FULL PAPER *Surgery*

Assessment of Trabecular Bone Mineral Density Using Quantitative Computed Tomography in Normal Cats

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ABSTRACT . The aim of this study was to assess age-related changes and anatomic variation in trabecular bone mineral density (tBMD) using quantitative computed tomography (QCT) in normal cats. Seventeen normal cats were included in this study and divided into the following 3 age groups:<6 months (n=4), 2–5 years (n=10) and >6 years (n=3). A computed tomographic scan of each vertebra from the 12th thoracic to the 7th lumbar spine and the pelvis was performed with a bone-density phantom (50, 100 and 150 mg/cm^3, calcium hydroxyapatite, CIRS phantom®). On the central transverse section, the elliptical region of interest (ROI) was drawn to measure the mean Hounsfield unit (HU) value. Those values were converted to equivalent tBMD (mg/cm^3) by use of the bone-density phantom and linear regression analysis (r^2 >0.95). The mean tBMD value of the thoracic vertebrae (369.4 ± 31.8 mg/cm^3) was significantly higher than that of the lumbar vertebrae (285 ± 58.1 mg/cm^3). The maximum tBMD occurred at the T12, T13 and L1 levels in all age groups. There was a statistically significant difference in the mean tBMD value among the 3 age groups at the T12 (P<0.001), T13 (P<0.001) and L4 levels (P=0.013), respectively. The present study suggests that age-related changes and anatomic variation in tBMD values should be considered when assessing tBMD using QCT in cats with bone disorders.

KEY WORDS: bone mineral density, feline, quantitative computed tomography, trabecular bone.

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Bone mineral density (BMD) decreases in accordance with normal aging and metabolic or systemic disease. The turnover rates for cancellous bone are 4 to 8 times greater than for cortical bone, so cancellous bone is more sensitive to disease-induced changes [25]. Therefore, in humans with osteoporosis, BMD has been primarily investigated in cancellous bone.

On the basis of this relationship, several techniques have been developed to noninvasively measure bone mineralization at various skeletal sites. These include radioabsorptiometry (RA), single photon absorptiometry (SPA), dual photon absorptiometry (DPA), quantitative ultrasound (QUS), dual-energy x-ray absorptiometry (DEXA), quantitative computed tomography (QCT) and peripheral QCT (pQCT). Among these, DEXA, QCT and pQCT have become the established methods for evaluating skeletal status, assessing osteoporosis, determining fracture risk and monitoring metabolic diseases and therapies in humans [8, 10–13].

Feline diseases affecting BMD include mucopolysaccharidosis, osteopetrosis, osteogenesis imperfect and osteopenia related to primary hyperparathyroidism and secondary to renal or nutritional hyperparathyroidism. Reports in cats have been limited to the studies for BMD evaluation using DEXA [17] and a few peer-reviewed studies that measured BMD in clinically normal cats and abnormal cats using DEXA [3, 23]. DEXA has been accepted as the gold standard method of BMD measurement, and it requires less X-ray exposure for the patient and is cheaper, faster and more accurate than computed tomography [1]. However, soft tissues cause a slight overestimation of DEXA measurements, and area density integrates the amount of mineral but also indirectly the dimensions of bone, such as bone diameter and cortical width [19, 20]. In contrast, QCT has advantages over DEXA, because it can discriminate trabecular bone and cortical bone and measure trabecular bone mineral density (tBMD) alone [26]. Therefore, QCT is an ideal modality for measurement of tBMD. However, there is a lack of reference data for feline tBMD measured by QCT. The purpose of this study was to assess age-related changes and anatomic variation of tBMD using QCT in normal cats.

