NOTE *Internal Medicine*

Changes in Plasma Bone Metabolic Markers in Periparturient Dairy Cows

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ABSTRACT. We examined fluctuations in plasma tartrate-resistant acid phosphatase isoform 5b (TRAP5b) measured using fluorometry in conjunction with those in calcium (Ca) and other bone metabolic markers from 2 weeks prepartum to 2 weeks postpartum in 7 primiparous and 18 multiparous pregnant cows. The plasma Ca concentration decreased temporarily on the day of calving in multiparous cows only. Plasma TRAP5b peaked on the day of calving in primiparous and multiparous cows and was significantly lower in multiparous cows than in primiparous cows 2 weeks before and after parturition. Plasma hydroxyproline increased 1 week postpartum in multiparous cows. Bone-specific alkaline phosphatase and osteocalcin tended to decrease after parturition in primiparous and multiparous cows. These results suggest that bone resorption increases around parturition in healthy parturient cows from the viewpoint of the TRAP5b activity.

KEY WORDS: bone metabolic marker, bone resorption, dairy cow, parturition, tartrate-resistant acid phosphatase isoform 5b (TRAP5b).

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Most dairy cows experience varying degrees of hypocalcemia during parturition while they adapt to the calcium (Ca) demands of lactation [6, 9]. Enhancement of bone Ca resorptive and intestinal Ca absorptive processes is the major homeostatic response to hypocalcemia [6, 9]. However, it has been described that the Ca homeostasis of parturient cows depends only on intestinal Ca absorption soon after calving because bone resorption is delayed for 1 week or more [20]. To describe the changes in bone metabolism from pregnancy to lactation in dairy cows, many studies have reported the changes in the levels of bone metabolic markers in blood and urine [10, 12, 14, 17]. Plasma tartrate-resistant acid phosphatase (TRAP) isoform 5b (TRAP5b) and urinary hydroxyproline (HYP), pyridinoline (PYD) and deoxypyridinoline (DPD) excretion serve as bone resorption markers, and bone specific alkaline phosphatase (BALP) and osteocalcin (OC) serve as bone formation markers.

TRAP, a family of acid phosphatase isoenzymes, in plasma or serum can be classified into three types, TRAP 5b, TRAP isoform 5a and non-type 5 TRAP; TRAP5b is a lysosomal enzyme secreted by activated osteoclasts, whereas TRAP isoform 5a and non-type 5 TRAP are thought to be derived from activated macrophages and erythrocytes and platelets, respectively [22]. Kurosaki *et al.* [14] reported that serum TRAP5b did not change around parturition in cows in a study in which they measured its activity spectrophotometrically using para-nitrophenyl phosphate (pNPP) as a substrate. Recently, an assay using pNPP was reported to have low specificity for TRAP5b because non-type 5 TRAP was released through hemolysis during blood collection and clotting and consequently interfered with the end-products of pNPP hydrolysis [22]. As an alternative method, fluorometry using naphthol-ASBI-phosphate (N-ASBI-P) as the substrate has been shown to be capable of measuring the plasma TRAP5b activity with a high degree of precision and reproducibility, without interference from hemolysis in bovine blood specimens [22]. Accordingly, the necessity exists to evaluate the diagnostic significance of TRAP5b measured using fluorometry in periparturient dairy cows.

Here, we examined the physiological fluctuations in the plasma TRAP5b activity measured using fluorometry from 2 weeks prepartum to 2 weeks postpartum in dairy cows in conjunction with those in the plasma Ca concentration and the bone metabolic markers HYP, BALP and OC.

Twenty-five parturient Holstein cows that did not develop parturient paresis during the periparturient period were housed in free-stall barns at a commercial dairy farm in Iwate (Japan). They were assigned to primiparous (n=7, 2.1 ± 0.1 years) and multiparous (n=18, 4.0 ± 1.0 years) groups. For 3 weeks prepartum, the respective groups were fed grass hay, grass silage and concentrate [0.3% Ca, 0.3% P and 0.13% Mg in dry matter (DM)] or grass hay, corn and grass silages and concentrate (0.3% Ca, 0.2% P and 0.13% Mg of DM). After parturition, all cows were fed a total mixed ration of grass hay, corn and grass silages and concentrate (0.5% Ca, 0.3% P and 0.05% Mg of DM). The prepartum dietary cation-anion difference (DCAD) calculated from the dietary minerals was 20.9 and 25.4 mEq/100 g of the dietary DM in the primiparous and multiparous groups, respectively. The postpartum DCAD was 14.7 mEq/100 g of the dietary DM. The study protocol was approved by the Iwate University Laboratory Animal Care and Use Committee.

