# Association between Changes in Plasma Calcium Concentration and Plasma Tartrate-Resistant Acid Phosphatase Activity in Periparturient Cows

Jun SATO[1], Reeko SATO[1], Aiko TAKAGI[1], Taichi GOTO[2]*, Keiji OKADA[3], Jun YASUDA[3] and Yoshihisa NAITO[1]

[1]*Departments of Clinical Veterinary Medicine and* [3]*Veterinary Hospital, Faculty of Agriculture, Iwate University, Ueda, Morioka, Iwate 020–8550 and* [2]*Koiwai Farm Co., Ltd., 26-Jiwari Maruyachi, Shizukuishi-chou, Iwate 020–0507, Japan*

ABSTRACT. Eight periparturient Holstein Friesian cows were examined for plasma tartrate-resistant acid phosphatase (TRAP) activity to assess the degree of bone metabolic activity and to evaluate the association between the change in calcium (Ca) concentration and bone metabolism during the periparturient period. Milk fever occurred in 1 of 8 cows just after parturition. Plasma TRAP activities did not markedly change in 5 of 8 cows during the experimental period. The changing rate of Ca between preparturition and just after parturition was under –20% in 3 of 8 cows, and low TRAP activities were observed in 2 of these 3 cows. This study suggests that cows with a low TRAP activity are at risk of developing milk fever in comparison to cows with high TRAP activity. Temporary increases of parathyroid hormone were observed in 7 cows, but not in the cow with milk fever.
KEY WORDS: bovine, milk fever, TRAP.

Many methods have been reported for the prevention of milk fever [8, 9], including the implementation of a low calcium (Ca) diet or the regulation of the dietary anion cation difference (DCAD) in the prepartum period [7, 9, 13, 16, 25]. Both of these methods aim to prevent milk fever by activating bone resorption and increasing Ca mobilization from the bones [7, 9, 13, 16, 25]. The importance of bone metabolism in milk fever has been established [8, 9] and many reports have evaluated bone metabolism in milk fever prevention studies [4, 5, 7, 13].

In recent years, studies on human bone metabolism with regard to osteoporosis have advanced [15]. The regulation, function and features of osteoclasts and osteoblasts, and the components and metabolism of the bone matrix in humans have been clarified [15]. Blood and urine markers of bone metabolism have been identified and include bone specific alkaline phosphatase [1, 3, 21], osteocalcin [3, 21], type 1 procollagen propeptide [1], tartrate-resistant acid phosphatase (TRAP) [1, 3, 21, 23], hydroxyproline (Hpr) [3, 21], pyridinoline (Pyr) [24], deoxypyrdinoline (D-Pyr) [24] and type 1 collagen cross-linked c-terminal teropeptide [3, 23, 24]. The levels of these markers positively correlate with the degree of bone metabolic activity [15], and are therefore used in the diagnosis and monitoring of treatment of patients with metabolic bone disease [1, 3, 24]. Many of these markers are evaluated by urinary concentration [15], but it is difficult to collect urine from cows, especially in the field. Hpr concentration in the blood and urine are usually used for the evaluation of bovine bone metabolism [4, 5, 17]. But measuring Hpr concentration is a complicated and time-consuming process [2, 12]. Recently, Liesegang *et al.* [14] evaluated bone metabolic activity by measuring the urinary concentration of Pyr and D-Pyr in postparturient cows, but

again, this procedure is complicated. We investigated bone metabolic markers in the serum or plasma, and found that TRAP is an easily measured and useful bone metabolic marker in cows [19].

In this study, to investigate the degree of bone metabolic activity and the association between change in plasma Ca concentration and bone metabolism, TRAP activity, Ca and parathyroid hormone (PTH) plasma were measured in periparturient cows.

Eight Holstein Friesian cows were examined during the periparturient period. All of the cows were clinically healthy and ranged from 3 to 6 years of age. A total of 7,300–10,600 kg of milk was collected over 305 days of the previous milking period. The cows were fed total mixed ration and dried timothy, with a 0.67% Ca content and a Ca/P ratio of 1.59. Clinical observation and blood sampling were performed 1 day before (pre.), just after (0d.) and 1 day (1d.) and 2 days (2d.) after calving. But sampling was more frequent when calving was imminent. Plasma was separated by centrifugation and was stored at –20°C until analysis. Plasma TRAP activity was measured enzymatically [19]. The plasma concentration of Ca was measured by atomic absorption spectrophotometry [18]. The plasma concentration of PTH was measured using a two-site immunoradiometric assay with human intact PTH standards [6]. The Ca change rate between pre. and 0d. was calculated as follows.

