NOTE

Effect of Pregnancy, Lactation and Weaning on Bone Mineral Density in Rats as Determined by Dual-Energy X-ray Absorptiometry

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Abstract. To elucidate the effect of pregnancy, lactation and weaning on bone mineral density (BMD) in rats, a longitudinal study was done on the same individuals measuring BMD by dual-energy X-ray absorptiometry (DXA) and comparing their profiles with those of nonpregnant controls. Twenty-seven pregnant Wistar rats which had been mated at 11 weeks old (baseline), lactated during the three weeks postpartum period and weaned thereafter. Twenty-four rats of the same age served as nonpregnant controls. BMDs in lumbar spine, distal femur and caudal spine of all rats were measured weekly from 11 to 22 weeks except for the week of parturition (14th week). During pregnancy, BMDs of the three sites increased significantly from the baseline values, but no significant difference was observed in comparison with the control. After parturition and during lactation, BMD of the three sites decreased significantly from the pregnant values and decreased even from baseline values. All the BMD values of the pregnant group were significantly lower than those of the control group. After weaning, BMDs of the three sites increased gradually and caught up to the control group at 22 weeks in the lumbar spine and the femur and at 21 weeks in the caudal spine.

In conclusion, pregnancy in itself does not significantly affect maternal BMDs of rats, although the significant bone mineral loss during lactation is not completely restored until at least 5 weeks after weaning.

Key Words: Rat, Pregnancy, Lactation, Bone mineral density, Dual-energy X-ray absorptiometry

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PREGNANCY and lactation in mammals induce considerable changes in the hormonal status as well as in the mineral metabolism. Previous studies have shown decreased mineral content of trabecular bones of lactating animals, particularly in rats [1-3]. These lines of evidence are consistent with the results of several human studies including ours, which have shown mineral loss in lactating women [4-8]. Furthermore, an ovariectomized rat that is lactating has been used as a good model for experimental osteoporosis [9]. However, several important elementary questions remain unanswered even in rat studies: whether pregnancy in itself affects bone mineral metabolism, when and how the decrease in bone mineral in a lactating animal occurs, and when the bone mineral deficit is normalized after weaning. Moreover, the differences in methodology should be taken into consideration, when it comes to inferring the results of animal studies to human. In rat studies, the invasive methods, such as gravimetric and histologic methods, have been generally used, because they provide an accurate way to quantitate bone mineral density (BMD). On the other hand, noninvasive methods have been predominantly used.
Bone mineral measurement in the lumbar spine or the proximal femur by dual-energy X-ray absorptiometry (DXA) now constitutes the most important noninvasive tool for diagnosis and follow-up of osteoporosis, because BMD can be estimated with considerable accuracy by this means [10]. But BMD of lumbar spine in pregnant women can not be measured by DXA, to avoid exposing the fetus to X-rays. In addition, conventional gravimetric and histologic methods have several disadvantages, including the excision of the skeletal site of interest, thus preventing longitudinal follow-up assessment of BMD. Although the acceptable precision of DXA measurement was also confirmed in BMD studies on small animals such as rat [11, 12], there are few studies on the profile of changes in rat BMD throughout pregnancy, lactation and weaning period.

In our overall objective of elucidating the effect of pregnancy, lactation and weaning on rat BMD, we measured the BMD by DXA in three skeletal sites throughout pregnancy, lactation and weaning period, and compared the profiles with those of nonpregnant controls.

Materials and Methods

Animals

Ten week old female Wistar rats obtained from Japan SLC Co. (Shizuoka, Japan) were acclimated for at least 7 days which housed in a 12-h/12-h light-dark schedule with room temperature set at 23 ± 5°C and humidity of 50 ± 5%. Rats were given water and standard laboratory rat chow (MF, Oriental Kobo, Japan) ad libitum. This feed contains 1.15% calcium and 0.88% phosphorus by weight, as well as 0.8 IU/g of vitamin D3. The stage of the estrous cycle was checked by vaginal cytology according to the usual methods. Rats not exhibiting a normal 4-to 5-day estrous cycle were excluded from the study. At 11 weeks of age, the BMD and body weight (baseline value) of all rats were measured. Thirty rats were then cohabited with males for mating overnight and the presence of sperm in the vagina was checked the next morning. The day of sperm confirmation was termed day 0 of pregnancy (P0). Twenty-four rats served as the nonpregnant controls (control group). Subsequently, 27 rats were confirmed to be pregnant and were designated as the pregnant group. Data from the mating-but-nonpregnant rats were excluded from the study. All pregnant rats delivered 9-13 pups at the 21st or 22nd day of pregnancy, and lactated for three week period.