Heparinized blood samples were obtained from the coc-

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cygeal vein once a week beginning 2 weeks before the predicted parturition date until 2 weeks postpartum and on the day of calving. The blood was centrifuged immediately to separate plasma, which was then frozen at –50°C until analysis. To evaluate the fluctuation in biochemical parameters around parturition in each group, the sampling days were regarded as follows for this investigation: 2 weeks (17 to 13 days) prepartum, 1 week (10 to 6 days) prepartum, the day of calving, 1 week (4 to 8 days) postpartum and 2 weeks (11 to 15 days) postpartum.

The plasma Ca concentrations were measured by applying an orthocresolphthalein complexone method [4], and the plasma TRAP5b activity was measured using a previously reported fluorometry [22]. Plasma HYP was measured spectrophotometrically using the method of Dabev and Struck [5], while plasma BALP was measured with a spectrophotometric method using wheat germ lectin [1]. Plasma OC was measured using an enzyme immunoassay (Gla-Type Osteocalcin EIA kit; Takara Bio, Shiga, Japan) 2 weeks prepartum, on the day of calving and 2 weeks postpartum only because of a technical error. To minimize the interference of hemolysis on measurements of bone parameters, plasma was utilized for this study.

All numerical data are expressed as means ± standard deviation. Either one-way repeated measures analysis of variance (ANOVA) or Friedman's test following the Kolmogorov-Smirnov normality test was used to analyze the changes in each parameter for each group. Dunnett's multiple comparison method was used to determine the significance of values in comparison with the value on the day of calving. Either the Student's *t*-test or the Mann-Whitney *U*-test, after the normality test, was performed to compare the difference between groups for the same period. The level of significance was set at $P<0.05$. These analyses were performed using statistical software (SigmaStat for Windows version 3.5; Systat Software, San Jose, CA, U.S.A.).

The plasma Ca concentration in the multiparous cows decreased temporarily at parturition and was lower than in primiparous cows throughout the periparturient period, except 1 week postpartum (Fig. 1). The plasma Ca concentration has been reported to decline slightly in multiparous cows and to not change in primiparous cows [12, 13], which is consistent with our findings. Therefore, the Ca homeostatic processes were considered to respond properly in our experimental cows because no parturient paresis occurred.

The plasma TRAP5b peaked on the day of calving in both primiparous and multiparous cows and was significantly lower in the latter than in the former 2 weeks before and after parturition (Fig. 2). To the best of our knowledge, this is the first report concerning application of plasma TRAP5b activity measured by fluorometry in veterinary medicine. Collectively, these data suggest that bone resorption was activated at parturition in the periparturient cows. This accelerated bone resorption may result from the effect of parathyroid hormone secreted from parathyroid glands due to acute Ca demand after calving [9], especially in multiparous cows, because the multiparous cows in the present

