Short communication: Serum osteoprotegerin concentrations in periparturient dairy cows

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ABSTRACT

Dairy cows experience hypocalcemia at the onset of lactation; however, the contribution of bone metabolism to circulating Ca concentrations during the periparturient period is not well understood. Osteoprotegerin (OPG) protects against loss of skeletal mass by regulating osteoclastogenesis. We previously reported that the bone resorption increased around parturition in dairy cows from the perspective of bone markers in blood. The aim of this study was to explore osteoclastic bone resorption in periparturient dairy cows by measuring changes in serum OPG concentrations around calving. The OPG concentrations were evaluated in primiparous ($n = 9$) and multiparous ($n = 9$) cows at 21 d precalving and over 5 d after calving. Based on mixed model analyses, the multiparous cows had a higher serum OPG concentration than primiparous cows at precalving and exhibited a significant decline in serum OPG and Ca concentrations after calving. In contrast to multiparous cows, primiparous cows did not exhibit these changes. Therefore, we hypothesized that multiparous cows preserve their bone mineral content during late pregnancy and activate the osteoclastic bone resorption after calving. Based on our serum OPG data, we identified the different periparturient osteoclastic bone resorption patterns in healthy primiparous and multiparous dairy cows.

Key words: dairy cow, osteoprotegerin, parturition

Short Communication

Calcium mobilization from bone is termed osteoclastogenesis, differentiation into multinucleated osteoclasts

that degrade bone. Osteoclastogenesis is regulated by the receptor activator of nuclear factor- κ B ligand (RANKL), a member of the tumor necrosis factor superfamily, and its receptor (Hofbauer et al., 2000). Binding of RANKL to the receptor on precursor osteoclasts initiates gene transcription of osteoclastogenesis (Hofbauer et al., 2000). Osteoprotegerin (OPG), a regulator of bone resorption, inhibits osteoclastogenesis by acting as a decoy receptor for RANKL (Simonet et al., 1997). In general, dairy cows are susceptible to hypocalcemia at the onset of lactation. Because of the slower hormonal response, older cows have a higher risk of clinical hypocalcemia around the time of parturition (parturient paresis, also termed milk fever; Reinhardt et al., 1988). However, the contribution made by bone metabolism to Ca homeostasis remains incompletely understood in cattle. The activity of tartrate-resistant acid phosphatase 5b (TRAP5b), a bone resorption marker exocytosed by osteoclasts, correlates well with the number of osteoclasts (Halleen, 2006). We previously found that the activity of TRAP5b in the blood of dairy cows began to increase 1 wk before parturition, suggesting that osteoclast numbers had risen before the fall in Ca concentration (Devkota et al., 2015). However, to the best of our knowledge, circulating OPG concentrations in dairy cows have not been studied. The aim of our study was to explore osteoclastic bone resorption in primiparous and multiparous cows by measuring sequential changes in peripartum serum OPG concentrations.

Blood samples were collected from 9 primiparous and 9 multiparous Holstein Friesian cows in the farm of Obihiro University of Agriculture and Veterinary Medicine. They were fed a TMR with ad libitum hay (Table 1). All cows underwent normal calving and were declared clinically healthy 5 d after parturition. The average number of lactations was 2.7 in multiparous cows (range = 2 to 4). Body condition scores of cows were 3.00 to 3.50 and BW during the close-up period

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Table 1. Ingredient and nutritional composition of the prepartum and postpartum rations during the study involving 9 primiparous and 9 multiparous dairy cows

Item	Prepartum ration ¹	Postpartum ration ²
Ingredient (% DM, unless noted)		
Grass silage	33.6	32.3
Corn silage	29.1	15.5
Orchard hay	12.0	0.0
Soybean meal	6.4	0.0
Beet pulp	0.0	3.8
Ground corn	0.0	5.4
Concentrate mix	18.7 ³	42.8 ⁴
Vitamin and mineral premix ⁵	0.1	0.1
Magnesium oxide	0.1	0.1
Vitamin E	0.1	0.04
DM (%)	41.6	42.5
Nutrition composition		
CP (% DM)	15.6	15.4
ADF (% DM)	23.5	20.9
Ca (% DM)	0.4	0.5
P (% DM)	0.4	0.4
K (% DM)	1.9	1.6
Mg (% DM)	0.2	0.3
S (% DM)	0.2	0.3
Na ⁶ (% DM)	0.01	0.1
Cl (% DM)	0.4	0.4
DCAD (mEq/100 g of DM)	23.4	15.3

¹Prepartum ration (from -28 to -1 d prepartum).

²Postpartum ration (after calving to mid-lactation).

³Dry Base 17 (nutrient composition on a DM basis: 17.0% CP, 2.0% crude fat, 10% crude fiber, 0.05% Ca and 0.5% P; 71% TDN, including corn, lupin, wheat, bran, rapeseed cake, and so on; Hokuren Cooperative Feed Mills Co. Ltd., Hokkaido, Japan).

⁴Milk Trend 74 (nutrient composition on a DM basis: 18% CP, 2.5% crude fat, 11% crude fiber, 0.55% Ca and 0.4% P; 74.0% TDN, including corn, corn gluten feed, bran, corn distillers by-products, and rapeseed cake; Chubu Shiryō Co. Ltd., Nagoya, Japan).

