NOTE *Internal Medicine*

Changes in the plasma levels of several bone markers in newborn calves during the first two days of life

Kaoru HATATE[1)], Kazuhiko SHINYA[1)], Asako MATSUO-SATO[1)], Shuichi SASAKI[2)], Bhuminand DEVKOTA[1,3)], Masahiro TAKAHASHI[1)], Toichi HIRATA[2)] and Norio YAMAGISHI[1,4)]*

[1)]*Cooperative Department of Veterinary Medicine, Iwate University, Morioka, Iwate 020–8550, Japan*
[2)]*Field Science Center, Faculty of Agriculture, Iwate University, Shizukuishi, Iwate 020–0581, Japan*
[3)]*Center for Biotechnology, Agriculture and Forestry University, Rampur, Chitwan, Nepal*
[4)]*Current affiliation: Department of Clinical Veterinary Medicine, Obihiro University of Agriculture and Veterinary Medicine, Inada, Obihiro, Hokkaido 080–8555, Japan*

(Received 16 June 2015/Accepted 28 September 2015/Published online in J-STAGE 10 October 2015)

ABSTRACT. The fluctuations in the plasma levels of several bone markers were investigated in newborn calves. Experiment 1 monitored the postnatal changes in the plasma levels of tartrate-resistant acid phosphatase isoform 5b (TRAP5b), total alkaline phosphatase (t-ALP) and bone-specific alkaline phosphatase (BAP) in four calves. These markers increased significantly from 9−20 hr after the first colostrum-suckling compared with the values immediately after birth. Experiment 2 evaluated changes in the plasma TRAP5b, t-ALP, BAP and type I collagen cross-linked N-telopeptide (NTx) levels within 2 days post-birth in five calves with successful passive immunization via colostrum (non-deficient group) and five others with poor colostrum intake (deficient group). The non-deficient group had significantly higher plasma levels of the four parameters around 12 hr of life compared with the deficient group. The results suggest that the increase in plasma bone markers in calves in the first day of life is related to the colostrum intake.
KEY WORDS: blood, bone marker, calf, colostrum, neonatal period

doi: 10.1292/jvms.15-0358; *J. Vet. Med. Sci.* 78(2): 337–340, 2016

The changes in the plasma levels of various bone markers with bone turnover are used in the diagnosis of human skeletal diseases [23, 25], while the clinical use of bone markers in veterinary medicine is limited. In cattle, tartrate-resistant acid phosphatase isoform 5b (TRAP5b), a lysosomal enzyme reflecting the number of activated osteoclasts [17], is a circulating bone resorption marker that can be quantified using a sensitive fluorometric assay [26]. The age-related and periparturient changes in plasma TRAP5b activity and other bone markers have been described in beef and dairy cattle [4, 5, 10, 26]. The plasma total alkaline phosphatase (t-ALP) activity is a useful index of bone formation in subjects with normal liver function [20]. However, the bone-specific isoenzyme of alkaline phosphatase (BAP) provides a better index of bone formation than t-ALP in the presence of liver disease, due to an increase in the liver-derived isoenzyme [20]. In skeletally immature animals, circulating BAP tends to predominate the alkaline phosphatase (ALP) isoenzymes because of high bone turnover rates [1]. Interestingly, a transient increase in plasma t-ALP activity has been reported in calves within the first 12 hr of life [27]; to our knowledge, however, the information on plasma bone markers in newborn calves is limited. Hence, we examined the plasma changes in several bone markers in neonatal calves within a few days after birth.

This study involved two experiments in clinical healthy calves to examine the fluctuations in plasma bone markers after the first colostrum-suckling (experiment 1) and the relationship between plasma bone markers and colostrum intake (experiment 2). The Iwate University Laboratory Animal Care and Use Committee approved the study protocol and experimental design of experiment 1 (#A201433) and experiment 2 (#A201449).

Experiment 1 involved four Japanese Black calves (one male and three females; 28.0−32.2 kg body weight) born at the Veterinary Teaching Hospital of Iwate University. The calves were reared in pens with their dams to allow voluntary suckling. All four calves had accomplished the first suckling within 2 hr after birth. Blood was collected immediately after birth (0 hr) and 2, 4, 6, 9, 12, 16, 20, 24, 36, 48 and 60 hr after the first colostrum-suckling. Blood was withdrawn from the jugular vein into 6-m*l* heparinized blood collection tubes (BD Vacutainer; Becton, Dickinson and Co., Franklin Lakes, NJ, U.S.A.) for measurement of plasma TRAP5b, t-ALP and BAP activities.