MATERIALS AND METHODS

Animals: The study population consisted of clinically normal client-owned cats. Seventeen normal cats fed nutritionally balanced commercial pet food were included in

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this study, 12 domestic shorthair, 2 Siamese, an American shorthair, a Persian and a Turkish angora. There were 4 males, 3 neutered males, 8 females and 2 neutered females. The cats had no previous orthopedic injury or disease and no musculoskeletal abnormalities upon physical examination. They were healthy based on complete blood cell count, serum chemistry profile and survey radiographs of the thorax, abdomen and pelvis. Fat accumulation in the body may influence bone marrow fat content and may alternate trabecular bone mineral density, and so we used a 9-point scoring system to evaluate the body condition of each cat. The 9-point scoring system rated body condition from 1 to 9 (1, emaciated; 5, ideal; 9, grossly obese) [9]. The 17 cats were divided into 3 groups according to age as follows: less than 6 months old, 2 to 5 years old and over 6 years old for groups 1 to 3, respectively. Group 1 consisted of 4 cats, group 2 consisted of 10 cats, and group 3 consisted of 3 cats. The mean ages were 0.4 ± 0.1 years, 3.1 ± 1.3 years and 7.7 ± 1.5 years in groups 1, 2 and 3, respectively. The mean body weights were 1.9 ± 0.8 kg, 4.9 ± 1.0 kg and 4.7 ± 0.9 kg, respectively, in groups 1, 2 and 3. The mean body condition scores (BCS) were 4, 5.3 ± 1.5 and 5.7 ± 3.1 in groups 1, 2 and 3, respectively (Table 1). All experimental procedures were approved by the Institutional Animal Care and Use Committee at Chungbuk National University, and informed owner consent was obtained prior to study enrollment.

Anesthesia: Each cat was premedicated with a subcutaneous injection of 0.05 mg/kg atropine (Atropine®; Daewon Pharm Co., Ltd., Seoul, Korea). Anesthesia was induced with an intravenous bolus injection of 5 mg/kg propofol (Anepol®; Hana Pharmaceutical Co., Ltd., Seoul, Korea) and maintained using 2–2.5% isoflurane (Isoflurane®; Choong-Wae Pharma Corporation, Seoul, Korea) in oxygen after endotracheal intubation. During anesthesia, oxygen saturation, heart rate and end tidal CO_2 were monitored continuously.

Quantitative computed tomography (QCT): Cats were positioned in dorsal recumbency within the CT scanner (HiSpeed CT/e; General Electric, Medical Systems Department, Milwaukee, WI, U.S.A.) under general anesthesia. After acquisition of lateral and dorsoventral pilot views, transverse images of the vertebra from the 12th thoracic vertebra to 7th lumbar vertebra and pelvis were obtained. The mean CT numbers for three regions containing calcium hydroxyapatite (CIRS phantom®, Computerized Imaging Reference Systems, Inc., Norfolk, VA, U.S.A.) were regressed against their known calcium concentrations (50, 100 and 150 mg/cm^3 of calcium hydroxyapatite) to determine the slope and intercept of the calibration line for each image [5]. The scanning protocols were as follows: transverse scan, 120 kVp, 100 mA, 2 mm thickness, 1.3 pitch and a 512 × 512 pixel matrix. Beam hardening correction was not used, and the standard reconstruction was used.

Image analysis: All CT image data were imported into a PC workstation, and tBMD values were measured at the vertebral body and iliac wing and viewed in a constant window (window width, 1000; window level, 200). The elliptical ROI of the trabecular bone at the most central transverse slice for each vertebral body was drawn. Each elliptical ROI was drawn by an eFilm viewing software (eFilm™ Workstation version 2.1; Merge Healthcare, Milwaukee, WI, U.S.A.). Each ROI for the iliac wing was drawn by manual tracing on an independent console using the manufacturer's software, respectively (Fig. 1). To minimize the beam hardening effects, beam hardening regions were avoided when drawing the ROI. Each HU measurement was repeated three times by a single observer. The mean HU value for each ROI was converted to equivalent tBMD (mg/cm^3) by use of the bone-density phantom and linear regression analysis (Fig. 2).

Statistical analysis: All data were expressed as means ± standard deviation (SD). Statistical analysis was performed by using the SPSS statistical program (SPSS version 17.0; SPSS Inc., Chicago, IL, U.S.A.). The mean tBMD value for the ROI in each group was analyzed by one-way analysis of variance (ANOVA), and differences between each group were evaluated with post hoc analysis and Dunn's method. The level of significance was set at a value of $P<0.05$.

