—Original Article—

# Experimental investigation of bone mineral density in Thoroughbreds using quantitative computed tomography

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*Bone mineral density (BMD) is one of the indications of the strength and health. BMD measured by quantitative computed tomography (QCT) was compared with that measured by dual energy X-ray absorptiometry (DXA) and radiographic bone aluminum equivalence (RBAE). Limbs were removed from horses that had been euthanized for reasons not associated with this study. Sixteen limbs (left and right metacarpals and metatarsals) from 4 horses were used to compare BMD as measured by QCT with those measured by DXA and RBAE. There was a strong correlation between BMD values measured by QCT and those measured by DXA ($R^2$=0.85); correlation was also observed between values obtained by QCT and those obtained by RBAE ($R^2$=0.61). To investigate changes in BMD with age, 37 right metacarpal bones, including 7 from horses euthanized because of fracture were examined by QCT. The BMD value of samples from horses dramatically increased until 2 years of age and then plateaued, a pattern similar to the growth curve. The BMD values of bone samples from horses euthanized because of fracture were within the population range, and samples of morbid fracture were not included. The relationship between BMD and age provides a reference for further quantitative studies of bone development and remodeling. Quantitative measurement of BMD using QCT may have great potential for the evaluation of bone biology for breeding and rearing management.*

**Key words:** *bone mineral density, dual energy X-ray absorptiometry, quantitative computed tomography, radiographic bone aluminum equivalence, Thoroughbred*

**J. Equine Sci.**
**Vol. 26, No. 3**
**pp. 81–87, 2015**

In Thoroughbred breeding and rearing, careful management is required, including training, feeding, and the risk of accidents. In particular, musculoskeletal problems are a substantial economic liability in the equine industry. Successful management contributes to the production of fast racing horses.

In humans, bone mineral density (BMD) is considered an important health indication because it allows clinicians to evaluate the risk of fracture [8, 14]. It is well known that athletes who engage in high-impact sports tend to have higher BMD values than athletes who engage in low-impact sports, and fatigue fractures are seen frequently in low-impact sport athletes, particularly runners [16, 24]. The peak BMD in humans is reached in the early twenties and the occurrence of fatigue fractures reaches its peak in late adolescence. Excessive training before BMD reaches it peak is considered to lead to fatigue fractures.

In horses as well, low BMD increases the incidence of orthopedic problems and orthopedic injuries [10]. One possible explanation for bone-related injury in young Thoroughbreds is that many horses begin rigorous training when still skeletally immature. Indeed, bucked shins, which are related to fatigue fractures, are less common in Standardbred horses because they are subjected to less early physical stress than racing Thoroughbreds [16]. Equine BMD studies have revealed a correlation between BMD and mechanical properties determined by a 3-point bending test [20, 21] and have shown that, like in humans, loading exercise has a positive effect on BMD [3, 12, 18]. In other

Received: May 1, 2015
Accepted: August 5, 2015
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words, BMD has been used for evaluation of the effects of diet and exercise [17] and pasture rearing in weanlings [1].

Evaluation of BMD is considered important in the rearing of race horses, and several noninvasive methods for measuring BMD have been investigated, including ultrasound [7, 11, 12], X-ray [1, 2, 9, 10, 17, 18], single photon absorptiometry [6, 12], dual energy X-ray absorptiometry (DXA) [12, 22], and computed tomography (CT) [4, 23]. X-ray requires no general anesthesia or sedation when it is used on horses, and it costs less than other methods. DXA is the most widely used method of BMD measurement for the assessment of osteoporosis and fracture risk in humans. In humans, quantitative CT (QCT) is the most reliable BMD measurement technique for assessing the degree of osteoporosis. The purpose of this study was to evaluate the QCT for measuring BMD in horses and to examine the relationship between QCT and age to establish a quantitative reference for future studies on rearing protocols for optimal BMD.

This study consisted of the following two experiments:

1. An experiment investigating the usefulness of QCT by comparing DXA and RBAE

2. An experiment examining the relationship between age and quantitative BMD measured by QCT

We investigated the utility of quantitative BMD through these two experiments.

## Materials and Methods

*Sample materials for investigation of the usefulness of QCT*

The limbs used in these experiments were removed from horses that had been euthanized. The decision regarding euthanization was made by a veterinarian based on the clinical course. The protocol was approved by the Animal Experiment and Welfare Committee of Obihiro University of Agriculture and Veterinary Medicine. Sixteen limbs (left and right metacarpals and metatarsals) from 4 Thoroughbred horses (age: 1-day-old male, 1-year-old male, 12-year-old female, and 14-year-old female) were used.