All animal studies were conducted according to the Guidelines for Animal Experiments approved by the Animal Ethics Committee of Yamanashi Medical University.

Measurement of BMD and body weight

BMD and body weight of all rats were measured longitudinally, i.e., by a follow-up method at one week intervals from 11 weeks (before the day of mating for pregnant group) to 22 weeks of age except for 14 weeks when the pregnant rat underwent delivery. All pregnant rats ceased breast-feeding at 17 weeks of age. BMD was measured anteroposteriorly at the three sites: lumbar spine (L2-5), distal one third portion of left femur (distal femur) and caudal spine (C2-5) by dual-energy X-ray absorptiometry (DXA) using Hologic QDR 1000 (Hologic, Inc., Waltham, MA). An ultra-high resolution software program (available from Hologic) was used, which led to a point resolution increase of over sevenfold compared with a typical human scan [12]. At BMD measurement, the rats were anesthetized with 3% pentobarbital and their body weight was measured, and then they were fixed on a transparent acrylic plate of 8 mm thickness in prone position with hindlegs maintained in external rotation. Short-term reproducibility was determined in anesthetized rats by making three measurements on each anatomic site on the same day. Coefficients of variation (n=15) for lumbar spine, distal femur and caudal spine were 1.10%, 1.21% and 2.36%, respectively.

Statistical analyses

All statistical analyses of the data were performed with SPSS program software (SPSS Inc., Chicago, IL). Distributions of subject characteristics and results for each parameter in different groups were compared by unpaired t-test. Repeated measures analysis of variance (repeated measures ANOVA) was performed for the sequential BMD and body weight 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, paired t-test was used. Correlations between BMD and body weight were determined by simple regression analysis. Statistical significance was defined as $P<0.05$.

**Results**

Table 1 shows the baseline values of body weight and BMD of three measuring sites in pregnant and control groups. Although there were no significant differences at baseline in any of the parameters between the two groups, significant sequential changes in both of the groups were confirmed for all parameters by repeated measures ANOVA ($P<0.001$). Time courses of the percent change from the baseline in lumbar spine BMD, distal femur BMD, caudal spine BMD, and body weight for the pregnant and control groups are shown in Fig. 1-A, B, C and D, respectively. In short, the control rats showed gradual and significant increase in all parameters, but pregnant rats showed characteristic changes in the study period.

**Changes in BMD during pregnancy, lactation and weaning period**

During pregnancy (11–13 weeks), BMD of the three sites increased significantly compared to baseline values ($P=0.003$ for lumbar spine and $P<0.001$ for femur and caudal spine), but no significant difference was observed in comparison with the control.

After parturition and during lactation (14–16 weeks), BMDs of the three sites decreased significantly from pregnant values (12 and 13 weeks, $P<0.001$) and decreased even from baseline values ($P<0.001$) in the lumbar spine and the distal femur. All BMD values of the pregnant group were significantly lower than those of the control.

After weaning (which began at 17 weeks of age), BMD of the three sites increased gradually and significantly exceeded the baseline value at 21 weeks for the lumbar spine and the distal femur and at 19 weeks for the caudal spine, and caught up to the control at 22 weeks in the lumbar spine and the femur, and at 21 weeks in the caudal spine. The same results were obtained regarding the percent changes as shown in Fig. 1-A, B, C.

**Changes in body weight and correlation between body weight and BMD**

Control rats showed gradual and significant increase in BMD of all measuring sites during the study period. In the pregnant group, body weight increased markedly during pregnancy, and during lactation was even significantly higher than the control. After weaning, no significant difference was observed between the two groups (Fig. 1-D). To clarify the effect of the changes in body weight on BMD, the percent changes in the body weight and BMDs of the three measuring sites during the pregnancy period (11 and 13 weeks of age), the lactation period (14 and 16 weeks of age) and the weaning period (17 to 22 weeks of age) were calculated, and the degree of correlation of the percent changes in BMDs with those in body weight were determined. However, no significant correlation was observed either in the pregnant group or in the control group during these subdivided periods (data not shown).

