Bone modeling in bromocriptine-treated pregnant and lactating rats: possible osteoregulatory role of prolactin in lactation

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Suntornsratoot P, Wongdee K, Goswami S, Krishnamra N, Charoenphandhu N. Bone modeling in bromocriptine-treated pregnant and lactating rats: possible osteoregulatory role of prolactin in lactation. Am J Physiol Endocrinol Metab 299: E426–E436, 2010. First published June 15, 2010; doi:10.1152/ajpendo.00134.2010.—The lactogenic hormone prolactin (PRL) directly regulates osteoblast functions in vitro and modulates bone remodeling in nulliparous rats, but its osteoregulatory roles in pregnant and lactating rats with physiological hyperprolactinemia remained unclear. Herein, bone changes were investigated in rats treated with bromocriptine (Bromo), an inhibitor of pituitary PRL release, or Bromo+PRL at different reproductive phases, from mid-pregnancy to late lactation. PRL receptors were strongly expressed in osteoblasts lining bone trabeculae, indicating bone as a target of PRL actions. By using dual energy X-ray absorptiometry, we found a significant increase in bone mineral density in the femora and vertebrae of pregnant rats. Such pregnancy-induced bone gain was, however, PRL independent and may have resulted from the increased cortical thickness. Bone trabeculae were modestly changed during pregnancy as evaluated by bone histomorphometry. On the other hand, lactating rats, especially in late lactation, showed massive bone loss in bone trabeculae but not in cortical shells. Further study in Bromo- and Bromo+PRL-treated rats suggested that PRL contributed to decreases in trabecular bone volume and number and increases in trabecular separation and eroded surface, as well as a paradoxical increase in bone formation rate in late lactation. Uncoupling of trabecular bone formation and resorption was evident in lactating rats, with the latter being predominant. In conclusion, pregnancy mainly induced cortical bone gain, whereas lactation led to trabecular bone loss in both long bones and vertebrae. Although PRL was not responsible for the pregnancy-induced bone gain, it was an important regulator of bone modeling during lactation.

bone histomorphometry; hyperprolactinemia; ion chromatography; osteopenia; uncoupling

IN PREGNANT AND BREASTFEEDING WOMEN, massive calcium loss occurs for fetal development (~200–300 mg/day) and lactogenesis (~300–1,000 mg/day), respectively (4, 23, 36). A huge amount of calcium demand is accomplished, in part, by enhanced intestinal calcium absorption during these reproductive periods (9). Our recent studies in rats demonstrated that the lactogenic hormone prolactin (PRL), released from the anterior pituitary gland during pregnancy (~100–200 ng/ml) and lactation (~200–300 ng/ml), was the principal calcitropic maternal hormone, which was capable of stimulating calcium absorption in the small intestine and proximal large intestine (7, 21). Moreover, lactation-induced bone resorption provides additional calcium to match the increased calcium demand of the offspring, which in turn induces reversible osteopenia in mothers (20, 36).

In both humans and rodents, hormonal regulation of bone changes during pregnancy and lactation is not completely understood, but it is not directly regulated by the major calcitropic hormones, namely parathyroid hormone (PTH) and 1,25-dihydroxyvitamin D3 [1,25(OH)2D3] (9, 30, 36). Other hormones with elevated plasma levels, such as PRL, PTH-related peptide (PTHRP), calcitonin, and insulin-like growth factor (IGF)-I, might contribute to the maternal bone changes, perhaps with each exerting action at different times and in a bone site-specific manner (4, 20). Among these hormones, PRL is of special interest, since its plasma levels are increased ~10-fold and ~20-fold in pregnant and lactating rats, respectively (6). In addition, primary osteoblasts cultured from rat bones were found to strongly express mRNAs and proteins of PRL receptors (PRLR), suggesting that bone could be another target tissue of PRL actions (40).

