NOTE

Bone Metabolism after Human Parturition and the Effect of Lactation: Longitudinal Analysis of Serum Bone-Related Proteins and Bone Mineral Content of the Lumbar Spine

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Abstract. A prospective study was performed to investigate postpartum changes in human bone metabolism and the effects of lactation on them. The subjects consisted of two groups: 13 women who stopped breast-feeding within 3 months postpartum (short-term group) and 14 women who continued breast-feeding for more than 6 months postpartum (long-term group). Serum carboxyl-terminal propeptide of type I procollagen (PICP), carboxyl terminal cross-linked telopeptide of type I collagen (ICTP), and bone gla protein (BGP) were measured prepartum, and at 5 days, 1 month, 3 months and 9 months postpartum. Lumbar BMD was measured at 3–7 days, 3 months and 9 months postpartum. Between prepartum and 3 months postpartum, the values and variations in the markers were essentially the same in both groups. PICP was maintained at a constant and significantly higher level than the control value. In contrast, ICTP had increased markedly at 5 days postpartum, gradually decreasing thereafter. BGP was low prepartum and gradually increased. At 9 months postpartum, PICP and ICTP decreased to the control values in the short-term group. The postpartum time course of lumbar BMD showed a significant decrease in both groups at 3 months postpartum. Recovery to the puerperal level was seen at 9 months postpartum in the short-term group but not in the long-term group. In conclusion, bone resorption is stimulated by parturition as well as lactation resulting in postpartum loss of lumbar BMD.

Key words: Bone mineral content, Postpartum, Lactation, Propeptide of type I procollagen (PICP), Telopeptide of type I collagen (ICTP)

(RECENT studies have shown decreased mineral content of trabecular bones in lactating women [1–5]. These lines of evidence are consistent with the results of several animal studies which have shown mineral loss in the trabecular bones of lactating animals [6, 7], but details of the change in bone metabolism in postpartum and lactating women have not been clearly defined. Notably, several elementary and important questions remain unanswered: when and how the decrease in bone mineral in lactating women occurs, and whether breast-feeding alone has a deleterious effect on bone in postpartum women.

Bone is a complex tissue constantly undergoing a process of renewal and remodeling that involves many different factors and substances. Considerable advances in quantitative assessment of bone mineral content have been made in recent years. Bone mineral content can be estimated with acceptable accuracy and precision by using dual-energy X-ray absorptiometry (DXA) [8, 9], but bone
mineral content at the time of the examination does not reflect the actual status of bone metabolism at that time, but rather the outcome of past bone turnover. Anatomical and physiological changes in women are extensive and profound during the short span of human pregnancy and puerperium. It is therefore difficult to follow the changes in bone metabolism that occur during a relatively brief period, such as the puerperium, by using an image generation technique alone. A panel of biological markers of bone metabolism that could be measured in blood or urine would enable the status of bone at any given time to be clarified, and would thus be useful as a complement to DXA measurement.

Recently, several promising bone-specific proteins which might serve as biological markers of bone turnover have been purified, and reliable assay systems for these proteins have been developed. These potential markers include the carboxyl-terminal propeptide of type I procollagen (PICP), carboxyl terminal cross-linked telopeptide of type I collagen (ICTP), and bone gla protein (BGP). PICP reflects the rate of formation of type I collagen, the main constituent of bone matrix [10–12]. ICTP reflects the rate of degradation of type I collagen [10, 11, 13]. BGP is the most abundant noncollagenous protein in bone and may represent the bone formation rate [10]. These proteins are released into the serum at concentrations correlated with the rates of bone formation and resorption [14–16]. Bone metabolism may thus be assessed quantitatively with these markers. Recently, several investigators have pointed out that ICTP is not a sensitive marker for estimating bone resorption, because the performance of ICTP as a marker appears to be reduced by low serum levels, i.e., near the detection limit of the assay, in healthy European and American populations [17–19]. Nevertheless, our previous study demonstrated that serum ICTP is adequate for the detection of changes in bone turnover and is highly correlated with changes in postmenopausal Japanese women [20].

The present prospective study was performed in order to answer the above questions. We examined postpartum changes in the bone metabolism of healthy women by measuring the bone mineral content of the lumbar spine and these serum biological markers. Moreover, the effects of lactation on these changes are also determined by comparing the time courses of these parameters in women breast-feeding long-term with those in women breast-feeding short-term.