RESULTS

Quantitative tBMD in normal cats: The quantitative mean tBMD in the females was slightly higher than that of the males, while the mean tBMD of the ilium was higher in males, although there were no significant difference between genders (data not shown). The quantitative tBMD values for the 12th thoracic vertebra to 7th lumbar vertebra were 315.5 ± 35.3 mg/cm^3, 325.6 ± 39.9 mg/cm^3, 315.0 ± 26.0 mg/cm^3, 277.2 ± 19.6 mg/cm^3, 274.8 ± 33.4 mg/cm^3, 263.9 ± 38.0 mg/cm^3, 257.9 ± 21.9 mg/cm^3, 262.7 ± 19.4 mg/cm^3 and 274.9 ± 13.8 mg/cm^3, respectively, in group 1. In group 2, the quantitative tBMD values for the 12th thoracic vertebra

Table 1. Age, weight and body condition score (BCS) in all groups

	Group 1	Group 2	Group 3
Number (n)	4	10	3
Age (years)	0.4 ± 0.1	3.1 ± 1.3	7.7 ± 1.5
Body weight (kg)	1.9 ± 0.8	4.9 ± 1.0	4.7 ± 0.9
BCS	4	5.3 ± 1.5	5.7 ± 3.1

Abbreviations: yr, year; BW, body weight; BCS, body condition score.
Age, weight, BCS are shown as the mean±SD.
A 9-point scoring system was used to evaluate the body condition of each cat.
Body condition scores lower than 5, equal to 5 and over 5 represent cachectic and underweight condition, optimal conditions and overweight and obese conditions, respectively.

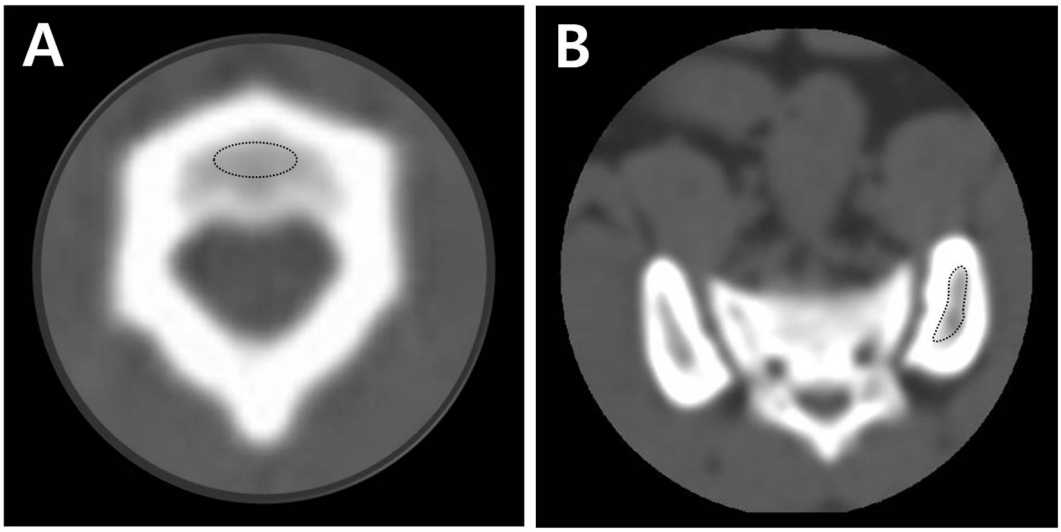

Fig. 1. Measurement of the mean Hounsfield unit (HU) value for each region of interest (ROI) at the level of the lumbar vertebra (A) and ilium (B). An elliptical ROI of trabecular bone at the most central transverse slice for each vertebral body was drawn. The ROI of trabecular bone at the level of the iliac wing was drawn by manual tracing, respectively.