A recent investigation in nulliparous rats with hyperprolactinemia induced by pituitary transplantation showed that PRL could induce both bone formation and bone resorption, with the latter being predominant, thereby leading to net bone loss and osteopenia (40). PRLR−/− mice also manifested an impairment of bone growth and mineralization of bone matrix (11). Moreover, pathological hyperprolactinemia in nonpregnant patients with prolactinoma or chronic uses of antipsychotic drugs (i.e., dopaminergic antagonists) may lead to progressive osteopenia and osteoporosis (27, 42). Such bone loss in rodents was apparent mainly in the primarily trabecular site (e.g., vertebrae) or the trabecular parts of the long bone (e.g., metaphysis or secondary spongiosa) but was rarely observed in the cortex (8, 44). The PRL-related bone loss may result from direct actions of PRL on osteoblasts as well as indirectly from the PRL-induced hypoeastrogenemia (8, 12, 27, 40).

Although pregnancy and lactation are considered a physiological hyperprolactinemic state (9), little is currently known regarding the effects of PRL on cortical and trabecular sites at different phases of the reproductive periods (e.g., early vs. late lactation). Generally, longitudinal studies of PRL actions on maternal bone metabolism are carried out in animals treated with bromocriptine (Bromo), an agonist of the PRL-inhibiting factor dopamine, which can inhibit pituitary PRL release by ~80–90% (3, 7, 35), in the presence or absence of exogenous PRL administration (43). The uses of PRL−/− or PRLR−/− mice are not possible due to infertility (2). Furthermore, disruption of PRL synthesis in the first week of pregnancy in rodents could lead to abortion (2). Thus, by using Bromo-treated lactating rats, the effect of PRL on the long bone could be demonstrated by densitometric analysis (43), but whether...
microstructural changes in the cortices and trabeculae were different remained to be investigated. Nevertheless, the aforementioned findings in nulliparous rats (40, 44) suggested that PRL could have action on maternal bone, particularly in the trabeculae.

Therefore, the objectives of the present longitudinal study were 1) to investigate macroscopic and microscopic bone changes by using dual energy X-ray absorptiometry (DEXA) and bone histomorphometry, respectively, in pregnant and lactating rats, from mid-pregnancy to day 15 postweaning, 2) to reveal the possible roles of PRL in regulating maternal bone changes, and 3) to demonstrate differential responses of maternal cortical and trabecular bones to PRL in different reproductive periods.

MATERIALS AND METHODS

Animals. Pregnant and age-matched nulliparous Sprague-Dawley rats (8 wk old) were obtained from the National Laboratory Animal Centre in Thailand. They were housed in the husbandry unit under a 12:12-h light-dark cycle (lights on at 0600) for at least 5 days prior to the experiments and were fed standard chow and distilled water ad libitum. The room had a temperature of 25 ± 2°C with average illuminance of 200 lux. This study was approved by the Animal Care and Use Committee of the Faculty of Science, Mahidol University. All animals were cared for in accordance with the principles and guidelines of the American Physiological Society’s “Guiding Principles in the Care and Use of Animals.”

Experimental design. Bone changes were investigated in different reproductive phases, i.e., mid-pregnancy (day 14, P14), late pregnancy (day 21, P21), early lactation (day 8, L8), mid-lactation (day 14, L14), late lactation (day 21, L21), and day 15 postweaning (PW). Since bone growth and calcium accretion are normally age dependent, we used age-matched nulliparous rats as a control group. Ages of P14, P21, L8, L14, L21, and PW rats were 10, 11, 12, 13, 14, and 16 wk old, respectively. Body weights of all rats were recorded weekly after delivery, litter size was adjusted to eight pups per dam for lactating groups, whereas in pregnant dams the number of pups per dam was maintained as the number of ovaries and corpora lutea. The six-point calibrations for calcium and magnesium were established in pregnant and lactating rats (7). Finally, all rats in each pregnant (P14 and P21) or lactating (L8, L14, and L21) group were killed on the same day, between 0800 and 1000.