*Sample materials for examination of the relationship between age and quantitative BMD measured by QCT*

Thirty-seven right metacarpal bones from 37 Thoroughbred horses (0 to 28 years old, 12 males and 25 females) were used, including seven from horses that were euthanized because of fractures (femur, pelvis, and sternum).

*QCT*

While the CT value indicates X-ray attenuation, BMD values measured by QCT reflect hydroxyapatite density. Images were obtained by multi-detector row CT (Asteion Super 4, Toshiba, Tokyo, Japan). Limbs and a phantom (B-MAS200, Fujirebio, Tokyo, Japan) were scanned in 5-mm slices for measuring BMD. Regions of interest (ROIs), including the whole bone (cortex and center), cortex, center, and phantom, on cross-sectional images at the level of the nutrition foramen were set by the image-processing software (Virtual Place, Aze, Tokyo, Japan). The ROIs for the whole bone, as well as the second and forth metacarpal/metatarsal bones were not included (Fig. 1). The calibration line was made from CT values in each ROI of the phantom and was used to calculate the BMD for bones.

*DXA*

DXA utilizes 2 levels of X-ray energy that are differentially impeded by bone, fat, and muscle. Software algorithms are used to calculate BMD. This method does involve radiation exposure, but it is a quick and simple method for mass screening. A DXA unit (Discovery, Hologic, Bedford, MA, U.S.A.) was used for measuring BMD at the level of the nutrition foramen of the metacarpal/metatarsal bones with human femoral neck parameter settings.

*Radiographic bone aluminum equivalence (RBAE)*

Radiographs of metacarpal/metatarsal bones and aluminum step wedges (1 mm/step, 20 steps) were acquired at 70 kV/1.5 mAs using a portable X-ray unit (TRB9020H, Mikasa, Tokyo, Japan) and read by a digital radiography system (AeroDR, Konica Minolta, Tokyo, Japan). The ROIs were set on the aluminum step, and the densities were measured to create a calibration line. The density of a ROI at the level of nutrition foramen was measured, and the RBAE was calculated from the calibration line.

*Statistical analysis*

Statistical analysis was performed using a statistical add-in software of Excel (Statcel 3, OMS Publishing, Saitama, Japan) for MS Excel (Microsoft, Redmond, WA, U.S.A.). BMD values measured by each method were assessed with the Mann-Whitney $U$ test. The peason correlation coefficient with $P$-value was used to examine the relationship between QCT and DXA, QCT and RBAE, and BMD and circumference of the metacarpal/metatarsal, respectively. A $P$-value less than 0.05 was considered as significant.

## Results

Comparison of BMD values as measured by QCT within ROIs placed over different metacarpal/metatarsal cortex regions revealed no significant difference among the dorsal, lateral, and medial cortex but significantly lower BMD values in the palmar/plantar cortex (Fig. 2). Measurements of BMD in ROIs of the whole bone, dorsal cortex, and center

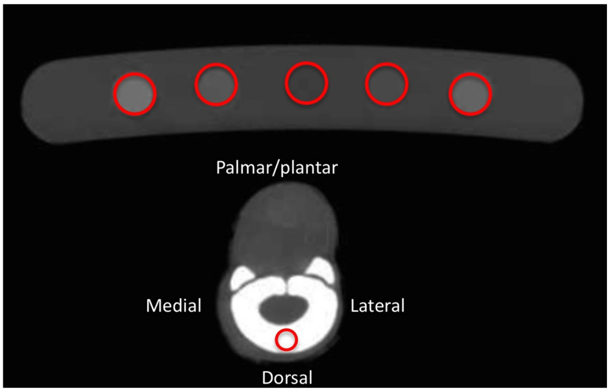


**Fig. 1.** Measurement of bone mineral density (BMD) by quantitative CT (QCT) using a phantom for calibration. Circles show regions of interest (ROIs) on a cross-sectional image at the level of the nutrition foramen of the metacarpal/metatarsal bone and areas of different densities of hydroxyapatite in the phantom.