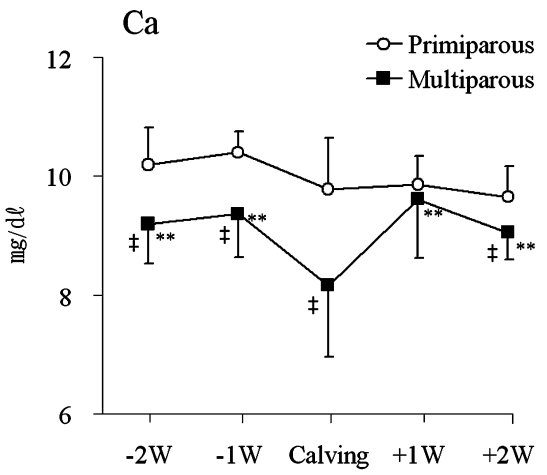

Fig. 1. Changes in the plasma calcium (Ca) concentration in primiparous (n=7) and multiparous (n=18) cows around parturition. Data are expressed as means ± SD. Significant differences were found between groups for the same period (‡ $P<0.01$). Significant differences were found compared with the data from the day of calving for each group (* $P<0.05$ or ** $P<0.01$). –2W: 2 weeks prepartum (17 to 13 days prepartum), –1W: 1 week prepartum (10 to 6 days prepartum), Calving: the day of calving, +1W: 1 week postpartum (4 to 8 days postpartum), +2W: 2 weeks postpartum (11 to 15 days postpartum).

study exhibited a temporal decline in plasma Ca concentration at parturition. In addition, the effect of corticosteroids on osteoclasts in increasing their life span and activity [11] may result in the acceleration of bone resorption in both primiparous and multiparous cows, because the plasma cortisol concentration increases at parturition in cows [8].

The plasma HYP concentration increased 1 week postpartum in both groups of cows (Fig. 2). Plasma HYP, an amino acid contributing to collagen orientation within the bone matrix, is used as a marker of bone resorption in cattle [19, 22]. Previously, we reported that plasma HYP was positively correlated (r=0.83) with the plasma TRAP5b activity using fluorometry in 65 healthy, nonpregnant, non-lactating Japanese Black cattle [22]. The clinical relevance of HYP is controversial because plasma and urinary HYP are affected by diet and the metabolism of non-bony collagens, such as those in muscle, skin and the liver [16]. Plasma HYP, PYD and DPD are thought to increase after parturition because they originate from the uterine collagen and are released into the bloodstream during several weeks after parturition [14]. In contrast, plasma TRAP5b is correlated with the number of osteoclasts and serves as a specific marker of osteoclastic activity and bone resorption [3]. Therefore, plasma TRAP5b appears to be a useful, specific alternative to plasma HYP for evaluating bone resorption in periparturient cows.

The bone formation markers decreased or tended to decrease in both the primiparous and multiparous cows; i.e.,