Ca change rate pre.-0d.=
(plasma Ca conc. at 0d.- plasma Ca conc. at pre.)/plasma Ca conc. at pre. $\times$ 100.

Milk fever was exhibited in 1 of 8 cows just after calving, but the cow was able to stand up immediately after intravenous administration of Ca borogluconate solution. The plasma Ca concentration of these cows ranged from 7.0–9.3 mg/d*l* at pre. (Fig. 1-a), temporarily decreasing on 0d. or 1d., but recovering by 2d., except for 1 cow (Fig. 1-a). However,

* PRESENT ADDRESS: GOTO, T., Iwate Breeding Service, Nishine-sakura 20, Kanegasaki-chou, Iwate 029–4503, Japan.
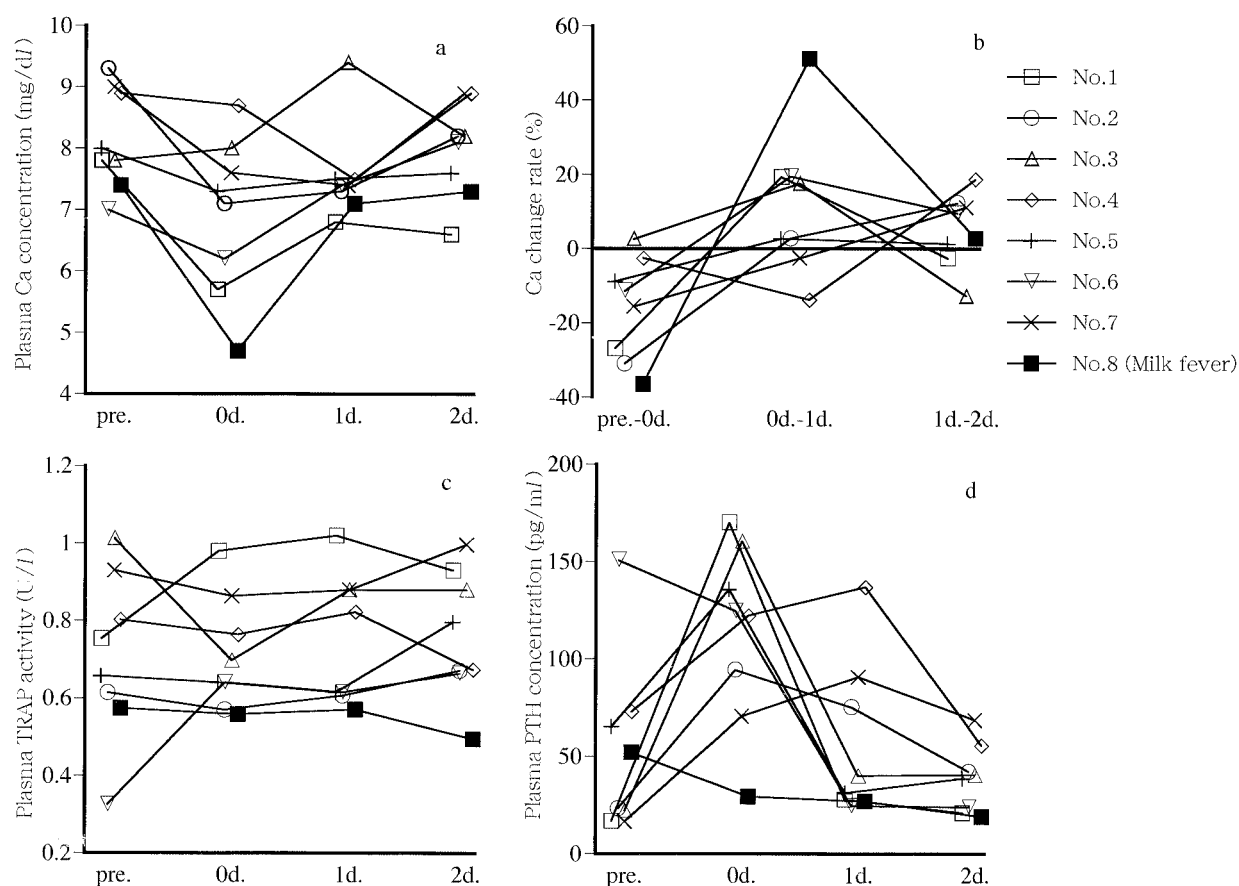
Fig. 1.    Variation of Ca concentration (a) and Ca change rate (b), TRAP activity (c) and PTH concentration (d) in the plasma of 8 peripar-
turient cows.