Experiment 2 involved 10 Japanese Black calves at the Iwate University Farm. The calves had been born in pens and reared with their dams to allow voluntary suckling. Since this farm is located ~25 km from our laboratory, calving was monitored using a web camera. Blood was withdrawn from the jugular vein of each calf 0.5−3 hr (day 0), 11−16 hr (day 0.5) and 42−53 hr (day 2) post-birth into 6-m*l* heparinized blood collection tubes for measurement of plasma TRAP5b,

*CORRESPONDENCE TO: YAMAGISHI, N., Department of Clinical Veterinary Medicine, Obihiro University of Agriculture and Veterinary Medicine, Inada, Obihiro, Hokkaido 080–8555, Japan.
e-mail: yamagishi@obihiro.ac.jp

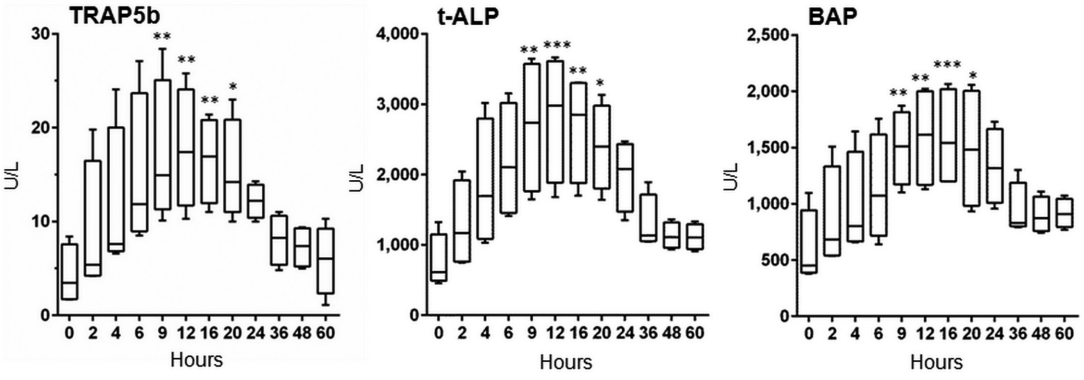

Fig. 1. Box-and-whisker plots (medians, interquartile ranges, maxima and minima) showing the plasma fluctuations in tartrate-resistant acid phosphatase isoform 5b (TRAP5b), total alkaline phosphatase (t-ALP) and the bone-specific isoenzyme of alkaline phosphatase (BAP) in four neonatal calves after the first colostrum-suckling. Significant differences compared with the levels immediately after birth (0 hr) for each parameter are shown: *P<0.05, **P<0.01, ***P<0.001.

t-ALP, BAP and type I collagen cross-linked N-telopeptide (NTx) levels, and a 3.5-m*l* serum-separating tube (BD Vacutainer; Becton, Dickinson and Co.) to determine serum total protein (TP) and γ-glutamyltransferase (GGT). The serum TP and GGT levels at days 0.5 and 2 were used as indices of colostrum intake according to the cut-off values (TP, 52 g/*l*; and GGT, 200 U/*l*) for the failure of passive transfer (FTP) of colostral immunoglobulins [12, 15, 22, 24]. The calves were then divided into two groups: one group consisting of five calves meeting the cut-off values for FTP, defined as the non-deficient group (two males and three females; 21.4−24.8 kg body weight) and the other group consisting of other five calves with the levels below the cut-off values, defined as the deficient group (five females; 19.0−40.9 kg body weight). Of all calves, three were unable to stand up and begin suckling by 12 hr after birth; therefore, they were assigned to the deficient group. In an interview to the labors of the farm, this colostrum deprivation did not seem to affect their subsequent growth for the calves in this experiment.