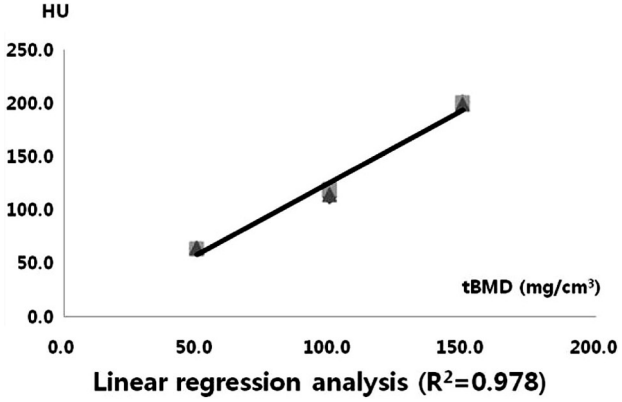

Fig. 2. Conversion of the mean Hounsfield unit (HU) value to trabecular bone mineral density (tBMD). For each region of interest (ROI), the mean HU value was converted to the equivalent tBMD by use of a bone-density phantom and linear regression analysis (R^2=0.978).

to 7th lumbar vertebra were 391.2 ± 43.1 mg/cm^3, 401.5 ± 40.2 mg/cm^3, 362.8 ± 48.1 mg/cm^3, 312.8 ± 35.5 mg/cm^3, 313.5 ± 49.1 mg/cm^3, 320.1 ± 41.7 mg/cm^3, 305.5 ± 36.9 mg/cm^3, 282.7 ± 56.8 mg/cm^3 and 302.1 ± 35.5 mg/cm^3, respectively. In group 3, the quantitative tBMD values for the 12th thoracic vertebra to 7th lumbar vertebra were 301.9 ± 39.2 mg/cm^3, 328.1 ± 16.3 mg/cm^3, 313.9 ± 29.9 mg/cm^3, 231.8 ± 17.9 mg/cm^3, 204.7 ± 62.3 mg/cm^3, 166.7 ± 45.2 mg/cm^3, 182.5 ± 76.0 mg/cm^3, 158.3 ± 64.9 mg/cm^3 and 213.4 ± 5.4 mg/cm^3, respectively. In all groups, there were significant differences in the mean tBMD values among vertebral bodies (P<0.001) (Fig. 3).

The overall quantitative tBMD values for the left ilium were 231.8 ± 72.6 mg/cm^3, 277.2 ± 75.5 mg/cm^3 and 239.2 ± 49.4 mg/cm^3 in group 1, 2 and 3, respectively. The overall quantitative tBMD values for the right ilium were 245.1 ± 43.7 mg/cm^3, 273.1 ± 93.3 mg/cm^3 and 235.2 ± 53.5 mg/cm^3 in group 1, 2 and 3, respectively. There was no significant difference in mean tBMD values between the right and left

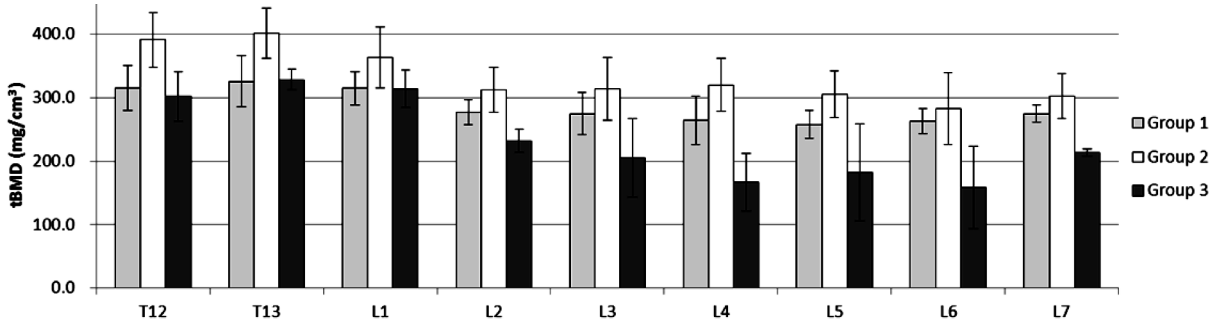

Fig. 3. Anatomic variation of the mean trabecular bone mineral density (tBMD) at the level of the vertebra. There were significant differences in mean tBMD values among vertebra in all groups (P<0.001). a) P<0.05 versus the mean tBMD value at T12, b) P<0.05 versus the mean tBMD value at T13, c) P<0.05 versus the mean tBMD value at L1.

ilium in each group. No significant difference in mean tBMD was observed among any groups (Fig. 4).

