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An evaluation of the effect of age and the peri-parturient period on bone metabolism in dairy cows as measured by serum bone-specific alkaline phosphatase activity and urinary deoxypyridinoline concentration

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ABSTRACT

Various biochemical markers help to evaluate the state of bone turnover in humans and could be used during the peri-parturient period in dairy cows when calcium (Ca) metabolism changes dramatically. To investigate this, the peri-partum characteristics of serum bone-specific alkaline phosphatase (BAP) and urinary deoxypyridinoline (DPD) were investigated. Both serum BAP activity and urinary DPD concentrations were increased and demonstrated wide variability in younger animals, and these findings were consistent with other bone turnover markers. Around the time of parturition, serum Ca concentration and serum BAP activity in multiparous cows were significantly lower than in primiparous cows, but urinary DPD concentration was unchanged.

The use of BAP as a bone formation marker appears to be valuable for evaluating bone remodelling status in cows, but the specificity of the test needs to be confirmed. The DPD/BAP ratio around parturition demonstrated a clear difference in bone turnover status between the two parity groups with multiparous cows demonstrating increased signs of bone resorption compared with primiparous cows, corresponding to the Ca requirement for milk production. In future studies, the applicability of the ratio of bone resorption marker to bone formation marker should be evaluated for bone turnover assessment.

Introduction

Hypocalcaemia in dairy cows is caused by pre- and post-partum abnormalities in calcium (Ca) metabolism and not only causes parturient paresis but around calving time is also related to peri-parturient diseases, including dystocia, retained fetal membranes, ketosis, and mastitis (Curtis et al., 1983). These conditions greatly reduce milk productivity and lead to severe economic losses in the dairy industry. Although many protocols have been proposed to prevent hypocalcaemia in dairy cows, no method completely eliminates this negative Ca balance. From late pregnancy to just after calving, the Ca metabolism of dams changes dramatically in order to facilitate fetal bone formation and milk production (Horst et al., 2005). Lactation causes an increase in Ca requirement, which generally cannot be adequately compensated for by increased Ca absorption from the intestinal tract in early lactation. As a result, the body begins to mobilize Ca from the bone. Hypocalcaemia in dairy cows often appears within 3 days of calving, because Ca mobilization from the bone does not react fully at the onset of lactation (Ramberg et al., 1984; Horst et al., 2005).

The skeleton is continually renewed by bone turnover, involving osteoclast-mediated bone resorption, osteoblast-mediated bone formation, and remineralisation of newly formed bone (Cremers et al., 2008). Various biochemical markers of bone metabolism have been used to evaluate the state of bone turnover in humans and animals, and can provide near real-time information about bone cell activity (Allen, 2003). The level of each bone turnover marker and its variability depend on uncontrollable factors such as age, sex, pregnancy, lactation and disease, as well as controllable factors such as circadian rhythm, season, diet and exercise (Hannon and Eastell, 2000). Markers may reflect changes in the bone turnover status of dairy cows during the peri-parturient when the Ca metabolism of dams dramatically changes. Osteocalcin

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(OC) and bone-specific alkaline phosphatase (BAP) have been used as indices of bone formation but the expression and secretion of OC and BAP in osteoblasts differ across stages of differentiation (OC, from middle to late; BAP, from early to middle). OC and BAP also differ with respect to blood half-life (OC, 20 min; BAP, 40 h) and main route of catabolism (OC, kidney; BAP, liver) (Cremers et al., 2008).

In this study, we used serum BAP activity as a bone formation marker instead of OC for several reasons. Firstly, BAP has been reported to demonstrate no significant circadian variation (Tobiume et al., 1997), although some studies have shown conflicting results (Hannon and Eastell, 2000). In contrast, OC has a clear circadian rhythm (Nielsen, 1994) and demonstrates seasonal variation (Woitge et al., 1998). BAP also has good stability for storage and freeze–thaw cycles (Gomez et al., 1995) while OC is relatively unstable (Peel and Eastell, 1993). Additionally, OC concentration is influenced by vitamin D administration (Markowitz et al., 1987) whereas BAP is not. Finally, the response of OC to pregnancy differs markedly from that of the other markers of bone formation (Hannon and Eastell, 2000).

Many bone resorption markers have been applied in cattle and humans. Hydroxyproline was widely used in the past (van Mosel and Corlett, 1990), while pyridinoline (PYD) (Liesegang et al., 2000), deoxypyridinoline (DPD), collagen type I cross-linked C-telopeptide (CTX) (Liesegang et al., 1998) and tartrate-resistant acid phosphatase (Yamagishi et al., 2009) have been used more recently. We selected urinary DPD concentration as a bone resorption marker in the present study for two main reasons. Firstly, Liesegang et al. (1998) concluded that DPD (as well as CTX) appeared to be a suitable indicator of bone resorption in dairy cows and, secondly, DPD has a higher specificity than PYD; PYD is incorporated into skin by type I collagen, while DPD is included only in bone (Eyre et al., 1984).

