

## Experimental assessment of bone mineral density using quantitative computed tomography in holstein dairy cows

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**ABSTRACT.** The aim of this study was to assess the measurement of bone mineral density (BMD) by quantitative computed tomography (QCT), comparing the relationships of BMD between QCT and dual-energy X-ray absorptiometry (DXA) and between QCT and radiographic absorptiometry (RA) in the metacarpal bone of Holstein dairy cows (n=27). A significant positive correlation was found between QCT and DXA measurements ( $r=0.70$ ,  $P<0.01$ ), and a significant correlation was found between QCT and RA measurements ( $r=0.50$ ,  $P<0.01$ ). We conclude that QCT provides quantitative evaluation of BMD in dairy cows, because BMD measured by QCT showed positive correlations with BMD measured by the two conventional methods: DXA and RA.

**KEY WORDS:** bone mineral density, dairy cow, dual-energy X-ray absorptiometry, quantitative computed tomography, radiographic absorptiometry

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Dairy cows are often affected milk fever at parturient, because of the decrease in serum calcium (Ca) concentration. Generally, when serum Ca is decreased, it is compensated by intestinal absorption and bone resorption. From this point of view, enhancing the intestinal Ca absorptive and bone Ca resorptive processes prevents milk fever [11]. Many reports have described the Ca homeostasis in parturient cows. One study reported that parturient cows depend only on intestinal Ca absorption, because bone resorption is delayed by 1 week or more [12]. Another study reported that the cows can depend on bone Ca absorption immediately after calving [11]. However, in these reports, bone mineral density (BMD) was not measured, and hence, bone Ca metabolism was still unknown.

In cows, changes in BMD with exercise [8] and parity [9] have been reported, but in these reports, BMD was not measured quantitatively, and the number of reports of cows is less than the number of humans and horses. It is reported that signs of osteoporosis is observed in beef cattle that were fed low amounts of phosphorus diets for a long period [13] and that bone mineral content of cattle has changed with dietary phosphorus [16]. Therefore, it is considered that quantitative computed tomography (QCT), which quantifies BMD, provides us with useful information about bone metabolism of cows. Furthermore, bone metabolism of cows at the periparturient may reveal the mechanism of milk fever. Thus, it was

postulated that QCT may be useful measurement of BMD when BMD by QCT is compared with BMD by dual-energy X-ray absorptiometry (DXA) [7] and BMD by radiographic absorptiometry (RA) [17], both of which are consistent with bone ash. The aim of this study was to assess the measurement of BMD by QCT, comparing the relationships of BMD between QCT and DXA and between QCT and RA.

Right metacarpals from Holstein cows (n=27, age 32–119 months) were excised and collected at slaughter. Twenty-seven metacarpals were scanned with a BMD phantom (B-MAS200, Fuji Rebio, Tokyo, Japan) by CT (Asteion Super4, Toshiba, Tokyo, Japan) operating at 120 kV, 150 mA and 5.0 mm slice thickness. The BMD phantom contained different calcium-hydroxyapatite concentrations (50, 100, 150, 200 and 250 mg/cm<sup>3</sup>). All CT imaging data were imported into an image processing workstation (Virtual Place Advance, AZE, Tokyo, Japan). And, BMD values were measured in the transverse sections at the level of distal foramen of the metacarpal, and the region of interest (ROI) was drawn manually on the cancellous bone at this sections (Fig. 1a). Circular ROIs were drawn into each calcium-hydroxyapatite concentration of the BMD phantom, and the BMD values were calculated. A calibration line was made from the CT values in each ROI of the BMD phantom and was used to calculate the BMD. DXA utilizes 2 levels of X-ray energy that are differentially impeded calibration by bone, fat and muscle. Each metacarpal was scanned by DXA (Hologic, Bedford, MA, U.S.A) operating at 100 or 140 kV, and 25 mA. The setting of DXA was applied to the third lumbar vertebra of human. The ROI was selected to include the cancellous bone and the cortical bone at the level of the distal foramen (Fig. 1b). In RA, each metacarpal was exposed by X-ray (70 kV, 1.5 mAs and FFD 75 cm) and then imaged by digital radiography (AeroDR, KONIKA MINOLTA, Tokyo, Japan).