The plasma and serum were separated by centrifugation and stored at −80°C until analysis. The plasma TRAP5b activity was measured fluorometrically [9, 13]. Briefly, the plasma samples were added to substrate consisting of naphthol-ASBI-phosphate in sodium acetate buffer containing heparin adjusted to pH 6.6 and allowed to react for 30 min at 37°C. Reagent blanks were prepared for each plasma sample, and a standard calibration curve was constructed using acid phosphatase (ACP) of known concentration. Fluorescence was measured with an excitation wavelength of 405 nm and a peak emission wavelength of 535 nm. Plasma t-ALP and BAP activities were measured spectrophotometrically using a LabAssay ALP kit (Wako Pure Chemical Industries, Ltd., Osaka, Japan). Plasma BAP activity was measured using the heat-inactivation method [14]; plasma samples were incubated at 56°C for 15 min, and aliquots were removed for measurement of heat-insensitive ALP. The plasma BAP activity was calculated by subtracting the heat-insensitive ALP activity from the t-ALP activity. The plasma NTx concentration was measured using an enzyme-linked immunosorbent assay (ELISA) validated for cattle (Osteomark NTx serum; TECOmedical, Sissach, Switzerland). Serum TP and GGT levels were determined using an Accute TBA-40FR autoanalyser (Toshiba Medical Systems CO., Otawara, Japan).

All numerical data were expressed as medians, with the minimum and maximum values and interquartile range (IQR). In experiment 1, the biochemical data were analyzed using the Friedman test with Dunn's multiple comparisons to evaluate the fluctuation in each parameter. In experiment 2, the plasma parameters were analyzed using two-way repeated-measures (RM) ANOVA (group × day) and the Šídák multiple comparisons test to evaluate the difference between two groups and the within-group variation. Spearman's rank correlation was used to evaluate the correlation of serum TP or GGT at day 0.5 with plasma levels of TRAP5b, t-ALP, BAP or NTx at day 0.5. All statistical analyses were performed using Prism ver. 6 for Windows (GraphPad Software Inc., La Jolla, CA, U.S.A.). The level of significance was set at P<0.05.

In experiment 1 (Fig. 1), the plasma TRAP5b, t-ALP and BAP activities increased significantly from 9 to 20 hr after the first colostrum-suckling (P<0.05 to 0.001).

In experiment 2, the medians (minimum values-maximum values) of GGT and TP values at day 0.5 were 1,191 (1,064.1–4,765.1) U/*l* and 52.5 (49.4–68.3) g/*l* in the non-deficient group and 70 (6.6–399.9) U/*l* and 38.8 (27.1–43.9) g/*l* in the deficient group, respectively. Two-way RM ANOVA (group × day) revealed a significant interaction across group and day (P<0.01 to 0.001) and significant group (P<0.05 to 0.01) and day (P<0.001) effects in all parameters. In the non-deficient group, the plasma TRAP5b, t-ALP and BAP activities increased significantly (P<0.001) at day 0.5, and the NTx concentration increased significantly (P<0.001) at days 0.5 and 2, compared with the values at day 0 (Fig. 2). Similarly, the non-deficient group had significantly higher plasma levels (P<0.01 to 0.001) of TRAP5b, t-ALP and BAP at day 0.5 and NTx at days 0.5 and 2 compared with the deficient group. According to Spearman's rank correlation, the serum TP concentration was correlated with the TRAP5b