Preparation of bone samples. As previously described by Charoenphandhu et al. (8), femora (primarily cortical sites) and LS vertebrae (primarily trabecular sites) were cleaned and subjected to densitometric analysis. In some experiments, femoral length and dry and ash weights were recorded. Femora were dried in an oven at 80°C for 3 days and then ashed at 800°C overnight in a muffle furnace (model 48000; Thermolyne, Dubuque, IA). After bone ash was dissolved with 3.0 N HCl, the samples were diluted with a solution containing 0.38% wt/vol SrCl\(_2\) and 0.9% vol/vol HClO\(_4\) and were analyzed for total calcium and magnesium contents by atomic absorption spectrophotometry (for calcium only; model SpectrAA-300, Varian Techtron, Springvale, Australia) and ion chromatography (for calcium and magnesium).

Tibiae (primarily cortical sites) were also removed, cleaned, and fixed for PRLR expression study and histomorphometric analysis of their cortical and trabecular portions.

Immunohistochemical analysis of PRLR expression. Tibiae were dissected from P21 and L8 rats. After cleaning, they were fixed overnight in 0.1 M phosphate-buffered saline (PBS) containing 4% paraformaldehyde. Decalcification was later performed by immersing bone specimens in 15% wt/vol ethylenediaminetetraacetic acid (EDTA; Sigma) at 25°C for 3 wk. Decalcifying solution was replaced every 3 days. After being embedded in paraffin, bone specimens were cut longitudinally into 7-μm sections, which were later incubated at 37°C for 30 min in antigen retrieval solution (0.01 mg/ml proteinase K, 50 mM Tris-HCl, pH 8.0, and 5 mM EDTA). To inhibit background endogenous peroxidase activity, sections were incubated for 1 h with 10% H\(_2\)O\(_2\). Nonspecific binding was blocked for 2 h by 4% bovine serum albumin, 10% normal goat serum, and 0.7% Tween-20 in PBS. Thereafter, sections were incubated at 4°C overnight with 1:50 rabbit polyclonal primary antibody against PRLR (catalog no. sc-30225; Santa Cruz Biotechnology, Santa Cruz, CA). After being washed with 0.7% Tween-20 in PBS, sections were incubated for 1 h at room temperature with 1:500 biotinylated goat anti-rabbit IgG (catalog no. 656140; Zymed, South San Francisco, CA) followed by incubation for 1 h with streptavidin-conjugated horseradish peroxi-dase solution (Zymed) and 3,3′-diaminobenzidine chromogen (Fierce, Rockford, IL). As for the negative control, sections were incubated with 0.7% Tween-20 in PBS in the absence of PRLR primary antibody. Sections were counterstained with hematoxylin and visualized under a light microscope (model BX51TRF, Olympus, Tokyo, Japan).

Measurement of bone calcium and magnesium contents by ion chromatography. A modular ion chromatographic system (Waters, Milford, MA) comprised a model-600 controller, model-600 pump, model-432 conductivity detector, and a cation column containing silica gel with sulfonic acid group (125 × 4 mm inner diameter, 5 μm particle size, model Nucleosil SSA; Metrohm, Herisau, Switzerland). All components were connected through the Waters SAT/IN module to a Millennium 32 workstation, which performed system control, acquisition, and data analysis. The eluent for simultaneous separation of the divalent cations consisted of 4 mM tartaric acid, 0.5 mM citric acid, and 3 mM ethylenediamine in 5% vol/vol acetone and were degassed for 5 min before use. Eluent flow rates were adjusted at 1.5 ml/min, and the column was equilibrated in eluent for 45 min prior to sample charging. The analytic condition was optimized for isocratic elution using a 100-μl injection loop and total run time of 10 min. Calcium and magnesium were eluted at 5.9 and 7.0 min, respectively. Standard stock solutions were prepared by dissolving appropriate amounts of CaCl\(_2\) or MgCl\(_2\) in ultrapure Milli-Q water to obtain a final concentration of 1 M and kept at −20°C. Working standard solutions were prepared fresh by diluting the stock solutions to 10, 20, 50, 100, 150, and 200 μM with Milli-Q water (pH ~2.5–3.5 adjusted with 2 M HNO\(_3\)) for six-point calibration. The detection limit of both calcium and magnesium at a signal-to-noise ratio of 3:1 appeared to be ~0.02 and ~0.04 μM, respectively. The six-point calibrations for calcium and magnesium showed percent residual standard deviations of 0.9 and 0.5 and correlation coefficients of 0.9989 and 0.9993, respectively. A reproducibility test was performed by injecting standard solutions 10 times on the same day as well as on different days. The relative standard deviations of peak area of calcium and magnesium were varied by less than 0.5% and 3–5%, respectively, during intraday vs. interday tests.

