Fluctuation in Plasma Bone Metabolic Markers in Multiparous and Primiparous Holstein Cows during Early to Peak Lactation

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ABSTRACT. Because the role of bone turnover during early to peak lactation in dairy cows is unclear, plasma levels of osteoclast- or collagen-specific bone resorption and osteoblast-specific bone formation markers from postpartum week 3 (W+3) to W+7 were compared with prepartum W-3 levels in multiparous (n=15) and primiparous (n=13) Holstein cows. The multiparous group showed a consistently low level of osteoclast-specific bone resorption markers, while collagen-specific resorption and osteoblast-specific formation markers decreased at postpartum W+7 and from W+5, respectively. In the primiparous group, the plasma levels of all three markers decreased from postpartum W+3. These results suggest that the calcium demand during the transition from early to peak lactation in dairy cows is less affected by bone turnover.

KEY WORDS: bone metabolic marker, dairy cow, early to peak lactation.

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The blood calcium (Ca) level in healthy adult cows is maintained at approximately 8.5 to 10 mg/dl. This means that a 600-kg cow has approximately 3 g and 8 to 9 g of Ca in the plasma and extracellular pools, respectively [3]. In dairy cows, the amount of Ca excreted in the milk, particularly from early to peak lactation, far exceeds that present in the body pools, resulting in a severe challenge to Ca homeostasis. Physiological bone turnover, which comprises bone formation and resorption, plays an important role in maintaining Ca homeostasis. In periparturient dairy cows, it is understood that effluence of Ca from the bone via bone resorption and its increased absorption from the intestine are the major responses to Ca homeostasis [7, 12, 15, 16]. Dairy cows are considered to be in early lactation from 8 to 50 days and peak lactation from 51 to 110 days postpartum followed by mid and late lactation [17]. Midlactational dairy cows are essentially and totally dependent on intestinal Ca absorption to replenish blood Ca lost to lactation [3]. However, whether the high level of Ca excreted through the milk during transition from early to peak lactation in dairy cows is affected by bone turnover or dietary absorption is unclear.

In the bovine, osteoclast-specific enzyme tartrate-resistant acid phosphatase 5b (TRAP5b) and collagen-specific hydroxyproline (HYP) were used as biomarkers of bone resorption, and osteoblast-specific noncollagenous enzyme bone-specific alkaline phosphatase (BALP) was used as an

indicator of bone formation [1, 12, 13, 18]. HYP is a predominant amino acid in collagen and can also be derived from sources other than bone; thus, TRAP5b is considered to be the more specific marker of bone resorption in cows, because of its specificity to osteoclasts [18]. The plasma levels of these biomarkers decline as the animal ages [18]. To clarify the role of bone turnover in maintaining Ca homeostasis during transition from early to peak lactation, plasma TRAP5b, HYP and BALP were evaluated from postpartum week 3 (W+3) to W+7 in multiparous and primiparous Holstein cows. Moreover, plasma nonesterified fatty acid (NEFA) and total cholesterol (T-Cho) were determined to evaluate the negative energy balance and feed intake states, respectively.

The study was conducted from July 2011 until February 2012, and its protocol was approved by the Iwate University Laboratory Animal Care and Use Committee. A total of 28 normally parturated Holstein cows (15 multiparous cows at 4.9 ± 1.4 years of age and 13 primiparous cows at 2.1 ± 0.15 years of age) from a commercial farm in Iwate, Japan were used. In this farm, the multiparous and primiparous cows were housed together in free-stall barns and given a similar prepartum (dry-up to close-up) and postpartum feed. The average dry-up period started from 64 days prepartum, and the close-up period started from 18 to 28 days. The cows were fed plenty of grass hay with 1 kg/day of concentrate [0.59% Ca, 0.47% phosphorus (P) and 0.24% magnesium (Mg) in dry matter (DM)] during the dry-up period, and the amount of concentrate was gradually increased to 3 to 4 kg/day together with 30 g of Ca sulphate as an additive during the close-up period. After parturition, all cows were fed a total mixed ration of grass silages and concentrates with plenty of grass hay (0.87% Ca, 0.49% P and 0.26% Mg of DM). The