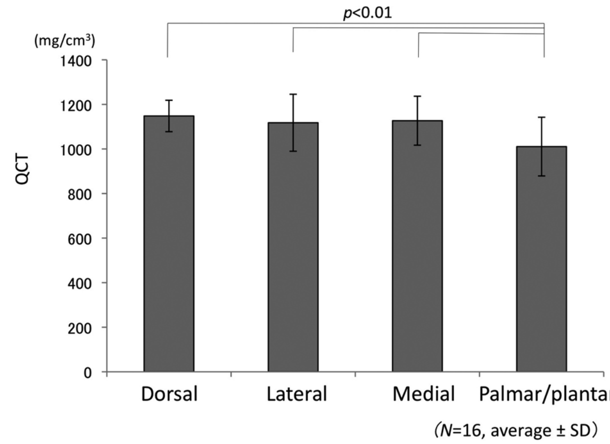


**Fig. 2.** Comparison of BMD values as measured by QCT among ROIs in cortical bone. The BMD values were significantly lower for the palmar/plantar cortex than for other regions of the cortex.

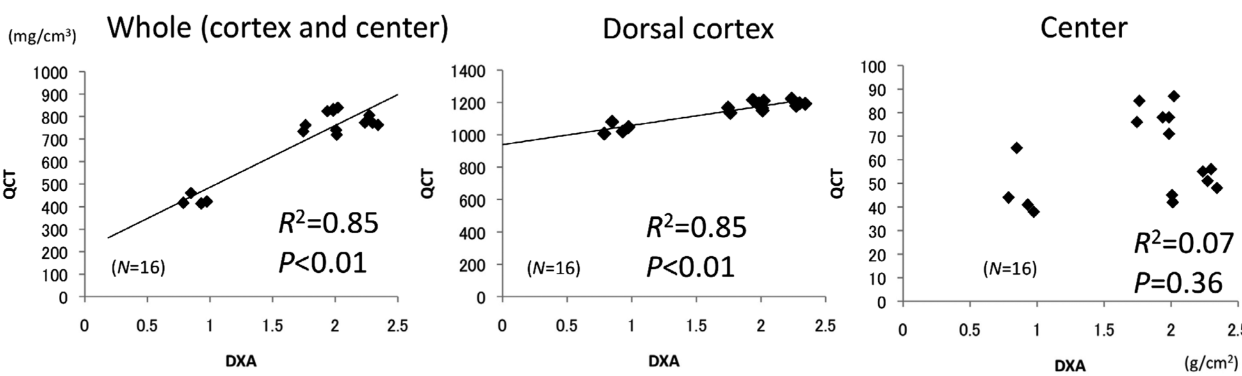


**Fig. 3.** Comparison of BMD values as measured by QCT and dual energy X-ray absorptiometry (DXA). There were strong correlations for the whole bone (cortex and center) and dorsal cortex, but not for the center.

using QCT and DXA revealed strong correlations between modalities in the whole bone ($R^2$=0.85, $P$<0.01) and dorsal cortex ($R^2$=0.85, $P$<0.01) but not in the center ($R^2$=0.07, $P$=0.36) (Fig. 3). Comparison of the whole bone BMD as measured by QCT and RBAE also revealed a significant but weaker correlation ($R^2$=0.61, $P$<0.01) (Fig. 4).

The bones of the Thoroughbred horses (14 years, 12 years, 1 year, and 1 day) exhibited decreasing BMD values with age as measured by QCT (whole bone) and DXA. In contrast, RBAE could not differentiate 12-, 14-, and 1-year-old bone (Fig. 5).

The BMD of the metatarsal bone was significantly higher than that of the metacarpal as measured by RBAE but not when measured by the other modalities (Fig. 6). To clarify the reason for these differences in BMD among measurement methods, BMD and circumference were compared.