For the analyses of bone calcium and magnesium contents, each bone sample was dissolved in 3 N HCl and diluted 100-fold with distilled water. Thereafter, all samples were filtered through 0.22-μm membranes prior to injection into the column. All samples were analyzed in duplicate and reported as millimoles per gram of dry weight of bone.
Expression of PRLR proteins in bone. Prior to the investigation of the PRL effects on bone, the expression of PRLR proteins in bone was determined by immunohistochemical technique. As shown in Fig. 1, brownish signals of PRLR proteins were observed in flat osteoblasts lining bone trabeculae in the tibiae of nulliparous, pregnant (P21), and lactating (L8) rats. Several hematopoietic cells in bone marrow also showed positive PRLR signals, whereas no signal was observed in osteocytes embedded in bone trabeculae and cortices.

Densitometric analyses of femora and L5 vertebrae of pregnant and lactating rats. At a macroscopic level, bone densitometric analysis using DEXA in femora (a primarily cortical site) and L5 vertebrae (a primarily trabecular site) demonstrated that BMD was markedly increased in mid- (P14) and late pregnancy (P21) but was decreased in late lactation (L21) compared with the age-matched controls (Fig. 2, A and B). In femora, an increase in BMD was maintained until early lactation (L8; Fig. 2A). Fifteen days after weaning, femoral BMD was restored, whereas vertebral BMD remained lower than the control level. Although whole bone BMC was also increased during pregnancy, BMC of L21 lactating rats was comparable to that of age-matched control rats (Fig. 2, C and D).

Neither exogenous PRL nor Bromo affected femoral and vertebral BMD during pregnancy (Fig. 2, A and B). On the other hand, Bromo restored femoral BMD but not vertebral BMD in L21 rats (Fig. 2, A and B). However, PRL administration did not decrease femoral BMD in Bromo-treated L21 rats (Fig. 2A). Interestingly, femoral BMDs in L8 and L21 nonsuckling rats were greater than that in age-matched control rats (Fig. 2A).

Femoral length, ash weight, and total contents of calcium and magnesium. Femoral length was significantly increased during pregnancy and lactation (Fig. 3A). Thereafter, the femoral elongation slowed down, as the femoral length of post-weaning rats was comparable to that of control rats (Fig. 3A). Femoral ash weight was increased only in pregnancy, but not in lactation, compared with age-matched control rats (Fig. 3B). Neither Bromo nor PRL altered femoral length and ash weight in pregnant rats. However, Bromo completely abolished the lactation-induced increase in femoral length. Exogenous PRL administration partially reversed the Bromo effect on femoral length in L21 rats (Fig. 3A).
Since PRL was reported to inhibit in vitro matrix mineralization (39), we further investigated the total calcium and mineral contents normalized by the dry weight of the femora. Normally, bone dry weight includes dry weight of protein matrix and weight of minerals (e.g., calcium, magnesium, and phosphate). Therefore, if the studied conditions do not change the stoichiometry of mineralized bone matrix (i.e., relative quantities of minerals per weight of protein matrix), the total calcium and magnesium contents normalized by the dry weight should be constant. As demonstrated by ion chromatography, these parameters were not altered in pregnant and lactating rats and remained constant after Bromo or Bromo+PRL administration (Fig. 4). In addition, calcium content in bone ash was greater than magnesium content by 40-fold. A constant total calcium content normalized by dry femoral weight was also confirmed by atomic absorption spectrophotometry (Supplemental Fig. S2).