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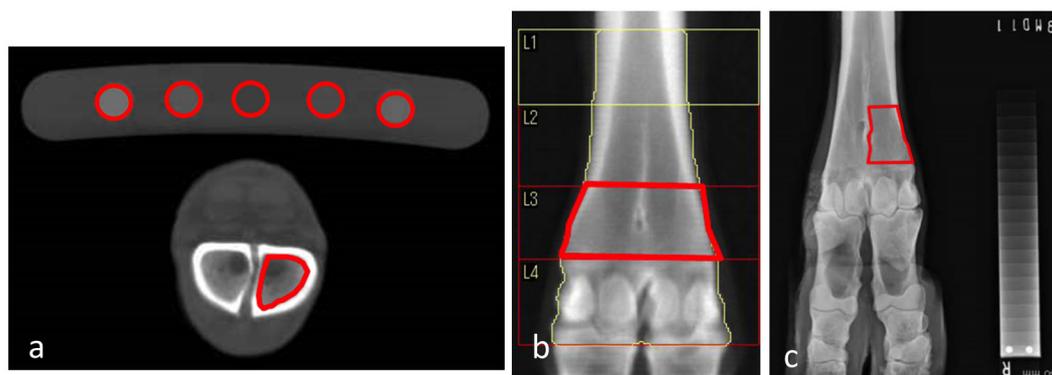


Fig. 1. Measurement of the BMD value for QCT ; (a), DXA; (b) and RA; (c). Each region of interest is drawn by red line. Circles (a) show the areas of different densities of hydroxyapatite in the phantom. From left to light, each density is 250, 150, 50, 100 and 200  $\text{mg}/\text{cm}^3$  of hydroxyapatite.

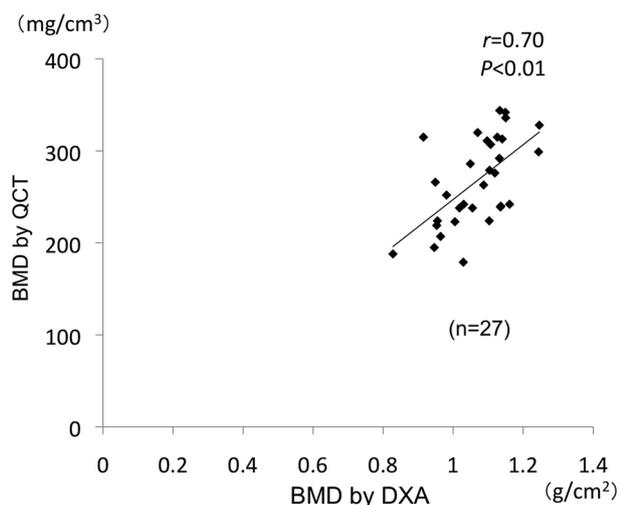


Fig. 2. Correlation between BMD by QCT and BMD by DXA.

An aluminium (Al) step wedge was exposed simultaneously in each radiograph as a reference standard. Digital imaging data were transferred to an imaging processing workstation. The ROI was selected to include cancellous bone at the level of the distal foramen (Fig. 1c). Quadrangular ROIs were drawn into each Al step (3, 6, 9, 12, 15 and 18 mm), and the BMD values were calculated. A calibration line was made from the density of each ROI in the Al step and was used to calculate the BMD. Statistical analysis was performed using the JMP®8 program (SAS Institute Inc., Cary, NC, U.S.A.). Shapiro Wilk test was used to compare changes in each parameter for each group. After confirming that each data set was normally distributed, Pearson's correlation test was performed to evaluate the correlations between the BMD values measured by QCT and those measured by DXA and RA. The level of significance was set at  $P<0.05$ . A significant positive correlation was found between QCT and DXA ( $r=0.70$ ,  $P<0.01$ ) (Fig. 2). This was consistent with the results of the research in humans [2]. A correlation was