⁵Yokabita M [contents per gram: 5,000 IU of vitamin A, 1,000 IU of vitamin D₃, 2 mg of vitamin E, 0.2 mg of vitamin K₃, 0.5 mg of vitamin B₁, 1 mg of vitamin B₂, 0.1 mg of vitamin B₆, 1 µg of vitamin B₁₂, 6 mg of nicotinic acid, 2 mg of chlorine chloride, 10 mg of pantothenic acid, 156 µg of Mn (MnSO₄), 700 µg of Zn (ZnSO₄), 550 µg of Fe (FeSO₄), 139 µg of Cu (CuSO₄), 325 µg of I (Ca(IO₃)₂), 38 µg of Co (CoSO₄), 1 mg of methionine, 0.5 mg of lysine hydrochloride and 50 ng of ethoxyquin; Snow Brand Seed Co. Ltd., Sapporo, Japan].

⁶A lack of Na intake was compensated by mineral salt brick.

was 594 ± 86.9 and 729 ± 49.5 kg (mean $\pm$ SD) in the primiparous and multiparous groups, respectively. The average milk production of the primiparous and the multiparous cows during the 5-d postcalving period was 19.8 and 28.1 kg/d, respectively. Dry matter intake of the primiparous and the multiparous cows, predicted by the NRC (2001) equations, were 10.1 and 14.2 kg/d for the close-up period and 10.8 and 13.6 kg/d for the 5-d postpartum period, respectively.

Coccygeal blood samples were withdrawn 3 wk before calving (-21 d) and 4 times after calving [immediately after calving (0 d), 12 h (0.5 d), and 2 and 5 d later]. The experimental design was approved by

the Obihiro University of Agriculture and Veterinary Medicine Laboratory Animal Care and Use Committee (approval #28-156).

Serum Ca concentrations were measured by a biochemical autoanalyzer (TBA-120FR, Toshiba Medical Systems, Otawara, Japan). Serum OPG concentrations were measured using a commercial kit (Bovine OPG ELISA kit, NeoScientific, Cambridge, MA) according to the manufacturer's instructions (<https://neobiolab.com/elisa-kits/bovine-opg/bo0027>). This ELISA is a quantitative competitive immunoassay that uses an antibody against bovine OPG. After incubation with horseradish peroxidase at 37°C for 60 min, OPG concentrations were measured by recording absorbances at 450 nm. The TRAP5b concentrations in plasma were measured by a fluorometric method using naphthol-ASBI-phosphate (Wako Pure Chemical Corp., Osaka, Japan) as the substrate. We used a modified version of the Janckila method (Yamagishi et al., 2009; Matsuo et al., 2014), where 10-µL amounts of plasma were added to a 0.25-mmol/L naphthol-ASBI-phosphate solution containing 100 mmol/L of sodium acetate and 50 mmol/L of sodium tartrate (Wako Pure Chemical Corp.). Following incubation at 37°C for 30 min, fluorescence was detected (excitation wavelength = 405 nm, emission wavelength = 535 nm) using a multilabel counter (ALVO MX/ Light 1420; PerkinElmer, Waltham, MA). The intra- and interassay coefficients of variation were 11.3 and 9.7% for the OPG ELISA and 5.6 and 7.8% for TRAP5b fluorometry, respectively.

All data were analyzed in the mixed model for repeated measures using SAS enterprise guide (ver. 7.1, SAS Institute Inc., Cary, NC). The fixed effects (group, day, and group $\times$ day) with the random effect of individual cows were assessed, followed by Tukey's post-hoc test. The Spearman's rank correlation analysis was done by for nonparametric distributions. All numerical values are shown as mean $\pm$ standard error of the mean and the level of significance was set at $P < 0.05$.

Figure 1 shows the changes in serum Ca concentrations around the time of parturition in primiparous and multiparous dairy cows. We observed no apparent differences in serum Ca concentrations between groups. The multiparous group decreased the serum Ca concentrations significantly ($P < 0.01$) from -21 d to 0 d (2.5 ± 0.04 to 2.1 ± 0.08 mmol/L), and returned to the prepartum concentrations at 5 d (2.4 ± 0.04 mmol/L).

Figure 2 shows the changes in serum OPG concentrations and plasma TRAP5b activities around the time of parturition in primiparous and multiparous dairy cows. The serum OPG concentrations were significantly higher ($P < 0.01$) in the multiparous compared with the primiparous group at -21 d (7.5 ± 0.4 vs. $5.0 \pm$

Table 2. Spearman’s rank correlation coefficient (ρ) and P -values between the serum osteoprotegerin (OPG) and Ca concentrations in 18 dairy Holstein parturient cows

Item	−21 d	0 d	0.5 d	2 d	5 d
ρ	0.06	0.45	0.04	0.29	0.14
P -value	0.81	0.06	0.87	0.25	0.57

0.6 ng/mL). Compared with −21 d, the OPG concentration fell significantly ($P < 0.01$) at 0 d (3.9 ± 0.3 ng/mL) and remained low until 5 d ($3.7\text{--}4.4$ ng/mL) in the multiparous group, but not in the primiparous group (mean = $5.0\text{--}5.6$ ng/mL). The plasma TRAP5b activities peaked at 0 d in both the multiparous and the primiparous groups (2.1 ± 0.2 and 3.1 ± 0.3 U/L). The TRAP5b concentrations in the primiparous group during the experimental period were significantly ($P < 0.01$) higher than those in the multiparous group. We found no significant correlations between serum OPG and Ca concentrations at each time point (Table 2).