Cortical thicknesses of tibiae and L5 vertebrae. Goldner’s trichrome staining of tibial and vertebral sections revealed that the thicknesses of tibial and vertebral cortical shells as well as of trabeculae were markedly increased during pregnancy (Figs. 5A and 6, A and B). The pregnancy-induced increase in cortical thickness was not dependent on PRL (data not shown). In L14 lactating rats, the thickness of tibial cortical shell, but not vertebral cortical shell, remained greater than that in the control groups (Figs. 5B and 6, C and D). In contrast, bone trabeculae in both tibiae and L5 vertebrae became thinner during lactation (Fig. 5B).

Histomorphometry of trabecular microstructure in pregnant and lactating rats. Further investigation by bone histomorphometry in the trabecular portion of tibiae demonstrated that trabecular bone volume and trabecular number were not changed during pregnancy compared with the age-matched controls but were markedly decreased after mid-lactation, which lasted until day 15 postweaning (Fig. 7, A and B). Trabecular thickness was modestly altered during pregnancy and lactation with a significant increase only at day 15 postweaning (Fig. 7C). Trabecular separation was significantly increased after mid-lactation (Fig. 7D), consistent with a decrease in trabecular bone volume during the same period.
Regarding bone formation-related parameters, although osteoblast surface was decreased during mid-pregnancy (Fig. 8A), double labeled surface, mineral apposition rate, and bone formation rate were significantly increased (Fig. 8B–D) compared with the corresponding age-matched control groups. However, these parameters were relatively constant or modestly changed between late pregnancy and mid-lactation but were later drastically increased in late lactation and postweaning (Fig. 8A–D). Mineralizing surface (Supplemental Fig. S3) was consistent with bone formation rate (Fig. 8D).

Interestingly, despite having net bone gain during pregnancy, as demonstrated by DEXA, osteoblast surface and eroded surface in tibial trabeculae had already increased from mid-pregnancy and lasted until late lactation (Fig. 9A and B). These results suggested that trabecular bone formation and resorption were uncoupled during these reproductive periods, thereby leading to bone gain and bone loss in pregnant and lactating rats, respectively. In other words, “bone modeling” may be present, since bone formation was not proportionally coupled to bone resorption (13). Both osteoclast surface and eroded surface returned to the control values postweaning (Fig. 9A and B).

Most histomorphometric parameters, with the exception of osteoblast surface, double labeled surface, and bone formation rate, were not altered in Bromo-treated or Bromo+/PRL-treated pregnant rats (Figs. 7–9), suggesting that PRL did not have much influence on trabecular bone changes during pregnancy. On the other hand, during the lactation period, especially in late lactation, changes in several parameters related to both bone formation and resorption, e.g., bone volume, trabecular number, trabecular separation, osteoblast surface, double labeled surface, mineral apposition rate, bone formation rate, osteoclast surface, and eroded surface, were regulated by PRL (Figs. 7–9). Multinucleated or activated osteoclasts were more abundant in Bromo+/PRL-treated L21 rats than in Bromo-treated L21 rats (data not shown). However, PRL-induced changes in these parameters showed different times of responses as reflected by nonuniform responses to exogenous...
It is noteworthy that trabecular thickness in the rat tibiae was relatively constant from late pregnancy until late lactation, and also not responsive to both Bromo and PRL (Fig. 7C).

In nonsuckling rats, which had no hyperprolactinemia and the suckling-induced PRL surge (1, 34), most parameters with the exception of trabecular thickness were not much different from those of the age-matched control rats (Figs. 7–9).