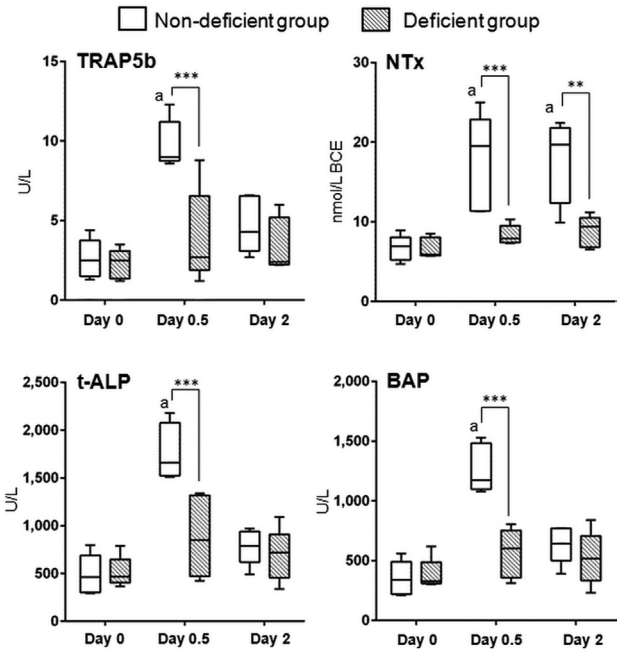

Fig.2. Box-and-whisker plots (medians, interquartile ranges, maxima and minima) showing the plasma changes in tartrate-resistant acid phosphatase isoform 5b (TRAP5b), type I collagen cross-linked N-telopeptide (NTx), total alkaline phosphatase (t-ALP) and the bone-specific isoenzyme of alkaline phosphatase (BAP) in the non-deficient (n=5) and deficient (n=5) groups. The letter "a" indicates a significant (P<0.001) difference from the level at day 0 within the non-deficient group. Asterisks indicate significant differences between the two groups at the same time point: **P<0.01, ***P<0.001.

(r=0.83, P=0.0047), t-ALP (r=0.94, P=0.0002), BAP (r=0.94, P=0.0002) and NTx (r=0.87, P=0.0022) levels; the serum GGT value was correlated with the TRAP5b (r=0.76, P=0.0149), t-ALP (r=0.94, P=0.0002), BAP (r=0.94, P=0.0002) and NTx (r=0.85, P=0.0029) levels.

The plasma t-ALP activity is reported to increase transiently after colostrum intake, suggesting the absorption of colostral ALP [7, 27]. Experiment 1 in our study revealed a remarkable transient elevation of the plasma TRAP5b and BAP activities within 1 day of the first colostrum-suckling, and the fluctuations coincided with the increase in plasma t-ALP activity. In experiment 2, there was marked elevation of the plasma TRAP5b, BAP and NTx levels similar to that of t-ALP within 1 day of life in the non-deficient group, and the plasma levels of these parameters at day 0.5 were strongly correlated with the serum indices (TP and GGT) of colostrum intake in neonatal calves. These observations indicate that the postnatal increase in plasma bone markers is due to the colostrum intake by calves. However, the present research lacked the precise data of the amount of colostrum taken by each calf, so that more definitive relationship between colostrum intake and the plasma bone marker levels would remain to be elaborated in our future study.

Although the ALP activity is highest in the first colostrum of cattle, and decreases thereafter [27], the precise levels of bone markers in bovine colostrum remain unknown. It was reported that ALP was localized in plasma membranes of epithelial and myoepithelial cells in the mammary glands of lactating rats [11]. Sato *et al*. [19] demonstrated that the ALP isoenzyme in bovine milk whey closely resembled BAP because of the similarities in the migration pattern on polyacrylamide gel disk electrophoresis and lectin affinity. Boyd [3] reported that the ACP activity was higher in bovine colostrum whey than that in the serum of calves after feeding colostrum. Recently, Bouroutzoglou *et al*. [2] reported that human breast milk contained NTx at concentrations almost equal to that in maternal serum. It has not been elucidated so far whether colostrum contains TRAP5b or not. However, our preliminary data of colostrum and milk from 6 dams in experiment 2 indicated TRAP5b, BAP and t-ALP levels in colostrum corrected at day 0.5 were significantly higher than that of milk corrected at day 2 (the medians of TRAP5b, BAP and t-ALP at day 0.5 were 2.7 U/*l*, 1,358.1 U/*l* and 3,322.9 U/*l*, respectively, which decreased to 1.01 U/*l*, 344.7 U/*l* and 940.9 U/*l* at day 2).

In experiment 2, the increase in plasma NTx concentration in the non-deficient group was prolonged until day 2, while the other bone markers showed a transient elevation at day 0.5. Circulating NTx is excreted in urine [8, 18], unlike TRAP5b and BAP, which are degraded mainly in the circulation [16, 21]. The glomerular filtration rate of calves is lowest during the first day of life [6]. Therefore, we postulate that this prolonged elevation of plasma NTx was due to immature renal function in newborn calves.

