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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-κB 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

[1]Prepartum ration (from −28 to −1 d prepartum).

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

[3]Dry 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).

[4]Milk 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 Shiryo Co. Ltd., Nagoya, Japan).

[5]Yokabita M [contents per gram: 5,000 IU of vitamin A, 1,000 IU of vitamin D_3, 2 mg of vitamin E, 0.2 mg of vitamin K_3, 0.5 mg of vitamin B_1, 1 mg of vitamin B_2, 0.1 mg of vitamin B_6, 1 μg of vitamin B_{12}, 6 mg of nicotinic acid, 2 mg of chlorine chloride, 10 mg of pantothenic acid, 156 μg of Mn ($MnSO_4$), 700 of μg Zn ($ZnSO_4$), 550 μg of Fe ($FeSO_4$), 139 μg of Cu ($CuSO_4$), 325 μg of I ($Ca(IO_3)_2$), 38 μg of Co ($CoSO_4$), 1 mg of methionine, 0.5 mg of lysine hydrochloride and 50 ng of ethoxyquin; Snow Brand Seed Co. Ltd., Sapporo, Japan].

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

was 594 ± 86.9 and 729 ± 49.5 kg (mean ± 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 × 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 ± 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 ±

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