Serum Activities of Tartrate-Resistant Acid Phosphatase and Bone Specific Alkaline Phosphatase as Indices of Bone Metabolism in the Cow

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ABSTRACT. The correlation between the serum hydroxyproline concentration and serum activity levels of TRAP and BALP was examined in 41 cows. The correlated coefficient (r) was 0.6391 for TRAP and 0.3147 for BALP, respectively. Judging from the significant correlation to the serum hydroxyproline concentration, serum TRAP activity is an index for bone metabolism in cows. Serum TRAP activity was therefore measured in 205 healthy cows (2–9 years old) in order to observe the changes in bone resorption with aging and milk production. TRAP levels differed slightly between group A (≤4 yrs) and B (5 yrs) at the same stage of lactation. The activity levels rose slightly at the height of lactation stage and during the dry stage.

NOTE [Internal Medicine]

Milk fever (hypocalcemia) occurs due to the acute depletion of blood Ca during the rapid onset of milk production [8]. Goff and Horst reported that milk fever was successfully prevented by stimulating bone resorption through the regulation of dietary minerals [3, 5]. Moreover, Yarrington et al. reported that severe milk fever occurred in periparturient cows administered a bone resorption depressor over long periods [27, 28]. The importance of Ca homeostasis in Ca mobilization from the bone to prevent milk fever has been recognized [3–5, 27, 28].

Bone metabolic indices in the serum or urine have been utilized for evaluations in humans and animals [1, 9, 11, 17, 21, 23]. Serum activities of tartrate-resistant acid phosphatase (TRAP) originating from osteoclasts and bone specific alkaline phosphatase (BALP) from osteoblasts have been recognized as general indices for the diagnosis and monitoring the effectiveness of the treatment of metabolic bone disorders in humans [1, 10, 11, 21]. In cattle, collagen metabolite (i.e. hydroxyproline) concentrations in serum and urine have been utilized to evaluate bone metabolism [3–5, 15, 16], however measuring collagen metabolites is complicated, time-consuming process [2, 23]. In this study, we evaluated TRAP and BALP in serum, both easily measured, as bone metabolic indices in the cow.

Blood samples were obtained from 41 healthy Holstein Friesian cows (2–6 years old). The blood was incubated at 37°C for 1 hr and centrifuged to separate the serum. Serum samples were frozen at –20°C until analyzed. Milk samples were obtained from 6 healthy cows, mixed with 1N HCl to lower the pH to 4.6, and centrifuged at 4°C to separate the whey. The TRAP activity in serum was measured by Lau’s method [14]. Heparin (23,000 U/l) was added to the reaction mixture and the pH was adjusted to 6.6 with 1N NaOH to measure the TRAP from osteoclasts specifically [18]. BALP activity in the serum and whey was measured by Rosalki’s method [22]. The serum hydroxyproline concentration was measured by Dabev and Struck’s method [2].

Pearson’s correlation coefficients between hydroxyproline concentration and activities of TRAP and BALP were then evaluated. Significance of the correlation coefficients was determined by correlation analysis, and values of p<0.05 were regarded as significant.

Serum TRAP activity was measured in 205 cows to examine the changes in bone resorption with aging and milk production. Two to 9 years old, healthy cows from 8 farms were used in this study. Milk fever was rare in those 8 farms. The 205 cows were divided by age (group A; younger than 4 and group B; older than 5 years old) and lactation stage (early; 8–50 days after calving, height; 51–110 days, middle; 111–220 days, late; day 221–dry, and dry). The results are expressed as means ± standard deviation.

Comparisons of means at same stage between the two groups, and at different stages in each group were assessed using Student’s t-test and one-way ANOVA, respectively. Values of p<0.05 were regarded as significant.

Correlated coefficient between hydroxyproline concentration and activities of TRAP and BALP was 0.6391 (p<0.001) and 0.3417 (p<0.05), respectively (Fig. 1). Serum TRAP activity correlated to hydroxyproline concentration more closely than BALP, making TRAP a potential index for bone metabolism in the cow. The BALP activity in whey was 387.6 ± 203.2 U/l on average.

The average activity of TRAP in the serum at each stage of lactation in groups A and B, and all cows were showed in Table 1. TRAP levels differed slightly between the two groups at the same stage of lactation, and were slightly higher at the height of lactation and during the dry stage.

Serum BALP activity correlated less well to the serum hydroxyproline concentration than did TRAP activity. BALP has been recognized as a bone-specific isoenzyme [22], however high activity of alkaline phosphatase (ALP) was observed in the whey by Rosalki’s method that be able
to measure BALP from the osteoblast specifically. It seems that ALP originating from the mammary gland affects greatly the serum BALP activity in dairy cows. It was difficult to determine which serum BALP originated from osteoblasts or mammary glands. Therefore, serum BALP activity is not suitable as a bone metabolic index in the dairy cow.

Tartrate-resistant acid phosphatase (TRAP) is one of the most abundant enzymes in osteoclasts, and is recognized as band 5 acid phosphatase on PAGE [12, 20]. TRAP activity in serum has been utilized as a bone resorption marker for diagnosis and monitoring of the effectiveness of treatment of metabolic bone disorders in humans [1, 21, 24, 26]. Lau et al. isolated TRAP from bovine bone matrix, and reported that it resembled human TRAP originating from osteoclasts in biological character [13]. TRAP from bovine bone matrix was utilized for an assay of human TRAP activity as a recovery test [14]. Serum TRAP is composed of two isoforms: band 5a and band 5b [6, 18], which is reported to be contained in osteoclasts and to reflect the level of osteoclast activity [6, 7, 18]. Several methods have been developed to measure serum TRAP for evaluation of bone resorption [6, 7, 14, 18]. In the presented study, we measured TRAP band 5b using sodium tartrate and heparin to repress the activity of other isoenzymes [18].

Levels of TRAP activity in the serum in this study were about 1/6 of that in previous studies on healthy adult humans [1, 21, 24, 26], and serum TRAP activity was significantly correlated with the serum hydroxyproline concentration. From these results, TRAP activity is a useful index of bone resorption even though the activities were low. The method proposed here is easy to perform and multiple samples can be assayed at once. And bovine serum TRAP activity can be used to evaluate bone resorption and diagnose bovine cases of metabolic bone disorders in cattle.

Serum TRAP activity increased slightly at the height of lactation and during the dry stage. It was thought that bone resorption increased in the former period because a large amount of Ca was consumed for milk production. The rate of bone resorption is reported to be affected by many dietary elements (Ca, phosphorus vitamin D and protein etc.) [25]. Demand of dietary element is calculated by its body weight and amount of milk production in dairy cattle [19]. In this study, demands and uptake of dietary element were not investigated in every cow. It was necessary for evaluating...
bone resorption to investigate demands and uptake of dietary elements.

Nakanishi *et al.* [18] reported on the changes of human serum TRAP activity by aging. TRAP activities were various in young, adult and old women [18]. Compared to adult woman (20–49 years old), TRAP activities were observed 6–7 times in young (younger than 14 years old) and about 1.5 times in old (older than 60 years old) [18]. In 20–49 years old woman, bone resorption suggested to be inactive and invariable [18]. Serum TRAP activities were not showed any differences between group A and B in this study. States of bone resorption in cows used in this study probably thought to be correspond to those in adult woman.

REFERENCES