In the multiparous cows, the serum OPG concentration was higher than that of primiparous cows at −21 d and decreased after calving. In primiparous cows, the serum OPG concentration was stable during the peripartum period; OPG prevents bone resorption and protects ovariectomized rats against excessive skeletal loss (Simonet et al., 1997). Multiparous cows

Figure 1. Changes in serum Ca concentrations around the time of parturition in the primiparous ($\square$; $n = 9$) and multiparous ($\bullet$; $n = 9$) groups (−21 to 5 d relative to calving; means $\pm$ SEM). **Significant decreases from the concentrations at −21 d in the multiparous group ($P < 0.01$).

use the dry period to replenish the bone mineral content lost during the last lactation (Reinhardt et al., 1988). Primiparous cows are often still growing and, as such, bone growth and bone remodeling is more active

Figure 2. Changes in serum osteoprotegerin (OPG) concentrations and plasma tartrate-resistant acid phosphatase 5b (TRAP5b) activities around the time of parturition in the primiparous ($\square$; $n = 9$) and multiparous ($\bullet$; $n = 9$) groups (−21 to 5 d relative to calving; means $\pm$ SEM). Different letters (A,B) indicate significant differences between groups (B: $P < 0.01$, A: $P < 0.05$). Asterisks denote a significant difference from the value at −21 d in the multiparous group (** $P < 0.01$, * $P < 0.05$); daggers indicate a significant difference from the value at −21 d in the primiparous group ($\dagger\dagger P < 0.01$).

through gestation than would be expected in mature nongrowing multiparous cows, in which OPG protects their bone from excessive loss during late pregnancy (Wathes et al., 2007; Yamagishi et al., 2009).

Most postpartum dairy cows experience varying degrees of hypocalcemia as lactational Ca demand increased (Reinhardt et al., 1988). We found that multiparous cows exhibited an acute decline in the serum Ca concentration at calving, with lower serum OPG concentrations compared with the prepartum values. However, primiparous cows did not exhibit any marked difference in either serum Ca or OPG concentrations from prepartum values. These data suggest that bone resorption only increased immediately postpartum based on TRAP5b data in multiparous cows compared with 3 wk prepartum. This contrasted primiparous cows, whose OPG concentrations remained constant over the sampling period but showed a higher and more sustained evidence of increased bone resorption postpartum based on TRAP5b. Our correlation analysis suggests this OPG-induced Ca mobilization was not the simultaneous response to Ca insufficiency. However, due to the lack of the sequential changes in the serum OPG concentrations toward calving, it is still unknown how the circulating OPG regulates the sudden Ca loss in calving cows.

At the day of calving, dairy cows exhibited elevated levels of TRAP5b but only multiparous cows reduced serum Ca levels, implying the osteoclasts increased irrespective of those resorbing capacity, as TRAP5b is secreted by osteoclasts at various stages of maturity (Halleen, 2006). In osteoporotic rats, TRAP5b in the osteocytes and osteoblasts act together with RANKL/OPG when regulating osteoclastogenesis (Solberg et al., 2015). Moreover, *TRAP5b* gene expression was elevated during osteocytic bone remodeling in lactating mice (Qing et al., 2012). Therefore, plasma TRAP5b activity may be influenced by complicated bone-resorbing interactions among osteoblasts, osteoclasts, and osteocytes. Further studies are needed to understand the precise mechanism underlying osteocytic osteolysis and the biochemical significance of blood TRAP5b activity in bone resorption.

Some studies reported the changes of other bone resorption markers in multiparous cows during the transition period. Liesegang et al. (2007) observed the serum carboxyterminal telopeptide of type-I collagen (ICTP) concentration restrained before calving and increased during early postpartum period. Taylor et al. (2009) reported a similar result using serum deoxypyridinoline (DPD), as the inhibition of bone resorption had continued around parturition. Devkota et al. (2015) revealed the increase in the number of bone-resorbing

cells before parturition; on the other hand, Teramura et al. (2015) found the acute decline of the serum cross-linked N-telopeptide of type I collagen (NTx) on the day of calving.

In conclusion, we found that the serum OPG concentration in multiparous cows was high at 3 wk prepartum, but lowered after calving to the same level as that in primiparous cows, suggesting the different pattern of peripartum bone metabolism between primiparous and multiparous cows. In contrast to primiparous cows, multiparous cows seemed to activate their osteoclastic bone degradation at calving. Based on our pilot study with the small sample size, we suggest that the further studies are needed to clarify how the changes in circulating OPG concentrations associated with its Ca levels in periparturient dairy cows with various health status.

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