**Subjects and Methods**

**Subjects**

A total of 30 healthy pregnant women at 38–39 weeks of pregnancy between October 1995 and February 1996 were selected as the subjects of this study. The criteria for inclusion in this study were 1) an uncomplicated pregnancy with a single fetus, 2) normal menstrual cycle before pregnancy, 3) no previous fractures, spinal deformities or any disease known to affect calcium metabolism, 4) planning to breast-feed long term (at least 6 months) or short term (0 to 3 months), and 5) intention to delay the next pregnancy for at least 9 months postpartum. All participants were native Japanese, non-smokers, and ranged in age from 21 to 35 years. During the study period, there were no restrictions on diet or physical activity. The only prohibition was against the use of drugs known to affect bone metabolism, including oral contraceptives. Although 16 women had planned on long-term breast-feeding and 14 on short-term breast-feeding, breast-feeding practices were modified at the participants’ discretion, and it was therefore necessary to record either the time of cessation of routine breast-feeding (at least twice a day, consecutively) or resumption of menstruation. When women continued breast-feeding beyond the end of the study period, detailed inquiries were made by telephone by the first author.

All subjects had an uneventful vaginal delivery between 38 and 41 weeks of pregnancy and breast-fed their infant for at least 12 days postpartum. All infants were healthy, and weighed 2.6–3.8 kg, but three women, two who had planned short-term breast-feeding and one who had planned long-term breast-feeding withdrew from the study between 1 and 3 months postpartum for personal reasons. Another woman, who had anticipated long-term breast-feeding, had to discontinue breast-feeding at 3 weeks postpartum due to insufficient lactation. Ultimately, 27 subjects completed the study. Fourteen women breast-fed their infants exclusively for more than 6 months postpartum.
and 13 ceased breast-feeding completely within 3 months. We defined the former as the long-term breast-feeding group (long-term group) and the latter as the short-term breast-feeding group (short-term group). None of the women became pregnant or suffered diseases and/or injuries for which hospitalization was required at any time during the study period.

Twenty-three female volunteers, 22-35 years of age, served as nonpregnant controls. None were pregnant or had become pregnant within a 2-year period prior to the measurements of bone mineral content and serum markers. In accordance with the Helsinki II Declaration, all participants gave their fully informed consent. The research protocols were approved by the Ethics Committee of Yamanashi Medical University.

Venous blood was collected from all subjects between 0900 h and 1000 h in the morning after entry into the study, and at 5 days, 1 month, 3 months and 9 months postpartum. Serum was immediately separated by centrifugation and stored at -40 °C until analyzed. The bone mineral content of the lumbar spine of all subjects was measured during puerperium (3-7 days postpartum), and at 3 months and 9 months postpartum.

**Serum sample measurement**

PICP and ICTP were measured with a radioimmunoassay kit [12, 13] obtained from Orion Diagnostica (Oulunsalo, Finland). The concentration of intact BGP was determined with a two-site immunoradiometric assay kit [21] obtained from Mitsubishi Yuka (Tokyo, Japan). The intra- and interassay CVs were 3.2% and 5.6%, respectively, for PICP, 3.9% and 6.1% for ICTP, and 2.9% and 7.3% for BGP. To exclude subjects with renal or hepatic insufficiency, urea nitrogen (BUN), creatinine, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were assayed in all samples by standard methods. All assays were performed within four weeks of collecting the serum samples.

**Bone mineral measurement**

Bone mineral content of the lumbar spine (L2-L4) was measured antero-posteriorly by dual-energy x-ray absorptiometry (Hologic QDR 2000, Hologic, Inc., Waltham, MA). Bone mineral content is expressed in terms of bone mineral density (BMD: g/cm²). The coefficient of variation (CV) inherent to the equipment was found to be 0.28% for a spine phantom and 0.76% for healthy volunteers by our radiologists. On the basis of these data, our radiologists defined the minimal significant difference (MSD) for two BMD measurements in a single individual to be 2.5%.

**Statistical analysis**

All statistical analyses of the data were performed with SPSS program software (SPSS Inc.; Chicago, IL). The distributions of subject characteristics and the results for each parameter in different groups were compared by the unpaired t-test or the unpaired Wilcoxon rank sum test, as appropriate. Repeated measures analysis of variance (repeated measures ANOVA) was performed for the sequential BMD and serum marker data to evaluate the statistical significance of changes in each parameter. For the within-group comparison of the results for each parameter at different measurement points, the paired t-test or the paired Wilcoxon rank sum test was used, as appropriate. For statistical comparison of means, data were expressed as means ± SD unless otherwise indicated. Statistical significance was defined as P<0.05.

**Results**

The duration of breast-feeding and amenorrhea were 1.5 ± 0.7 months and 2.8 ± 1.1 months in the short-term group and 11.7 ± 3.8 months and 12.3 ± 3.7 months in the long-term group, respectively. Three women in the long-term group resumed menstruation before cessation of breast-feeding, but no significant between-group difference was observed in the intervals between cessation of breast-feeding and resumption of menstruation.