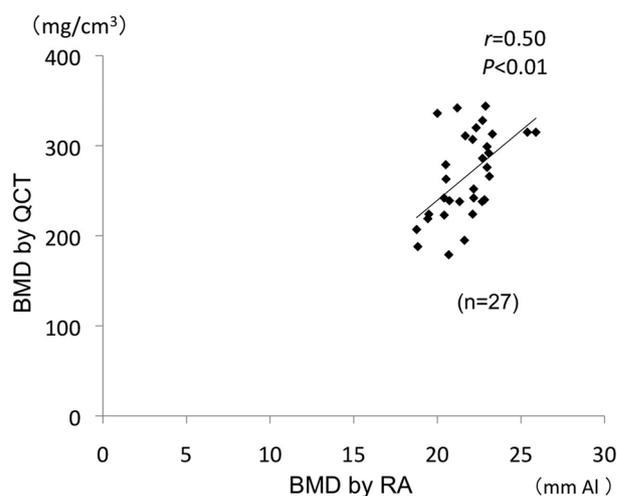


Fig. 3. Correlation between BMD by QCT and BMD by RA.

also found between QCT and RA ( $r=0.50$ ,  $P<0.01$ ) (Fig. 3). The correlation between QCT and RA was weaker than the correlation between QCT and DXA, because DXA exposes dual-energy X-ray and soft tissue absorption is attenuated by X-rays of two energy levels [15].

In humans, QCT, DXA and RA have been used as non-invasive measurement for BMD [5, 6]. DXA is rapid [14], and RA is inexpensive [5]. However, DXA and RA measure BMD by area ( $\text{g}/\text{cm}^2$ ), while QCT measures BMD by volume ( $\text{g}/\text{cm}^3$ ). Thus, BMD by DXA and RA are semiquantitative, while BMD by QCT is quantitative because it is less affected by bone size. In addition, QCT is the only method that can discriminate the cortical and cancellous bones, because of a cross-sectional image [2, 3].

BMD values varied with measurement site; therefore, it is important that setting landmark would be necessary to achieve reproducible measurement. It is important to choose a measurement area where BMD is sensitive to metabolic change. Changes of BMD appear preferentially in cancel-

lous bone, because cancellous bone is more sensitive than cortical bone to changes in bone metabolism [10]. Thus, to assess the changes in BMD accurately, it is necessary to quantify the BMD of cancellous bone. The metacarpal used in this study is the most weight bearing bone in cows [4] and thus is sensitive to BMD change. In case of living cows, the metacarpal is the first option for BMD measurement because it is accessible for CT scan to evaluate the BMD by QCT, and the distal foramen is a suitable landmark. It is considered that the cross-sectional image at the level of the metacarpal distal foramen is the most suitable measurement area for BMD by QCT in dairy cows.

The coefficients of variation (CV) were measured by 5 observers (A.M., M.I., K.N., M.O. and K.Y). These CV were shown as percentage CV values ( $100 \times \text{S.D.}/\text{mean}$ ). The CV measured by the 5 observers ranged between 1.6% and 4.3%. The CV repeated 10 times by the 5 observers was 3.3%. Therefore, it was considered that BMD measured by QCT had high reproducibility.

We concluded that QCT has high reproducibility and utility for BMD measurement in dairy cows because the BMD measured by QCT showed positive correlations with the BMD measured by the two conventional methods: DXA and RA.

We did not measure the biomarkers of bone metabolism in this experiment, because we used the isolated metacarpals. Biomarkers of bone metabolism provide current information on bone metabolism [1]. Further investigations are needed to clarify bone metabolism in periparturient that is measured by time course BMD change and biomarker.

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