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Fig. 1. Fluctuations in the plasma levels of the bone resorption markers tartrate-resistant acid phosphatase 5b (TRAP5b) and hydroxyproline (HYP), and the bone formation marker bone-specific alkaline phosphatase (BALP) in multiparous ($n=15$) and primiparous ($n=13$) Holstein cows at prepartum weeks W-3 and W-1 and postpartum weeks W+3, W+5 and W+7. Each marker indicates the mean value, and the corresponding vertical bar indicates the standard deviation. Significant differences are indicated by the symbols # for $P<0.05$ within the group with W-3 as a control and by * and ** for $P<0.01$ and $P<0.05$, respectively, between the groups at the same time point.

milk production in the multiparous group, recorded once per month, was 35.8 ± 8.2 and 37.2 ± 12.5 kg/day in the first and second months of lactation, respectively, and was 34.4 ± 4.7 and 33.9 ± 4.7 kg/day, respectively, in the primiparous group.

In the final gestational weeks (prepartum W-3 and W-1) and during transition from early to peak lactation in the postpartum weeks (W+3, W+5 and W+7 with a ± 3 -day span in each week), heparinized blood samples were obtained by coccygeal venipuncture. Plasma was separated within 3 hr of collection after centrifugation at 3,000 rpm for 20 min and stored at -50°C until analysis. The body condition score (BCS) of each cow was also recorded (range: 1–5 for thin to fat [2]) at prepartum W-3 and postpartum W+3 and W+7.

The plasma NEFA and T-Cho levels were measured using an automated clinical chemistry analyzer (ACCUTETM; Toshiba Medical Systems Co., Tochigi, Japan). Using the autoanalyzer, the plasma Ca concentrations were measured by applying an o-cresolphthalein complexone method [12]. The plasma TRAP5b activity was determined fluorometrically, and HYP and BALP levels were measured spectrophotometrically [18].

All numerical data are expressed as means $\pm$ standard deviation. Using Sigmasat for Windows version 3.5 (Systat Software, San Jose, CA, U.S.A.), fluctuations in plasma levels of nutritional metabolic parameters, minerals and bone resorption and formation markers were analyzed by repeated-measures ANOVA and Dunnett's tests using W-3 levels as controls. Student's *t*-test was used to compare the differences between the groups for the same period. A value of $P<0.05$ was considered to indicate statistical significance.

The changes in plasma TRAP5b, HYP and BALP levels are shown in Fig. 1. The plasma TRAP5b and BALP lev-

els at prepartum W-3 and W-1, BALP at postpartum W+3 and W+7 and HYP throughout the study period in the primiparous group were significantly higher than those in the multiparous group, while there was no difference between the groups in plasma TRAP5b during the postpartum study period. Moreover, all bone markers evaluated were constantly low in the multiparous group throughout the study period. When compared with prepartum W-3 levels, the multiparous group showed a significant decrease in plasma HYP at postpartum W+7 and BALP at W+5 and W+7. In addition, plasma TRAP5b did not show any fluctuation in the multiparous group, maintaining a low level until postpartum W+7. In the primiparous group, plasma TRAP5b and BALP increased significantly at prepartum W-1 and then decreased during the postpartum study period, whereas HYP decreased at W+7.

The fluctuations in plasma Ca, NEFA and T-Cho and the changes in BCS are shown in Fig. 2. Plasma Ca level at postpartum W+3 was significantly lower in the multiparous group compared with the primiparous group; however, it was maintained from 8.7 to 10.9 mg/dl in all cows. The pre- and postpartum fluctuation patterns of the plasma NEFA and T-Cho levels were almost identical in the multiparous and primiparous groups. Although a higher plasma NEFA level was shown at prepartum W-1 and postpartum W+3 in the multiparous group compared with the primiparous group, it started declining from postpartum W+3 in both groups. On the other hand, the T-Cho level in both groups increased steadily from W+3 to W+7. The BCS decreased at postpartum W+3 and W+7.

Consistent with some early reports [12, 13], the primiparous group showed more active prepartum bone turnover than the multiparous group in terms of higher levels of bone

Fig. 2. Fluctuations in the plasma levels of calcium (Ca), nonesterified fatty acid (NEFA) and total cholesterol (T-Chol) and in the body condition score (BCS) of multiparous ($n=15$) and primiparous ($n=13$) Holstein cows at prepartum weeks W-3 and W-1 and postpartum weeks W+3, W+5 and W+7. Each marker indicates the mean value, and the corresponding vertical bar indicates the standard deviation. Significant differences are indicated by the symbols # for $P<0.05$ within the group with W-3 as a control and by ** for $P<0.05$ between the groups at the same time point.

resorption and formation markers at W-3 or W-1. Older animals have a lower number of receptors for parathyroid hormone [3, 4], probably due to passive bone turnover in multiparous cows. Moreover, the relatively higher Ca demand in the primiparous cows due to their body weight gain and mammary gland development together with fetal bone growth was probably not replaced by intestinal Ca absorption during the prepartum weeks, requiring Ca mobilization from maternal skeletal reserves [14].