Table 1 shows the data for age, parity, height, weight, increase in body weight during pregnancy, birthweight of the neonate, and lumbar BMD during the puerperium in the short-term group and the long-term group. The control group data are also shown. There were no significant differences in age, parity, or height among the three groups.
Table 1. Clinical and background characteristics of the subject and control groups

<table>
<thead>
<tr>
<th></th>
<th>Short-term (n=13)</th>
<th>Long-term (n=14)</th>
<th>Control (n=23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>30.6 ± 3.7</td>
<td>29.7 ± 3.7</td>
<td>29.3 ± 4.4</td>
</tr>
<tr>
<td>Parity</td>
<td>1.6 ± 0.7</td>
<td>1.8 ± 0.7</td>
<td>1.6 ± 1.1</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>159.2 ± 6.0</td>
<td>157.4 ± 3.5</td>
<td>157.5 ± 4.2</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td></td>
<td></td>
<td>51.4 ± 4.8</td>
</tr>
<tr>
<td>Weight at 3–7 days pp (kg)</td>
<td>54.0 ± 4.4</td>
<td>54.7 ± 7.1</td>
<td></td>
</tr>
<tr>
<td>Weight at 3 months pp (kg)</td>
<td>51.0 ± 4.4</td>
<td>51.5 ± 7.5</td>
<td></td>
</tr>
<tr>
<td>Weight at 9 months pp (kg)</td>
<td>49.8 ± 4.5</td>
<td>51.1 ± 8.0</td>
<td></td>
</tr>
<tr>
<td>Weight increase during pregnancy (kg)</td>
<td>9.1 ± 1.4</td>
<td>9.6 ± 1.9</td>
<td></td>
</tr>
<tr>
<td>Weight of neonate (g)</td>
<td>3124 ± 328</td>
<td>3096 ± 374</td>
<td>1.008 ± 0.113</td>
</tr>
<tr>
<td>BMD (g/cm²)</td>
<td>0.989 ± 0.118</td>
<td>1.011 ± 0.103</td>
<td></td>
</tr>
<tr>
<td>BMD at 3–7 days pp (g/cm²)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

pp, postpartum.

The body weight of the subject groups, whether determined in the puerperium, or at 3 months, or at 6 months postpartum, was never significantly different from the control values, although the puerperal values were significantly higher ($P<0.001$) than at 3 and 9 months postpartum in both subject groups. Lumbar BMD determined during the puerperium in the subject groups was essentially the same as the control value. No significant difference between the two subject groups was observed in any parameter.

Changes in serum markers of bone metabolism

None in the all samples had abnormal serum BUN (>7.0 mmol/L of urea), creatinine (>80 μmol/L), ALT (>40 U/L), and AST (>40 U/L) values. The time courses of serum PICP, ICTP, and BGP in the short-term and long-term groups, the differences between these and the control values, and the differences between the subject groups are shown in Table 2. The variations over time in these markers analyzed by repeated measures ANOVA, and ICTP and BGP values at each different point differed significantly ($P<0.001$) in both groups.

Between prepartum and 3 months postpartum, the values and those variations in the markers were essentially the same in both groups. PICP was maintained at a constant and significantly higher level than the control value during the study period. ICTP changed noticeably. Although prepartum ICTP was already higher than the control level, it had increased to more than five fold the prepartum and 10 fold the control level by 5 days postpartum, gradually decreasing thereafter. ICTP levels at 3 months were still significantly higher than the control level. BGP was low prepartum, and undetectable (less than 1 ng/ml) in four (30.7%) and six (42.8%) samples from the short-term and long-term groups, respectively. Postpartum, BGP gradually increased until 3 months, and the levels determined at 1 and 3 months were significantly higher than the control level.

At 9 months postpartum, between-group differences were observed in the levels of serum markers. PICP and ICTP decreased to the control values in the short-term group but not in the long-term group. BGP in the short-term group was significantly lower than that in the long-term group.