although the cow with milk fever (No. 8) showed hypocal-
cemia (4.7 mg/d$l$) at 0d., plasma Ca concentration recovered
to 7.1 mg/d$l$ after treatment by 1d.  The Ca change rate pre.-
0d. (Fig. 1-b) of these cows ranged from −36.5 to 2.6%, with
a positive change in only one cow.  The Ca change rates 0d.-
1d., and 1d.-2d. were positive in all but 2 cows.  The plasma
TRAP activity (Fig. 1-c) of these cows ranged from 0.33–
1.01 U/$l$ at pre., increasing to more than 0.5 U/$l$ by 0d., 1d.
and 2d.  The plasma TRAP activity of the cow with milk
fever (No. 8) was markedly low (< 0.6 U/$l$) during the exper-
imental period.  The plasma PTH concentration (Fig. 1-d) of
these cows ranged from 16–150 pg/m$l$ at pre., temporarily
increasing from pre. to 1d. in 7 cows, but not in cow No. 8.

The plasma TRAP activity did not change remarkably in
5 of the 8 cows during the duration of this study.  Liesegang
*et al.* [14] reported that the concentrations of urinary Pyr and
D-Pyr, and serum Hpr did not increase within 2 days after
calving, suggesting that bone metabolism remains stable in
the peripartum period.  The Ca change rate pre.-0d. of cows
No. 1, 2 and 8 were less than −20%, and the pre. plasma
TRAP activities were 0.75, 0.61 and 0.57 U/$l$, with mark-
edly low levels in cows No. 2 and 8.  Sechen *et al.* [20]
reported that the serum Hpr concentration in cows with milk

fever is lower than that of cows without milk fever during
the periparturient period, and suggested that bone metabo-
lism is inactive in cows with milk fever.  The present results
suggest that bone metabolism does not change in the peri-
parturient period, but the degree of bone metabolic activity
affects the plasma Ca concentration during this time.

In cow No. 8, the temporary increase of PTH observed in
other cows did not occur.  PTH increases bone and renal
resorption of Ca, and induces the renal enzyme to produce
the vitamin D metabolite, 1,25-dihydroxyvitamin D
[1,25(OH)$_2$D] [11].  1,25(OH)$_2$D stimulates the active trans-
port of dietary Ca across the intestinal epithelium [22].
Yamagishi and Naito [26] reported that the plasma
1,25(OH)$_2$D concentration increased following the tempo-
rary increase of PTH in periparturient cows.  This
1,25(OH)$_2$D increase did not occur in cow No. 8, presum-
ably because of inadequate PTH secretion, despite the pres-
ence of hypocalcemia.  It seems that the Ca regulating
organs were inactive in cow No. 8.  Cows with clinical signs
of milk fever have higher levels of PTH and 1,25(OH)$_2$D
than unaffected cows [10].  In such cases, it is thought that
the Ca regulating organs exhibit a slow or absent response to
PTH and 1,25(OH)$_2$D, and fail to maintain plasma Ca levels,

resulting in hypocalcemia [10]. However, Yamagishi *et al.* [27] reported that this absence of PTH increase was observed in only a few cows with milk fever. The No. 8 cow of this experiment was suspected to have a PTH secretion disorder. The lower levels of TRAP activities in cow No. 8 than in the other cows may be the result of inadequate PTH secretion.

Blood Ca concentration is not directly reflected by bone metabolism, because Ca homeostasis is regulated by a number of organs. In this study, low Ca change rates at pre.-0d. were observed in cows that exhibited low TRAP activity. This study suggests that cows with low TRAP activities are at increased risk of developing milk fever as compared with high TRAP activity, but further study with a large number parturient cows is required to confirm this.

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