**DISCUSSION**

How maternal bone adapts during pregnancy and lactation is crucial for growth, development, and calcium metabolism of the offspring. Herein, we provided evidence that bone changes during these periods were complex and time-dependent (pregnancy vs. lactation), and varied with sites of bone (femora/tibiae vs. vertebrae) as well as types of microstructure (cortical shells vs. trabeculae). Specifically, pregnancy predominantly induced femoral and vertebral bone gain in the cortical shells, whereas long-term lactation eventually led to trabecular bone loss. Lactation-induced trabecular bone loss, but not pregnancy-induced cortical bone gain, was found to be regulated by PRL. However, other hormones with elevated plasma levels, such as IGF-I and PTHrP, might also be responsible for bone changes during pregnancy and lactation, respectively (4, 28, 47).

In mid- and late pregnancy, the observed increases in femoral and vertebral BMD were due to an increase in cortical thickness, presumably by enhancing periosteal bone formation (4). Increased femoral bone length (or size), which probably results from pregnancy-enhanced endochondral bone growth (4), may also contribute to the increased femoral BMD in pregnant rats. Since bone size of pregnant rats was larger than that of their age-matched control rats, their whole bone BMC and ash weight were markedly increased during this period. However, the stoichiometry of calcium and magnesium in bone matrix remained unchanged. In contrast to the cortical parts, despite an increase in trabecular thickness in mid-pregnancy, changes in the trabecular microstructure in P14 and P21 rats were modest. Although P14 pregnant rats manifested the enhanced trabecular bone turnover, as indicated by the augmented bone formation and resorption, both processes were still coupled, and no change in trabecular bone volume was observed. These coupled processes in P14 rats could not be explained simply by osteoblast-induced bone formation, since osteoblast surface was indeed decreased in a PRL-dependent manner (Fig. 8A). It was hypothesized that surplus minerals supplied by the intestine might accelerate bone calcium acquisition (7, 9), as indicated by the twofold increase in the double labeled surface and mineral apposition rate in P14 rats (Fig. 8, B and C). On the other hand, uncoupling of trabecular bone formation and resorption, the latter of which being predominant, was evident in late pregnancy and continued throughout lactation. Therefore, the differential changes in cortical and trabecular structures (i.e., net bone gain and loss occurred at...

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**Fig. 5.** Goldner’s trichrome staining of tibiae (primarily cortical sites) and L5 vertebrae (primarily trabecular sites) dissected from P14 (A) and L14 rats (B) and their corresponding age-matched controls (n = 3–4 rats per group). Cortical shell (Ct; bone tissue between dashed lines in A), epiphysial plate (Ep), bone trabeculae (arrows), and bone marrow (M) were identified. Mineralized bone matrix, erythrocytes, and cytoplasm were stained green, orange, and red, respectively. Bars, 1,000 μm.

**Fig. 6.** Average thicknesses of cortical shells in proximal tibiae and L5 vertebrae of P14 (A–B) and L14 (C–D) rats. **P < 0.01, ***P < 0.001 vs. its respective control group. Nos. in parentheses represent nos. of experimental animals.
different parts of the same bone) indicated a process of bone modeling in this phase of the reproductive cycle (13).

It was postulated that pregnancy-induced bone gain was essential for the preparation of the maternal calcium pool during the last 5 days of pregnancy in rodents and the third trimester in humans for fetal skeletal mineralization and the upcoming lactogenesis (4). In addition, thickening of the cortical shells was important for maintaining bone shape and strength to compensate for the gradual increase in the trabecular separation during lactation (16). Such pregnancy-induced cortical adaptation was certainly under the regulation of hormone(s) other than PRL, such as progesterone and IGF-I (4, 17, 28). PRL might indirectly promote maternal bone gain through stimulation of intestinal calcium absorption to provide more calcium for mineralization (7). However, 1,25(OH)2D3 and PTHrP might not participate directly in this bone gain, because bone mass was found to be increased in the vitamin D receptor knockout mice (17) and the plasma PTHrP levels were significantly increased in late pregnancy (20), after BMD had already been increased.