Changes in lumbar BMD

The postpartum time course of the lumbar BMD in the short-term and long-term groups is shown individually in Fig. 1. At 3 months postpartum, a decrease in lumbar BMD was observed in all subjects and the decrease in 24 women (88.8%) was beyond the MSD. At 9 months postpartum, lumbar BMD had recovered to the puerperal level in 12 (92.3%) of 13 women in the short-term group, but in only 4 (28.5%) in the long-term group. The recovery in BMD was seen in all subjects more than 130 days after the resumption of menstruation.
Table 2. Time courses of biomarkers in the subject groups and comparison with control values and between groups

<table>
<thead>
<tr>
<th></th>
<th>Short-term group (n=13)</th>
<th>P vs control value</th>
<th>Long-term group (n=14)</th>
<th>P vs control value</th>
<th>Difference between groups (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PICP (µg/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control (n=23)</td>
<td>84.7 ± 23.8</td>
<td>0.005</td>
<td>105.8 ± 31.6</td>
<td>0.014</td>
<td>NS</td>
</tr>
<tr>
<td>prepartum</td>
<td>114.3 ± 28.9</td>
<td>&lt;0.001</td>
<td>123.0 ± 40.4</td>
<td>0.002</td>
<td>NS</td>
</tr>
<tr>
<td>5 days pp</td>
<td>135.2 ± 31.0</td>
<td>0.001</td>
<td>118.3 ± 30.1</td>
<td>0.008</td>
<td>NS</td>
</tr>
<tr>
<td>1 month pp</td>
<td>128.9 ± 34.6</td>
<td>0.002</td>
<td>130.0 ± 39.8</td>
<td>0.001</td>
<td>NS</td>
</tr>
<tr>
<td>3 month pp</td>
<td>131.7 ± 41.5</td>
<td>NS</td>
<td>131.0 ± 42.1</td>
<td>0.001</td>
<td>NS</td>
</tr>
<tr>
<td>9 month pp</td>
<td>105.4 ± 32.8</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>ICTP (µg/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control (n=23)</td>
<td>3.6 ± 1.1</td>
<td>&lt;0.001</td>
<td>5.3 ± 1.4</td>
<td>0.001</td>
<td>NS</td>
</tr>
<tr>
<td>prepartum</td>
<td>5.9 ± 1.1</td>
<td>&lt;0.001</td>
<td>35.3 ± 6.7</td>
<td>&lt;0.001</td>
<td>NS</td>
</tr>
<tr>
<td>5 days pp</td>
<td>33.9 ± 7.9</td>
<td>&lt;0.001</td>
<td>10.6 ± 4.1</td>
<td>&lt;0.001</td>
<td>NS</td>
</tr>
<tr>
<td>1 month pp</td>
<td>10.9 ± 3.2</td>
<td>&lt;0.001</td>
<td>5.6 ± 2.0</td>
<td>&lt;0.001</td>
<td>NS</td>
</tr>
<tr>
<td>3 month pp</td>
<td>4.6 ± 0.5</td>
<td>0.003</td>
<td>4.5 ± 0.9</td>
<td>0.013</td>
<td>0.008</td>
</tr>
<tr>
<td>9 month pp</td>
<td>3.6 ± 0.7</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>BGP (µg/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control (n=23)</td>
<td>5.0 ± 2.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>prepartum</td>
<td>2.6 ± 1.4*</td>
<td>&lt;0.001</td>
<td>2.2 ± 0.9*</td>
<td>&lt;0.001</td>
<td>NS</td>
</tr>
<tr>
<td>5 days pp</td>
<td>4.4 ± 1.2</td>
<td>NS</td>
<td>4.3 ± 2.0</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>1 month pp</td>
<td>8.7 ± 2.2</td>
<td>&lt;0.001</td>
<td>7.6 ± 3.0</td>
<td>0.010</td>
<td>NS</td>
</tr>
<tr>
<td>3 month pp</td>
<td>10.3 ± 2.4</td>
<td>&lt;0.001</td>
<td>10.4 ± 3.5</td>
<td>&lt;0.001</td>
<td>NS</td>
</tr>
<tr>
<td>9 month pp</td>
<td>7.6 ± 2.0</td>
<td>0.001</td>
<td>11.6 ± 4.5</td>
<td>&lt;0.001</td>
<td>0.007</td>
</tr>
</tbody>
</table>

BGP, bone gla protein; ICTP, telopeptide of type I collagen; PICP, propeptide of type I procollagen; NS, not significant; pp, postpartum. *, values in detectable samples (9 samples in the short-term group and 8 in the long-term group).

The data for lumbar BMD during the study period are summarized in Table 3. According to repeated measures ANOVA, the values at each measurement point differed significantly in both groups (P<0.001). The percentage of the decrease in lumbar BMD by 3 months postpartum was approximately the same in both groups.

Discussion

This study demonstrated that bone resorption reflected by serum ICTP is stimulated consistently and noticeably after human parturition and that the lumbar BMD in women is significantly decreased at 3 months postpartum regardless of the duration of breast-feeding. This study also confirmed the results of previous studies [1-5] which indicated that the accelerated bone turnover is maintained in lactating women, and that the significant bone mineral loss in lactating women is subsequently restored several months after resumption of menstruation.