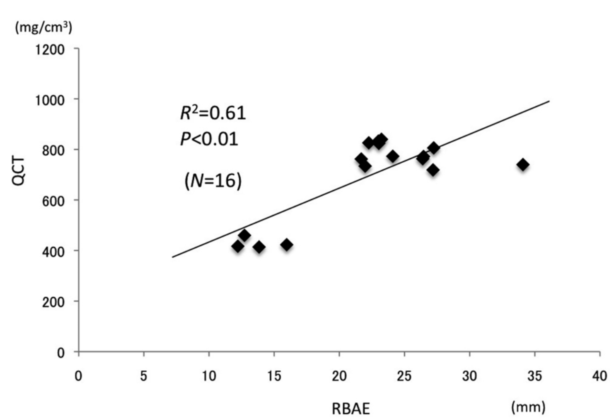


**Fig. 4.** Correlation of BMD values as measured by QCT and radiographic bone aluminum equivalence (RBAE).

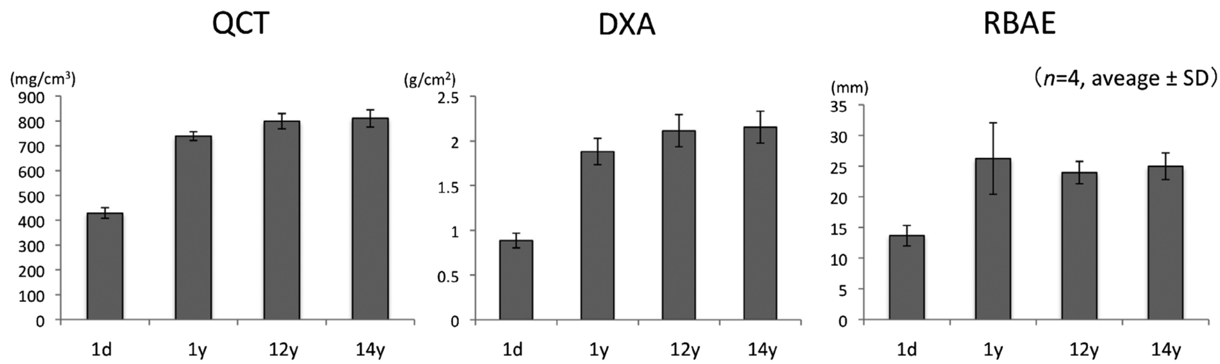


**Fig. 5.** BMD values at different ages (14, 12 and 1 year of age and 1 day of age) as measured by QCT, DXA, and RBAE. QCT and DXA measurements exhibited a similar pattern, but RBAE could not distinguish between 14-, 12- and 1-year-old bone.

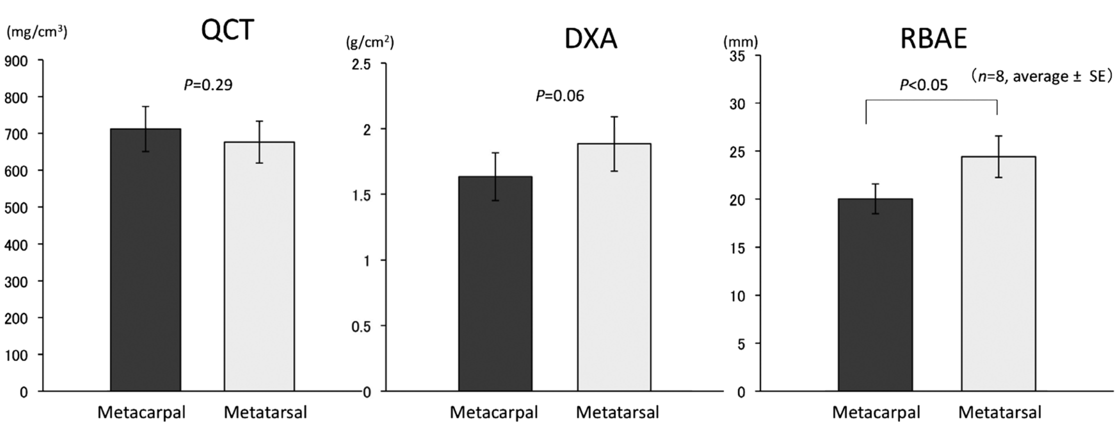


**Fig. 6.** Comparison of BMD values between metacarpal and metatarsal bones as measured by QCT, DXA, and RBAE. When measured by RBAE, the metatarsal BMD was significantly higher than the metacarpal BMD.

The results showed a strong correlation between measured BMD and circumference for all three methods (Fig. 7), but the coefficient of determination for QCT ($R^2$=0.82) was lower than that for DXA ($R^2$=0.96) and RBAE ($R^2$=0.86).

The BMD of the right metacarpal as measured by QCT increased dramatically with age until 2 years of age and then plateaued. None of the 7 bone samples from cases euthanized because of fracture was dramatically lower than the others and all were within the population range (Fig. 8).