Several studies have reported fluctuations in bone turnover during different lactational periods in multiparous and primiparous dairy cows. The higher bone formation activity in primiparous than in multiparous cows at postpartum W+3 and W+7 was in accordance with the results of a previous report [8]. Kim *et al.* [12] showed increased plasma activity of the bone resorption marker TRAP5b together with the bone formation marker BALP soon after parturition, followed by decreases within postpartum W+2 in both the multiparous and primiparous cows. Similarly, Kurosaki *et al.* [13] reported higher activities of TRAP5b or BALP at postpartum W+1 in primiparous than in multiparous cows. However, they did not assess bone turnover beyond postpartum W+2. The decreased plasma TRAP5b and BALP from postpartum W+3 onward and HYP at W+7 in primiparous cows we report here suggests decreased bone turnover during transition from early to peak lactation in younger cows.

In the multiparous group, the consistently low plasma

levels of bone metabolic markers were justifiable, because these levels decrease with increasing age of the cow [18]. While evaluating the lactational bone metabolic activity in multiparous cows, Liesegang *et al.* [15] showed an increased urinary HYP up to 60 days of lactation, which decreased thereafter compared with its antepartum day 14 concentrations. Because HYP is not specific to bone and is derived from nonskeletal collagenous and noncollagenous sources, they reasoned that uterine collagen during its involution might have caused increased HYP for a few weeks after parturition [15]. However, we did not find increased plasma HYP levels after postpartum W+3; instead, these levels decreased at W+7. Furthermore, our results showed consistently low plasma TRAP5b activity in multiparous cows throughout the study period, while plasma BALP decreased from W+5, when cows were approaching peak lactation. Sato *et al.* [17] reported increased serum TRAP5b and BALP activities from early to late lactation than during the dry period, but different animals with wide parity ranges and from different farms were used together during different lactational periods. In the present study, bone turnover from prepartum W-3 to postpartum W+7 was evaluated in individual animals. Moreover, cows were given a similar feed, and parity was considered to be an important criterion of age-related changes. Therefore, the present study provided more reliable information regarding bone turnover fluctuations in multiparous and primiparous cows.

One previous study reported higher bone resorption and formation rates in high-yielding cows than in those with a lower yield [15], and another study reported no direct relationship between milk yield and bone turnover [5]. A similar milk yield (>30 kg/day/cow) during the first 2 months of lactation was recorded in the multiparous and primiparous cows used in our study, but we did not evaluate the association between milk yield and bone turnover activity. In addition, dairy cows start ovarian cyclicity as early as postpartum W+3 [10, 11], and the estrus cycle fluctuation of estrogen hormone is related to TRAP5b activity [1]. Here, we could not monitor estrus cyclicity to determine its effect on TRAP5b activity.

Some early studies showed higher plasma Ca levels in primiparous cows around parturition than in multiparous cows [9, 12]. We also observed higher plasma Ca levels at postpartum W+3 in primiparous than in multiparous cows. However, the plasma Ca level was maintained within the normal range throughout the study period in all cows. Although the reason for the differences in plasma Ca levels between younger and older cows fed a similar diet was not clear, depletion of the receptors for 1,25-dihydroxyvitamin D in the intestine and subsequent reduction in Ca absorption ability in older cows was suspected [3, 6].

Overall, similar fluctuation patterns in plasma NEFA and T-Cho levels as well as BCS changes reported previously in metabolically normal periparturient multiparous [10] and primiparous [11] Holstein cows were observed in the present study. The difference in NEFA levels between the two groups indicates that older cows have more fat mobilization for energy around parturition than younger cows. Steadily increasing plasma T-Cho levels from postpartum W+3 onward suggest an increasing dry matter, and thus dietary Ca, intake.

In conclusion, the present data indicate decreased bone turnover in both multiparous and primiparous Holstein cows during the transition from early to peak lactation. The results suggest that Ca demand during transition from early to peak lactation in dairy cows is less affected by bone turnover and may be dependent on dietary Ca absorption.

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