The ratio of bone resorption markers to bone formation markers is thought to reflect the coupling status of bone remodelling in both humans (Nakayama et al., 1996) and horses (Lepage et al., 1998). A change in the ratio of CTX to OC was observed from 14 days pre-partum to 150 days post-partum in cows (Liesegang et al., 2000) and the ratio was not only independent of age and milk yield but markedly elevated at 14 days post-partum in parallel with the marked increase in bone resorption for lactation. This suggests that the CTX to OC ratio is a good indicator of bone turnover status in cows.

Additionally, biochemical markers of bone turnover are generally higher in juveniles than in adults (Hannon and Eastell, 2000). Thus in order to determine the applicability and establish the effective usage of such markers in dairy cows, the effect of age on serum BAP activity and urinary DPD concentration has to be established outside of the peri-parturient period. The objectives of the present study were: (1) to determine the relationships between age and serum BAP activity and urinary DPD concentration; (2) to quantify peri-parturient changes in BAP and DPD in primiparous and multiparous cows, and (3) to evaluate whether the DPD to BAP ratio is a useful indicator of bone turnover in peri-parturient dairy cows.

Materials and methods

Farm and management

This study was performed on a 740-head dairy farm in Hokkaido, Japan, over a 7-month period between March and December 2007. Suckling calves were housed separately in calf pens and fed milk replacer twice a day until 7 weeks after birth. Commercial calf starter was made available after 2 weeks of milk feeding. Cows were housed in a 4-row free-stall barn and kept separately in five groups according to age and/or milk production, namely, (1) fresh cows, (2) cows with low, medium, and high milk production, and (3) dry cows. The cattle were fed a total mixed ration consisting of corn silage, grass silage, Italian ryegrass hay, soybean meal, and beet pulp according to the standard of the National Research Council (2001) and had ad libitum access to water. No cows were fed an acidogenic diet during the peri-parturient period. Lactating cows were milked three times a day.

Animals

All animals were treated in compliance with the Guidelines for the Care and Use of Laboratory Animals of Azabu University School of Veterinary Medicine, Sagamihara, Japan (Approval number 110310-4). The cattle were observed daily for health status by the farm staff. All animals used for these experiments were clinically healthy Holstein dairy cattle as evaluated by physical examination.

To determine the relationship between age and bone turnover markers, blood samples were collected from 104 cattle (age range 11 days to 10 years) for the BAP assay, and urine samples were collected from 100 cattle (age range 1 day to 10 years) for the DPD assay. Samples were not collected from these cattle from 2 months prior to their estimated calving date until 2 months after calving. To observe peri-parturient changes in serum Ca concentration and BAP activity as well as urinary DPD concentration, we collected blood and urine samples from seven primiparous (age range 1.7–2.6 years) and seven multiparous (age range 3.8–8.1 years) cows at 14 and 7 days before the estimated calving date, again on the day of calving, and at 1, 2, 3, 7, 14, and 21 days after calving). Parities of multiparous cows were as follows: 3rd, $n = 3$; 4th, $n = 2$; 5th, $n = 1$, and 6th, $n = 1$.

Blood and urine sampling

Blood samples were collected from the jugular vein, incubated at 37 °C to allow clotting, and then centrifuged (1500 *g* for 20 min at 4 °C) within 30 min of sampling. Urine was collected from the bladder via sterile catheter. Isolated serum and urine samples were stored at −40 °C until analysis. All samples were collected on farm between 0900 and 1300 h, except on the day of calving (when samples were collected immediately after calving).

Laboratory analyses

Serum Ca concentration was measured by an autoanalyser (Hitachi 9000) using the *o*-Cresolphthalein Complexone method. Serum BAP activity was measured using the Osteolinks-BAP kit (DS Pharma Biomedical), and urinary DPD concentration was measured using the Osteolinks-DPD kit (DS Pharma Biomedical). Urinary DPD concentration was standardized according to creatinine (Cre) concentration, which was measured by an autoanalyser (Hitachi 9000) using the creatininase–sarcosine oxidase method.

Statistical analyses

Pearson's correlation analysis was performed to determine the relationships between cow age and values of bone turnover markers using Graph Pad Prism 3.03 for Windows. No instances of missing data occurred, and the balanced dataset enabled conventional analysis of variance (ANOVA). Therefore, each of the four analytes (serum Ca concentration, serum BAP activity, urinary DPD concentration, and DPD/BAP ratio) was subjected to repeated-measures ANOVA. A priori hypotheses of differences between parity at each time interval were made using the conventional test of simple main effects after repeated-measures ANOVA with Satterthwaite denominator degrees of freedom. Adjustment for multiple comparisons was made using the Bonferroni method. The type 1 error rate was set to 5% after adjustment for multiple comparisons. Statistical analysis was performed using Stata SE, Release 11.2 (StataCorp).

Results

Age-related changes in serum BAP activity and urinary DPD concentration

There was a significant negative correlation between age and serum BAP activity ($r = -0.64$, $P < 0.0001$) and between age and urinary DPD concentration ($r = -0.27$, $P < 0.01$) (Fig. 1). Both markers varied widely during the juvenile period.

Peri-parturient changes in serum Ca concentration, serum BAP activity, and urinary DPD concentration

The serum Ca concentrations of primiparous cows remained within the normal range throughout the experiment (Fig. 2A). In multiparous cows, serum Ca concentrations decreased markedly from the day of calving to 2 days post-partum, and at these