## Discussion

BMD is the major determinant of bone strength; therefore, studies have been performed to measure the accuracy of different modalities. Ultrasound is radiation free and yields information about bone architecture and mass. However, ultrasound is affected by soft tissue swelling and factors interfering with surface contact of the ultrasound probe [11]. RBAE is also easy to apply, even in the field, but the value is relative for BMD measurement. In human medicine, DXA is the most commonly used method because of its ease. In equine medicine, DXA is also thought to accurately measure BMD [13], but DXA is not portable and cannot be used for standing, conscious horses. Recently, the availability of CT in equine practice has increased [15, 19]. Even if general anesthesia is required, it is nonetheless a useful diagnostic modality and we expect CT to play an increasingly important role in the evaluation of BMD in living horses, as only one additional scan is required for BMD measurement when horses undergo CT examination. Therefore, the authors investigated the method of QCT and created reference data for QCT in this experiment.

Preliminary studies revealed that BMD values varied on the basis of the site of measurement. Therefore, it was concluded that setting landmarks, as is typically performed for human measurements (femoral neck or lumbar vertebral bone), would be necessary to achieve reproducible measurements. Weight-bearing bone measurements are

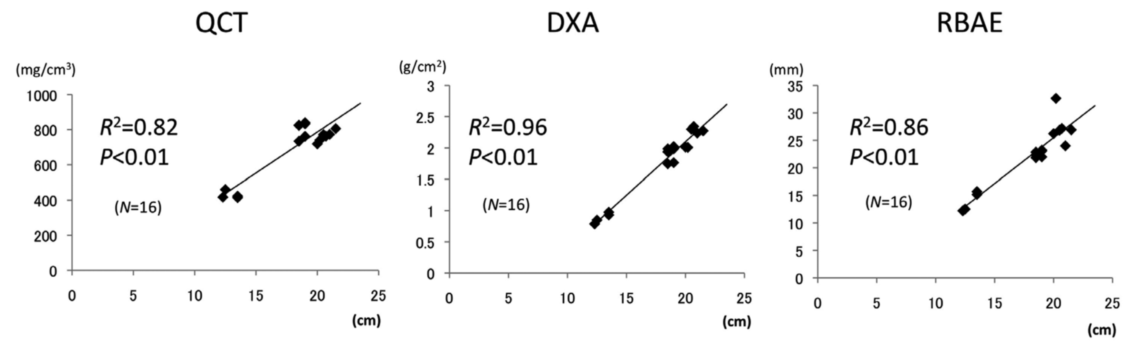


**Fig. 7.** Correlation between BMD and bone circumference of the metacarpal/metatarsal as measured by QCT, DXA, and RBAE. All methods showed strong correlations, but the correlation determination was lowest for QCT.

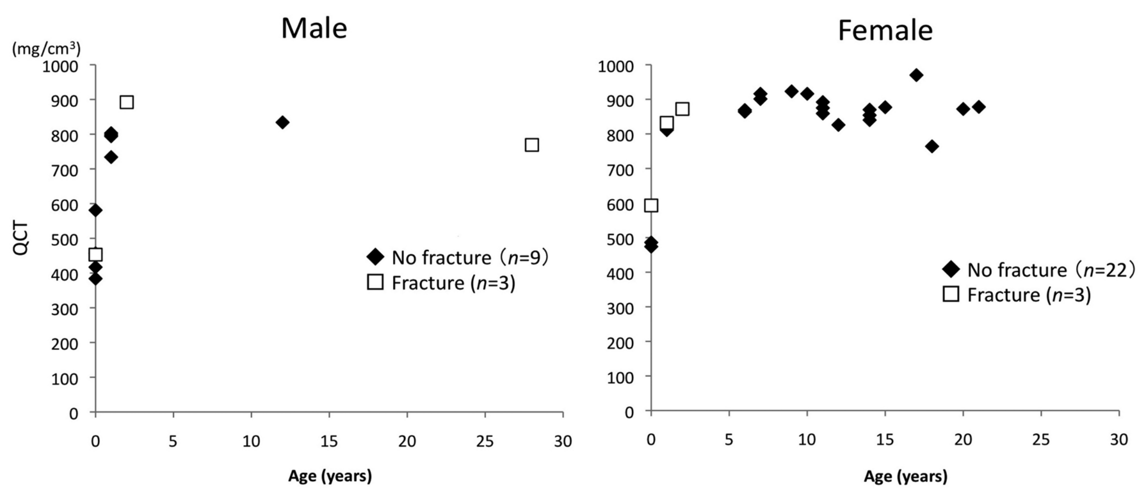


**Fig. 8.** Change in BMD with age in Thoroughbred horses ($N$=37, 12 male, 25 female, 0–28 years old). The BMD values of bones from horses euthanized because of fracture are within the population range.