During lactation, progressive bone loss was evident, especially in the trabecular portions of both long bones and vertebrae. Since the trabecular surface was much greater than the exposed cortical surface (13), the lactation-induced bone loss occurred mainly in bone trabeculae rather than in cortical shells, thereby leading to a significant decrease in BMD in late lactation. Nevertheless, the endocortical surface of the rat tibiae showed greater erosion during lactation compared with the end of pregnancy (25). Previous investigations in humans also demonstrated that calcium lost during breastfeeding may cause a 10% loss of the total body skeletal mass (22, 37, 41). In rodents nursing around six to eight pups, bone mass could be decreased by 30% in late lactation (5, 32, 47). Nevertheless, BMC and ash weight were not decreased, because bone size, as indicated by the femoral bone length, in lactating rat was actually larger than that of their age-matched controls. Similar to the pregnant rats, the stoichiometry of calcium and magnesium in bone matrix of lactating rats remained constant (Fig. 4), suggesting that the newly formed osteoid might be rapidly mineralized. It was possible that there was a sufficient amount

![Fig. 7. A, trabecular bone volume normalized by tissue volume (BV/TV). B, trabecular number (Tb.N); C, trabecular thickness (Tb.Th); D, trabecular separation (Tb.Sp) of the trabecular portion (secondary spongiosa) of tibiae obtained from age-matched control, pregnant (P14 and P21), lactating (L8, L14 and L21), and 15-day postweaning (PW) rats, as determined by bone histomorphometry. Some pregnant and lactating rats were administered Bromo for 7 days (4 mg·kg⁻¹·day⁻¹ sc) or Bromo+PRL. PRL doses for pregnant and lactating rats were 0.4 and 0.6 mg·kg⁻¹·day⁻¹, respectively. Some rats were permanently separated from their pups after parturition (nonsuckling). All values in line graphs and bar graphs are presented as means ± SE (n = 7–8 rats per condition). *P < 0.05 vs. age-matched control group; †P < 0.05 vs. corresponding lactating group; ‡P < 0.05 vs. Bromo group; ‖P < 0.05 vs. Bromo+PRL group.}
of mineral supply for mineralization, perhaps from the intestine and kidney (9); otherwise, a decrease in normalized bone calcium content would be apparent as in dietary calcium deficiency with osteomalacia (15, 24, 33, 38).

It was evident that trabecular bone loss in lactating rats was induced by PRL, the plasma levels of which were 200–300 ng/ml (normal levels 7–10 ng/ml) and could further elevate up to 600–800 ng/ml during suckling (1, 6, 7). The presence of PRLR in osteoblasts corroborated a direct effect of PRL on bone metabolism (12, 14, 40). Besides its direct action, PRL may indirectly induce bone resorption by suppressing ovarian estrogen synthesis (48). Furthermore, PRL may be responsible for calcium availability for bone mineralization, as it was the principal stimulator of intestinal calcium absorption in lactating rats (7, 9, 10).

Evidence that supported the PRL actions on bone in this study were based on the use of Bromo, which suppressed PRL release through stimulation of the pituitary D₂ dopaminergic receptors and in turn reduced circulating PRL levels by 80–90% (3, 7, 35). Since osteoblasts did not express D₂ receptors (43), Bromo should not exert a direct action on this cell type. Although all histomorphometric parameters related to osteoblast functions and bone formation in L21 rats (e.g., osteoblast surface and bone formation rate) were completely inhibited by Bromo and restored by exogenous PRL supplement, changes in some other parameters seemed to be partially PRL dependent (e.g., osteoclast surface in L21 rats). It was possible that other hormones, such as PTHrP from the mammary glands, also contributed to the lactation-induced trabecular bone loss as reported previously (46). On the other hand, the PRL-induced bone changes were not clearly observed at the macroscopic level with DEXA, presumably because the gross changes in femoral BMD required prolonged duration of 2–7 wk of PRL exposure (40, 44).