The overall quantitative tBMD values in group 1 were 327.87 ± 37.60 mg/cm^3, 279.73 ± 31.27 mg/cm^3 and 238.47 ± 58.99 mg/cm^3 at the thoracic vertebra, lumbar vertebra and ilium, respectively. The overall quantitative tBMD values in group 2 were 396.32 ± 41.60 mg/cm^3, 311.83 ± 46.65 mg/cm^3 and 275.19 ± 84.17 mg/cm^3 at the thoracic vertebra, lumbar vertebra and ilium, respectively. The overall quantitative tBMD values in group 3 were 314.99 ± 31.75 mg/cm^3, 210.20 ± 67.26 mg/cm^3 and 237.18 ± 28.17 mg/cm^3 at the thoracic vertebra, lumbar vertebra and ilium. In all groups, significant differences were observed between the mean tBMD value of the thoracic vertebra and that of the lumbar vertebra (P<0.05). However, there were no significant differences between the mean tBMD values of the lumbar vertebra and ilium in group 1 and group 2, between the mean tBMD values of the lumbar vertebra and ilium in group 3 or between the mean tBMD values of the thoracic vertebra and ilium in group 3 (Fig. 5).

Age-related changes in mean tBMD were significantly observed at the level of the thoracic vertebra and lumbar vertebra (P<0.001). The mean tBMD value of group 2 was significantly higher than that of group 1 and group 3 at the level of the thoracic vertebra. In addition, there were statistically significant differences in the mean tBMD values at the level of the lumbar vertebra among all groups. The mean tBMD of the lumbar vertebra in group 3 was significantly lower than that in group 1 and group 2 (P<0.05) (Fig. 6).

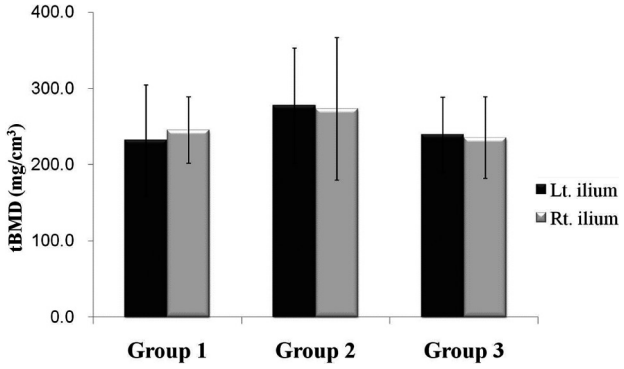

Fig. 4. Anatomic variation of the mean trabecular bone mineral density (tBMD) at the level of the ilium. There was no significant difference in mean tBMD values between the right and left ilium for each group. Also, a significant difference in mean tBMD was not observed among any groups.

DISCUSSION

With the advance of imaging techniques, several methods for quantification of BMD have been used to investigate diseases affecting BMD in humans and animals. Among these methods, RA, SPA, DPA and DEXA are inexpensive, easy to manage and relatively accurate, but they cannot measure the tBMD separately. In contrast to these methods, QCT is capable of measuring trabecular, cortical or integral bone at any site, centrally or peripherally. Also, QCT is so far the only method to measure the tBMD per unit volume and is a clinically established method for assessment of tBMD [6]. However, there are few published reports using QCT in normal cats. To our knowledge, there is only one report of the use of QCT to measure tBMD in adult cats [4]. Therefore, the tBMD values estimated by QCT in this study may contribute to establishment of normative reference values for tBMD obtained by QCT in healthy cats, to assessment of the severity of disease and to evaluation of therapeutic response. Therefore, tBMD values were measured using QCT for cats with various ages and anatomical sites in this study.