Fig. 1. Postpartum time course of lumbar BMD in the short-term group (A) and the long-term group (B).
Serum levels of the markers are reportedly influenced by renal and hepatic insufficiency, because the degradation of PICP occurs primarily via hepatic pathways [22], ICTP is apparently eliminated by the kidneys [13], and serum BGP is also affected by renal filtration [23]. We therefore examined hepatic and renal functions of all participants including the control subjects. Moreover, the samples were collected within a specified time, since all three biomarkers have been reported to have circadian rhythms [24, 25].

The three serum markers of bone turnover showed characteristic changes from the prepartum period to 9 months postpartum. PICP, a marker of bone formation, remained at a relatively constant level throughout the study period. In contrast, ICTP, a marker of bone resorption, changed noticeably. Although significantly high ICTP and PICP levels in pregnant women and lactating women had already been confirmed in previous studies including ours [26, 27], the noticeable increase in ICTP soon after parturition is a striking observation made in this study for the first time. The possibility that the postpartum increase in ICTP may reflect degradation of uterine collagen due to uterine involution should be considered, but the effect of uterine involution on postpartum ICTP levels may be small, since the postpartum levels of PICP, a type I collagen-related protein the same as ICTP, were not significantly different from the prepartum level. Prepartum BGP was low or undetectable. This finding is basically in agreement with the results of previous studies [28, 29]. The disappearance of serum BGP during pregnancy has been speculated to be attributable to trapping or destruction of BGP by the placenta [29]. Postpartum, BGP gradually increased and was maintained at a high level from one month postpartum to the end of the study. The continued increase in postpartum BGP is in agreement with earlier reports [1, 3] and suggests a high degree of bone formation during this period. Although the time courses of these markers were essentially the same in both groups up to 3 months postpartum, between-group differences in the serum markers were observed at 9 months. PICP and ICTP decreased to the control values in the short-term group, but higher levels than the control were maintained in the long-term group. The BGP level in the short-term group was also significantly lower than that in the long-term group. These biochemical findings were consistent with the significant decrease in lumbar BMD seen at 3 months postpartum in both groups and with the recovery of lumbar BMD at 9 months postpartum in the short-term group.

The results of this study indicate that bone resorption is greatly stimulated after parturition and suggest that parturition as well as lactation causes a bone loss. We consider this evidence to be important in elucidating the pathophysiology of post-pregnancy osteoporosis and in devising ways to prevent it. Parturition is therefore a risk factor of osteoporosis. This realization may help clinicians understand the clinical evidence indicating that post-pregnancy osteoporosis occurs soon after parturition and in women who breastfeed for only a short time [30, 31].

The characteristic change in estrogen secretion in postpartum women may explain why bone resorption is stimulated consistently after parturition regardless of lactation. Maternal serum estradiol levels rise throughout pregnancy until term, reaching 70 to 110 nmol/L, and then decline to approximately one-thousandth that level at 2 or 3 days postpartum [32]. A causal relationship between estrogen deprivation and bone loss is well known. A sharp and rapid increase in the rate of bone loss following oophorectomy has been reported in many studies, and the efficacy of estrogen replacement therapy in reducing bone loss after the cessation of ovarian function has been
well-documented [33]. The acute weight loss in the puerperium could be considered as another causative factor, because the positive correlation between vertebral density and body weight is also well known [34]. In this study, however, the percent BMD decline at 3 months postpartum was not correlated with the decrease in maternal weight (data not shown). The lost BMD is restored 4–5 months after the resumption of menstruation; i.e., 6–8 months after weaning. This is consistent with the results of earlier reports [1, 3, 5], and is generally attributed to physiological changes caused by the hyperprolactinemic hypoestrogenic state maintained by lactation [35].

Previous reports demonstrated no significant reduction in lumbar BMD over the period 2–3 days to 24 weeks postpartum in exclusively formula-feeding women or in the women in whom lactation was inhibited with bromocriptine, in spite of a 5.1–6.5% reduction in lumbar BMD in breast-feeding women [1, 3, 5]. The discrepancy between their results and ours can be explained mainly by differences in the measurement points and partly by the study population. This conclusion is based on the fact that lumbar BMD recovered to the puerperal level at 9 months postpartum in our short-term group and that lactation was not completely inhibited in our short-term group.

In conclusion, our data demonstrate that bone resorption is stimulated by parturition as well as lactation, resulting in postpartum loss of lumbar BMD. This bone loss is not reversed until several months after the resumption of menstruation.

Acknowledgments

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