superior to non-weight-bearing bone measurements for general evaluation of bone strength as weight-bearing bone is more sensitive to factors causing BMD changes (i.e., development or training). Similarly, cancellous bone is more sensitive than cortical bone. BMD changes are high in the epiphysis but low in the diaphysis because the epiphysis contains more cancellous bone. In human QCT, the femoral neck, a cancellous weight-bearing bone, is often used for determination of BMD [24]. In animals, bone shape and size vary widely, which influences BMD measurement accuracy and reproducibility. As reproducibility is important in equine studies as well, the metacarpal bone is the first option because of its accessibility for CT examination, and the nutrition foramen is a suitable landmark. The choice of ROI is also important. The BMD value of the palmar/plantar cortex was significantly lower than that of the other regions examined, in accordance with a previous report [23]. The BMD values of the whole bone and cortex as measured by QCT were strongly correlated with those measured by DXA, while that for the center was not because the center has relatively less cancellous bone. As the whole bone and cortex showed similar results, the authors selected the whole bone as the best measurement site. Indeed, the coefficient of variation of the whole bone using QCT was within 2% in raw data, underscoring the reproducibility of QTC and its suitability for evaluation of individual differences in BMD and changes with development or treatment. The effect of X-ray tube voltage on the BMD value was examined in a preliminary study. While attenuation did depend of tube voltage, there was no significant difference among 80, 120 and 135 kV (data not shown). The calibration line created by measurement of a phantom will enable consistent BMD measurement even in the case of different CT units, imaging settings, and facilities.

RBAE is also used for BMD evaluation, particularly in the field, and the values obtained were also correlated with QCT measurements. However, while QCT and DXA measured a similar pattern of BMD change with age, RBAE could not differentiate 14-, 12- and 1-year-old bone. Therefore, the accuracy of RBAE is considered to be inferior to that of DXA or QCT. There was no significant difference in BMD between the left and right sides (data not shown) as measured by QCT, DXA, and RBAE; however, hind limb BMD was significantly higher than front limb BMD when measured by RBAE. The BMD value was strongly affected by bone circumference. While QCT measurements were also affected by circumference, the coefficient of variation of QCT was lower than that of DXA and RBAE because QCT uses cross-sectional images that are less affected by bone size.

The BMD as measured by QCT increased rapidly with age until 2 years and then plateaued, which was similar to the equine growth curve. All 7 samples from horses that were euthanized because of fracture were within the population range. However, the collected samples in this experiment do not reflect whole population of horses, and those collected were from a breeding mare and a service horse with excellent racing records. Therefore, no case of morbid fracture was included. To summarize, the data regarding BMD changes with age provide a reference for further quantitative studies.

Women experience an accelerated phase of bone loss during menopause, and this accelerated bone loss is the dominant cause of postmenopausal osteoporosis [14]. Kobayashi described that in foals there was a difference in BMD based on the sex [10]. In this study, male horses and female horses showed similar changes with age; in other words, female horses did not show a dramatic decrease in BMD with age. However, horses have a reproduction period during their entire lives that differs from that of humans [8]. As isolated legs were used in this experiment, serum was not collected. The concentrations of estrogen, parathyroid hormone, or other biomarkers of bone metabolism may provide a reason for the stability of BMD in aging female horses [5]. Serum concentrations of hormones or biomarkers should be considered in future experiments.

Bell *et al*. reported that horses are stalled for several months before sale to improve hair coat and to prevent injuries. However, this practice could leave horses with weaker bones that are more prone to injury when training commences [1]. Thus, this suggests that BMD may decrease when horses are rested in a stall while recovering from injury, increasing the risk of skeletal injuries when training resumes. In addition, there was a high incidence of bone disorders, including splint, bucked shin, and sesamoiditis, when BMD did not change even though training had increased [10]. Therefore, QCT will be a useful modality for evaluating the effects of training and providing an estimate of fatigue fracture risk.

In conclusion, quantitative measurement of BMD using QCT may have great potential for the evaluation of bone biology for breeding and rearing management. *In vivo* investigations are warranted.

## Acknowledgment

This study was supported by a grant from the Equine Research Institute of the Japan Racing Association.

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