Furthermore, the results in nonsuckling rats confirmed that the factor(s), either PRL or PTHrP, that induced trabecular bone loss during lactation was related to suckling. Normally, the plasma PRL levels decreased rapidly within 1–2 days after cessation of breastfeeding (26, 34). However, the significant increases in BMD and BMC in nonsuckling rats suggested that...
the absence of suckling pups and normal weaning might differentially affect bone metabolism, because BMD and BMC continued to increase in nonsuckling rats (Fig. 2), although their trabecular histomorphometric parameters showed similar modest changes (Figs. 7–9). It was, therefore, speculated that an endocrine factor that increased cortical bone growth during pregnancy remained active after parturition, perhaps due to the lack of antagonistic signals triggered by suckling.

Previous investigations concerning the direct effect of PRL on osteoblast-induced bone formation at the cellular and molecular levels agreed with the present histomorphometric findings. Specifically, PRL was found to downregulate Runt-related transcription factor-2, alkaline phosphatase, and osteocalcin (12, 40). Such direct PRL actions explained the PRL-induced decreases in osteoblast surface in tibial trabeculae of P14 rats and double labeled surface and bone formation rate of L14 rats. Interestingly, in L21 rats there were paradoxical increases in all bone formation-related parameters, which could be prevented by Bromo and restored by exogenous PRL administration (Fig. 8). The exact explanation of this paradox is not known, but it might be related to the PRL-enhanced intestinal calcium absorption (7, 19) and/or the calcitonin-induced renal calcium reabsorption (20), both of which could provide extra calcium for mineralization. Alternatively, extensive bone resorption in late lactation might lead to substantial release of the embedded growth factors, thus in turn stimulating bone formation (13).

Regarding PRL effects on bone resorption, a previous study using human osteoblasts showed that PRL upregulated the expression of receptor activator of nuclear factor-κB ligand (RANKL), while concurrently downregulating the osteoprotegerin (OPG) expression (40), thereby increasing the number of active osteoclasts and their activities. The PRL-induced increase in the RANKL/OPG ratio explained the present findings that osteoclast surface and eroded surface were markedly increased in mid- and late lactation. Besides PRL, mammary gland-derived PThrP, which could directly stimulate osteoclasts, may also participate in maternal bone resorption, especially when its plasma levels were elevated from mid-pregnancy to late lactation (20, 46).

After weaning, BMD was restored by increasing trabecular bone formation (Fig. 8) and decreasing bone resorption (Fig. 9). BMD of the primarily cortical sites (e.g., femora) recovered faster than that of the trabecula-rich sites (e.g., L5 vertebrae). It was apparent that the repair of trabecular microstructure in rats could not be accomplished within 15 days postweaning, but might require up to 20 wk of recovery (4). Similarly, in humans, bone mass could be restored after cessation of breast-feeding, although some mothers might develop the so-called pregnancy- and lactation-induced osteoporosis featuring bone pain, height loss, and fragility fracture (29).

In conclusion, we demonstrated herein that pregnancy increased bone mass predominantly in the cortical shells of both long bones and vertebrae, whereas lactation decreased bone mass predominantly in bone trabeculae. Thus, trabecular structures were an important source of calcium for lactogenesis. Interestingly, bone modeling was discernible during these reproductive periods as a result of increased bone length and uncoupling of bone formation and bone resorption. Although the pregnancy-induced cortical bone gain appeared to be PRL independent, PRL still contributed to maternal bone changes during pregnancy since it could decrease osteoblast surface in P14 rats. On the other hand, suppression of bone formation in mid-lactation and stimulation of bone resorption in mid- and late lactation were clearly PRL dependent and could be explained by the direct PRL actions on osteoblasts (39, 40). The present in vivo data, therefore, provided further information on a dynamic, time-dependent, site-specific adaptation of maternal bone, as well as differential osteoregulatory roles of PRL during pregnancy and lactation.

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DISCLOSURES

No conflicts of interest are reported by the authors.

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