In summary, this study showed that newborn calves consuming sufficient colostrum showed a dramatic increase in plasma bone markers in the first day of life, suggesting the absorption of colostral bone markers. In neonatal calves, plasma bone metabolic markers were unlikely to represent the precise level of bone metabolism; however, it is suggested those values could be used as alternative indices of colostrum ingestion. Moreover, if these markers were transported via colostrum, monitoring the bone metabolic markers in milk could be helpful to evaluate the dam's bone metabolism around parturition. However, Boyd [3] reported a small increase in circulating t-ALP in newborn calves after consumption of ALP-free pasteurized milk. Therefore, further studies should examine the source of the transient increase in bone markers in calves after the first colostrum feeding to elucidate the clinical utility of bone metabolic markers in neonatal calves.

REFERENCES

1. Allen, M. J. 2003. Biochemical markers of bone metabolism in animals: uses and limitations. *Vet. Clin. Pathol.* **32**: 101–113. [Medline] [CrossRef]
2. Bouroutzoglou, M., Malamitsi-Puchner, A., Boutsikou, M., Marmarinos, A., Baka, S., Boutsikou, T., Hassiakos, D., Gourgiotis, D. and Briana, D. D. 2014. Biochemical markers of bone resorption are present in human milk: implications for maternal and neonatal bone metabolism. *Acta Paediatr.* **103**: 1264–1269. [Medline] [CrossRef]