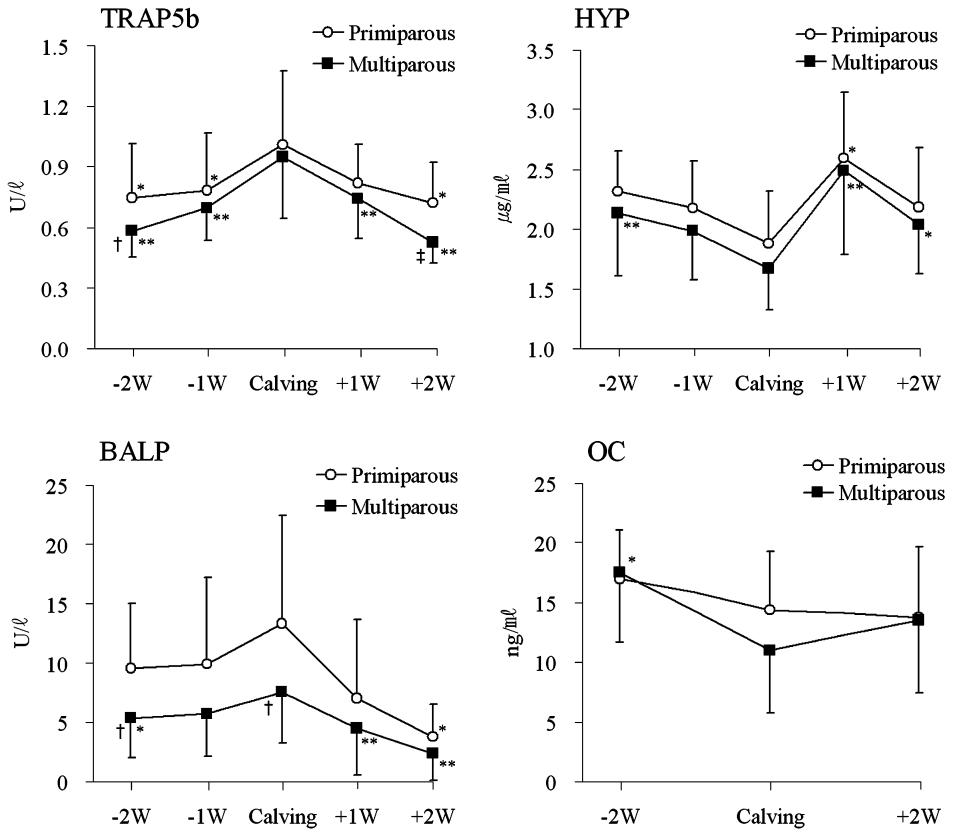

Fig. 2. Changes in plasma tartrate-resistant acid phosphatase isoform 5b (TRAP5b), hydroxyproline (HYP), bone-specific alkaline phosphatase (BALP) and osteocalcin (OC) in primiparous (n=7) and multiparous (n=18) cows around parturition. Data are expressed as means ± SD. Significant differences were found between groups for the same period († $P<0.05$ or ‡ $P<0.01$). Significant differences were found compared with the data from the day of calving for each group (* $P<0.05$ or ** $P<0.01$). –2W: 2 weeks prepartum (17 to 13 days prepartum), –1W: 1 week prepartum (10 to 6 days prepartum), Calving: the day of calving, +1W: 1 week postpartum (4 to 8 days postpartum), +2W: 2 weeks postpartum (11 to 15 days postpartum).

the plasma BALP decreased after parturition in both groups, and the plasma OC was higher 2 weeks prepartum compared with the day of calving in the multiparous cows (Fig. 2). This implied that the osteoblastic activity or function was depressed at parturition [7]. However, the plasma BALP peaked at parturition, which is in agreement with the report by Kurosaki *et al.* [14]. One possible reason for this status may be related to BALP being overestimated as a result of the cross-reactivity of lectin with corticosteroid-induced alkaline phosphatase (CALP) in measurement of BALP activity [18]. Since the plasma cortisol concentration increases from the day before parturition and peaks at parturition in cows [8], the higher plasma BALP on the day of calving may be due to the effect of CALP released from the liver. Another reason could be that the plasma BALP activity reflects osteoblast functions relating to the regulation of osteoclast activation and differentiation [15]. Nevertheless, the fluctuations in the plasma BALP and OC levels postpartum seemed to differ; i.e., the decrease in plasma OC may have occurred before the plasma BALP changes. Presumably, the two markers reflect different stages during osteoblastic differentiation [2].

In the present study, significant differences between the groups in plasma TRAP5b and BALP were observed prepartum. Previously, we reported that these bone markers are significantly higher in younger individuals, indicating that bone metabolism in younger animals is accelerated compared with that in older animals [22]. Nevertheless, it remains unclear whether the OC concentration correlates with the previous experience of parturition in periparturient cows. Kurosaki e*t al.* [14] reported that the OC levels in primiparous cows were higher than those in multiparous cows from 40 days before until 7 days after parturition, whereas Iwama *et al.* [10] found no difference 14 days postpartum. In our study, no difference was observed in the plasma OC concentrations between the primiparous and multiparous cows during the experiment.

The Ca homeostasis of parturient cows has been reported

to depend on intestinal Ca absorption immediately after calving because the bone resorption is delayed for 1 week or more [20]. However, our findings suggest that bone resorption is accelerated at parturition in both multiparous and primiparous cows from the viewpoint of the plasma TRAP5b activity. To resolve this disagreement between the previous and present studies, further investigation is needed to evaluate the participation of the accelerated osteoclastic function indicated by plasma TRAP5b in the amounts of Ca efflux from bone in parturient cows. In addition, further studies are also necessary to delineate the determinable relationship among the bone metabolic markers and factors relating to bone turnover such as estrogen, parathyroid hormone, cortisol and 1,25-dihydroxyvitamin D_3 around parturition in dairy cows. Our data indicate that the periparturient changes in plasma TRAP5b activity in conjunction with other markers of bone metabolism constitute additional background values for clinical guidance in bovine medicine and further investigations of parturient paresis.

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