartum; *, deoxypyridinoline/osteocalcin; wks, weeks

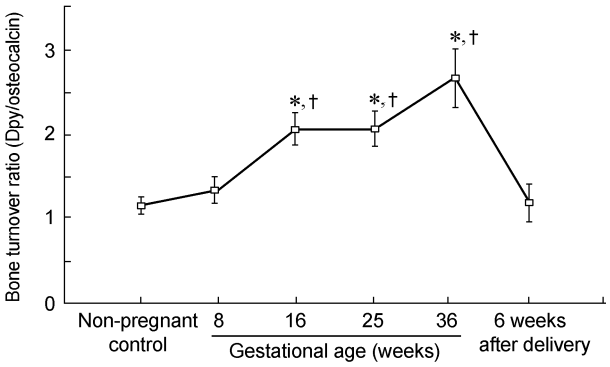

Fig. 2. Changes in bone turnover ratio (deoxypyridinoline/osteocalcin) during pregnancy and puerperium. Data are expressed as mean±SEM. *$p<0.05$ vs. non-pregnant control, Wilcoxon rank sum test; †$p<0.05$ vs. 8 weeks of pregnancy, repeated- measures ANOVA.

who used bottle-feeding exclusively. They also had higher Dpy and bone turnover ratios at 6 weeks postpartum. Changes in Dpy levels after parturition seemed greater in nursing women. However, the differences did not reach statistical significance, probably due to the small number of subjects.

DISCUSSION

The mechanism for calcium homeostasis is somewhat different during pregnancy from that during lactation. During pregnancy, increased calcium demand is maintained mainly by enhanced intestinal absorption (11). This reaches twice the normal non-pregnant and non-lactating levels at as early as 12 weeks of gestation and remains at a plateau throughout pregnancy (11, 12). This situation is mediated by elevated serum 1,25-dihydroxy-vitamin D level (13). The early increase in intestinal calcium absorption has been speculated to allow the pregnant mother to accrete calcium before the peak fetal demand (4). The renal excretion of calcium during pregnancy, however, is also increased (11). This is related to an increase in creatine clearance and glomerular filtration rate (GFR) (14). The 24-hr urine calcium excretion is increased at as early as the 12th week of gestation and remains elevated throughout pregnancy (300 mg in average in the third trimester) (6). Meanwhile, the importance of bone resorption in pregnancy has not been established yet (4). To compensate for the further increased calcium requirements for breast milk production, maternal adaptations include increased bone resorption as well as increased renal calcium conservation (4). Renal excretion of calcium is typically reduced to as low as 50 mg/24 hr, lower than in the non-pregnant state (11). In contrast, intestinal calcium absorption in lactating women falls from the high levels seen in pregnancy to non-pregnant levels (7, 12). These findings suggest a possible contribution by skeletal calcium release during pregnancy.

Our data has revealed that urine levels of Dpy, which reflect change in bone resorption during the previous several weeks, began to increase significantly from 16 weeks of pregnancy. This implies that bone resorption could play an important role in calcium homeostasis beginning in early pregnancy. In addition, significant and unexpected depression of serum osteocalcin levels were observed at 16 weeks of pregnancy, rather than any increase during pregnancy. There are some plausible explanations for this: increased renal degradation secondary to increased GFR (15), trapping or destruction of osteocalcin by the placenta (16) and pregnancy-induced hypervolemia.

Previous reports have suggested that significant changes in bone resorption occur only in late pregnancy. Cross et al. (11) reported that bone resorption markers (serum tartrate acid phosphatase and urinary Dpy) were significantly increased during the third trimester of pregnancy, compared with non-pregnant controls, whereas serum bone formation markers (osteocalcin and carboxy-peptides of collagen I) were decreased during pregnancy from the first to the third trimester. Yamaga et al. (17) reported that both bone formation and bone resorption markers were elevated in the third trimester of pregnancy as well as during puerperium.

Our results on bone markers are consistent with several recent reports describing changes in bone density. Cross-sectional (18) and longitudinal studies (15, 19)

have demonstrated a progressive decline in bone density during pregnancy.

Multiple pregnancy would presumably have a greater impact than singleton gestation on bone turnover (20). In our study, Dpy levels in five twin pregnancies showed a tendency for higher increments than those seen in singleton pregnancies (data not shown).

We also evaluated the changes in bone markers during puerperium. Dpy levels remained elevated, but osteocalcin levels rose sharply after completion of pregnancy. The bone turnover ratio then returned to the non-pregnant state. In general, bone loss associated with pregnancy recovers spontaneously after delivery and multiple parity is not related to later post-menopausal osteoporosis (21).

In line with previous observations (15, 22), our study has shown that lactating women have a tendency for higher bone resorption compared with the non-lactating group. Parathyroid hormone-related protein (PTH-rP), produced by mammary as well as placental tissues, must play a significant role in calcium homeostasis during pregnancy and lactation. Plasma concentrations of PTH-rP rise throughout pregnancy and continue to rise after birth for at least as far as 6 weeks into the postpartum period (4, 23). Sowers et al. (24) reported that, compared with bottle-feeding, the practice of full breast-feeding was associated with significantly elevated levels of PTH-rP. This may affect maternal calcium metabolism by increasing 1,25-dihydroxyvitamin D during pregnancy, and increasing skeletal resorption and renal tubular reabsorption of calcium during lactation (4, 23). Moreover, estrogen deficiencies associated with lactation-amenorrhea would exacerbate skeletal resorption (4). Serial measurements of bone density reveal a fall of 3-8% in bone mineral contents after 2 to 6 months of lactation at trabecular sites, with smaller losses at cortical sites (5-7). However, bone losses during lactation usually recover spontaneously after cessation of lactation and do not increase the risk of postmenopausal osteoporosis (21).

Pregnancy-associated osteoporosis, a rare complication of pregnancy and lactation, has been described in the literature (25). Bone losses associated with pregnancy might cause clinical problems in the spine or hip in susceptible women. Frequently repeated pregnancies might therefore increase the risk of osteoporosis in women with low bone density, especially if associated with prolonged lactation (26).

We conclude from this prospective study that skeletal calcium release might play a major role in calcium homeostasis during the entire period of pregnancy, as well as during lactation. Further prospective studies are necessary to define the changes in bone metabolism during pregnancy and puerperium.

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