3. Boyd, J. W. 1989. Serum enzyme changes in newborn calves fed colostrum. *Vet. Clin. Pathol.* **18**: 47–51. [Medline] [CrossRef]
4. Devkota, B., Takahashi, M., Sasaki, K., Osawa, T., Izaike, Y. and Yamagishi, N. 2013. Fluctuation in plasma bone metabolic markers in multiparous and primiparous Holstein cows during early to peak lactation. *J. Vet. Med. Sci.* **75**: 1257–1260. [Medline] [CrossRef]
5. Devkota, B., Takahashi, M., Sato, S., Sasaki, K., Ueki, A., Osawa, T., Takahashi, M. and Yamagishi, N. 2015. Plasma fluctuation in estradiol-17β and bone resorption markers around parturition in dairy cows. *J. Vet. Med. Sci.* **77**: 875–878. [Medline] [CrossRef]
6. Dratwa, A. 2006. Atrial natriuretic peptide and renal haemodynamics in newborn calves. *Acta Vet. Brno.* **75**: 477–483. [CrossRef]
7. Egli, C. P. and Blum, J. W. 1998. Clinical, haematological, metabolic and endocrine traits during the first three months of life of suckling simmentaler calves held in a cow-calf operation. *Zentralbl. Veterinarmed. A* **45**: 99–118. [Medline] [CrossRef]
8. Hanson, D. A., Weis, M. A., Bollen, A. M., Maslan, S. L., Singer, F. R. and Eyre, D. R. 1992. A specific immunoassay for monitoring human bone resorption: quantitation of type I collagen cross-linked N-telopeptides in urine. *J. Bone Miner. Res.* **7**: 1251–1258. [Medline] [CrossRef]
9. Janckila, A. J., Takahashi, K., Sun, S. Z. and Yam, L. T. 2001. Naphthol-ASBI phosphate as a preferred substrate for tartrate-resistant acid phosphatase isoform 5b. *J. Bone Miner. Res.* **16**: 788–793. [Medline] [CrossRef]
10. Kim, D., Yamagishi, N., Ueki, A., Miura, M., Saito, F., Sato, S. and Furuhama, K. 2010. Changes in plasma bone metabolic markers in periparturient dairy cows. *J. Vet. Med. Sci.* **72**: 773–776. [Medline] [CrossRef]
11. Leung, C. T., Maleeff, B. E. and Farrell, H. M. Jr. 1989. Subcellular and ultrastructural localization of alkaline phosphatase in lactating rat mammary glands. *J. Dairy Sci.* **72**: 2495–2509. [Medline] [CrossRef]
12. MacFarlane, J. A., Grove-White, D. H., Royal, M. D. and Smith, R. F. 2014. Use of plasma samples to assess passive transfer in calves using refractometry: comparison with serum and clinical cut-off point. *Vet. Rec.* **174**: 303. [Medline] [CrossRef]
13. Matsuo, A., Togashi, A., Sasaki, K., Devkota, B., Hirata, T. and Yamagishi, N. 2014. Diurnal variation of plasma bone markers in Japanese black calves. *J. Vet. Med. Sci.* **76**: 1029–1032. [Medline] [CrossRef]
14. Mohebbi, A., Khaghani, A. and Mohammadnia, A. 2010. Bone-specific alkaline phosphatase activity in dairy cows. *Comp. Clin. Pathol.* **19**: 33–36. [CrossRef]
15. Parish, S. M., Tyler, J. W., Besser, T. E., Gay, C. C. and Krytenberg, D. 1997. Prediction of serum IgG1 concentration in Holstein calves using serum gamma glutamyltransferase activity. *J. Vet. Intern. Med.* **11**: 344–347. [Medline] [CrossRef]
16. Posen, S. and Grunstein, H. S. 1982. Turnover rate of skeletal alkaline phosphatase in humans. *Clin. Chem.* **28**: 153–154. [Medline]
17. Rissanen, J. P., Suominen, M. I., Peng, Z. and Halleen, J. M. 2008. Secreted tartrate-resistant acid phosphatase 5b is a Marker of osteoclast number in human osteoclast cultures and the rat ovariectomy model. *Calcif. Tissue Int.* **82**: 108–115. [Medline] [CrossRef]
18. Rosen, H. N., Dresner-Pollak, R., Moses, A. C., Rosenblatt, M., Zeind, A. J., Clemens, J. D. and Greenspan, S. L. 1994. Specificity of urinary excretion of cross-linked N-telopeptides of type I collagen as a marker of bone turnover. *Calcif. Tissue Int.* **54**: 26–29. [Medline] [CrossRef]
19. Sato, J., Kanata, M., Yasuda, J., Sato, R., Okada, K., Seimiya, Y. and Naito, Y. 2005. Changes of serum alkaline phosphatase activity in dry and lactational cows. *J. Vet. Med. Sci.* **67**: 813–815. [Medline] [CrossRef]
20. Seibel, M. J. 2005. Biochemical markers of bone turnover: part I: biochemistry and variability. *Clin. Biochem. Rev.* **26**: 97–122. [Medline]
21. Shidara, K., Inaba, M., Okuno, S., Yamada, S., Kumeda, Y., Imanishi, Y., Yamakawa, T., Ishimura, E. and Nishizawa, Y. 2008. Serum levels of TRAP5b, a new bone resorption marker unaffected by renal dysfunction, as a useful marker of cortical bone loss in hemodialysis patients. *Calcif. Tissue Int.* **82**: 278–287. [Medline] [CrossRef]
22. Tyler, J. W., Hancock, D. D., Parish, S. M., Rea, D. E., Besser, T. E., Sanders, S. G. and Wilson, L. K. 1996. Evaluation of 3 assays for failure of passive transfer in calves. *J. Vet. Intern. Med.* **10**: 304–307. [Medline] [CrossRef]
23. Watts, N. B. 1999. Clinical utility of biochemical markers of bone remodeling. *Clin. Chem.* **45**: 1359–1368. [Medline]
24. Weaver, D. M., Tyler, J. W., VanMetre, D. C., Hostetler, D. E. and Barrington, G. M. 2000. Passive transfer of colostral immunoglobulins in calves. *J. Vet. Intern. Med.* **14**: 569–577. [Medline] [CrossRef]
25. Wheater, G., Elshahaly, M., Tuck, S. P., Datta, H. K. and van Laar, J. M. 2013. The clinical utility of bone marker measurements in osteoporosis. *J. Transl. Med.* **11**: 201. [Medline] [CrossRef]
26. Yamagishi, N., Takehana, K., Kim, D., Miura, M., Hirata, T., Devkota, B., Sato, S. and Furuhama, K. 2009. Fluorometric method for measuring plasma tartrate-resistant acid phosphatase isoform 5b and its application in cattle. *J. Vet. Med. Sci.* **71**: 1637–1642. [Medline] [CrossRef]
27. Zanker, I. A., Hammon, H. M. and Blum, J. W. 2001. Activities of γ-glutamyltransferase, alkaline phosphatase and aspartate-aminotransferase in colostrum, milk and blood plasma of calves fed first colostrum at 0-2, 6-7, 12-13 and 24-25 h after birth. *J. Vet. Med. A Physiol. Pathol. Clin. Med.* **48**: 179–185. [Medline] [CrossRef]