| Table 1. Body weight and BMD (mean±SD) in pregnant group and control group at baseline |
|---------------------------------|---------------------------------|-----------------|
|                                | Pregnant group (n = 27)         | Control group (n = 24) | $P$ |
| Body weight (g)                | 168.3±5.3                      | 170.1±4.7         | ns  |
| BMD (g/cm²)                    |                                |                  |
| Lumbar spine                   | 0.167±0.011                    | 0.166±0.011      | ns  |
| Femur                          | 0.209±0.011                    | 0.212±0.012      | ns  |
| Caudal spine                   | 0.195±0.009                    | 0.203±0.013      | ns  |

ns, not significant
Discussion

This study clearly demonstrates the characteristic changes in rat BMD throughout the pregnancy, lactation and weaning period compared with age-matched nonpregnant rats. To our knowledge, this is the first report assessing the changes in BMD during and after pregnancy of rats using the DXA technique. This study also elucidated several basic questions which had remained unanswered; pregnancy in itself does not affect maternal BMD significantly, the significant bone mineral loss in lactating rats is completely restored to the levels of nonpregnant rats with the same age at least by five weeks after weaning.

Nonpregnant control rats showed gradual and significant increase in BMD of all measuring sites during the study period, although percent changes in BMD were not significantly correlated with that in body weight. This age-and growth-related increase in BMD has been reported by Juhn et al. [13]. They indicated that age- and growth-related increase in dry weight and ash weight as well as BMD in the lumbar spine were observed in the rat until 32 months of age. The age-and growth-related increase in BMD was
also observed during pregnancy, but this increase was not significantly different from those in control animals. This means that hormonal or metabolic changes during pregnancy do not significantly affect BMD. Miller et al. [2] reported small but significant increase in wet bone weight, dry bone weight, ash weight, and calcium content in the rat femur during pregnancy. The discrepancy between their results and ours can be explained mainly by differences in the methods of study; longitudinal measurements by DXA in ours and cross-sectional measurements by the gravimetric method in theirs.

A significant decrease in BMD of all measuring sites after parturition and during lactation agrees basically with all previous studies by gravimetric and histologic methods [1-3] or by single photon absorptiometry [14]. The maximum difference in BMD from the control rats were 25.1%, 16.0% and 8.5% in the lumbar spine, the distal femur and the caudal spine, respectively, during lactation. The degree of decrease in the lumbar spine and the distal femur were almost the same as those obtained by gravimetric and histologic methods [1-3], but smaller than the values in the total femur (−28%) obtained by single photon absorptiometry [14]. The discrepancy between their results and ours can be explained mainly by differences in the method and site of the BMD measurement. The differences in the degree of decrease among the three measuring sites is explainable by the difference in percentage of trabecular bone component [2]. After weaning, BMD of the three sites increased gradually and caught up to the control level at 22 weeks in the lumbar spine and the femur and at 21 weeks in the caudal spine. Similar time-lag was observed in human longitudinal studies using DXA [5, 6, 8]. The time-lag is considered as the time required for reflection of change in bone metabolism on BMD.

The interspecific differences in pregnancy and lactation behavior are well known. Rats usually conceive and suckle litters of 8–12 or more pups while humans generally have only a single infant. In addition the drain of calcium is greatly increased by lactation in rats compared to humans, since the calcium content of rat milk is much higher than that of human milk [15]. However, the results obtained from this study correspond well with those from human studies using DXA; that is, pregnancy in itself does not significantly affect maternal BMD [8, 16], and the significant bone mineral loss during lactation is completely restored within several months after weaning [4, 5, 7, 8]. The mechanism controlling bone mineral loss in the lactating rat is not completely elucidated. Until recently, discussion of the regulation of bone metabolism has emphasized the role of circulating hormones, principally those traditionally involved in the regulation of calcium and phosphorus metabolism, but a number of explanatory factors have been ruled out. The bone loss can occur independent of vitamin D [17] and PTH [15], and perhaps independent of estrogens, calcitonin, and glucocorticoids [1, 9]. In recent human studies, parathyroid hormone related peptide (PTHrP) is considered as an alternative mechanism associated with bone loss during and subsequent to lactation [18]. PTHrP has been shown to be synthesized in lactating mammary tissues in animal studies including rat [19, 20]. But we cannot discuss this hypothesis from the results of this study and must await further investigation.

In conclusion, a BMD profile throughout pregnancy, lactation and weaning period in rats is clearly elucidated. Pregnancy in itself does not significantly affect maternal BMD of rats, and the significant bone mineral loss during lactation is not completely restored until at least 5 weeks after weaning. Furthermore, the present results demonstrate quite a similarity in bone metabolism during and after pregnancy between rats and human, and suggest that the rat is a good model for experimental research on human bone metabolism during and after pregnancy.

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References