In the present study, the numbers of cats in group 1 and group 3 were relatively small, and the breeds used in this study were inconsistent. We used clinically normal client-owned cats. So, it was quite difficult to recruit a sufficient number of volunteers, because our study design included the use of anesthesia. However, we found that there were significant differences in tBMD among groups, and we regarded this as a meaningful result. Also, most of the cats used in this study were relatively popular and common breeds and had similar body weights and body conditions. So, we did

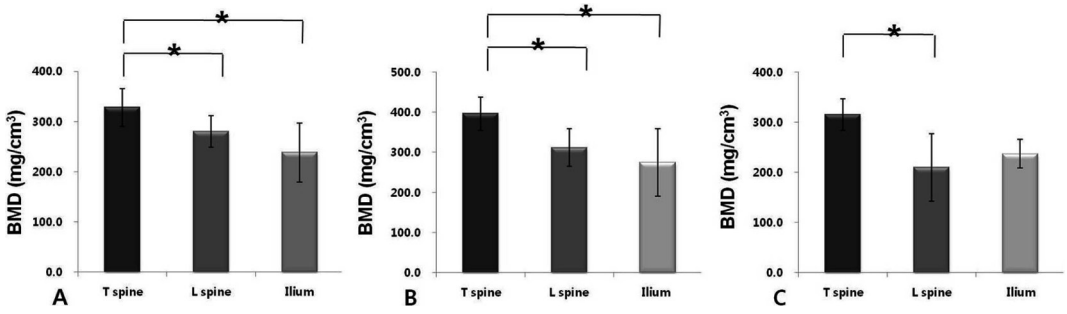

Fig. 5. Variation of mean trabecular bone mineral density (tBMD) according to anatomical site in Group 1 (A), Group 2 (B) and Group 3 (C), respectively. In all groups, significant differences were observed for mean tBMD values between the thoracic vertebra and lumbar vertebra. Also, there were significant differences between the mean tBMD values of the lumbar vertebra and ilium in group 1 and group 2 (P<0.05). However, there were no significant differences between mean tBMD values of the lumbar vertebra and ilium in any groups or between mean tBMD values of the thoracic vertebra and ilium in group 3. *P<0.05 (Dunn's method).

not regard it as a major concern that these breeds cannot represent the cat population.

In this study, there was no significant difference in tBMD between genders. This result might have been due to the small study population in this study. However, considering the previous report that there were no significant differences in tBMD between male and female dogs, our results may reflect a unique pattern of tBMD in animals, though we cannot extrapolate it to cat tBMD directly [22].

The vertebra and ilium were selected out of various skeletal sites as the anatomical sites for tBMD measurements. This selection of the anatomical sites to be investigated was based on the fact that the vertebra, and pelvis are skeletal sites rich in trabecular bone [24]. Clinical relevance was also considered, as the ilium is a standardized site for bone biopsy [2], and tBMD values for the lumbar vertebra and pelvis are measured to monitor metabolic diseases and evaluate the treatment effect of drugs in human and veterinary medicine [4, 12, 15]. However, the tBMD values of only the T12 and T13 vertebra were measured in the thoracic vertebra region in this study. This is because assessment of tBMD for clinical purposes may not require estimation of tBMD for the entire vertebra. It may also be helpful to reduce the time required to perform a QCT scan, to decrease radiation exposure and to decrease the duration of anesthesia in clinical application of QCT.

This study demonstrated the following order of mean tBMD values from highest to lowest: the thoracic vertebra, the lumbar vertebra and the pelvis. The thoracic vertebra and lumbar vertebra showed significant differences in all groups. Several studies have reported the differences in tBMD values throughout the lumbar, thoracic and cervical spine in human medicine. One study showed that mean tBMD values for women and men at the T12 vertebra (193.1/184.9 mg/cm^3) were higher than those at the L4 vertebra (186.2/180.1 mg/cm^3) [28]. Another study revealed that the mean tBMD value at the T1 vertebra was higher than that at the L2 through L4 vertebrae in healthy female adults [29]. The present study showed significantly higher mean tBMD values for the entire thoracic vertebra than for the entire lumbar vertebra and a significantly higher mean tBMD value for the T13 vertebra than that of the L1 through L7 vertebrae in each group. This result was consistent with a previous report in human medicine that the tBMD of the lumbar vertebral was lower than that of the thoracic vertebra and that age-related changes were greater in the lumbar vertebra than in the thoracic vertebra. It is generally accepted that the tBMD of the lumbar vertebra is lower than that of the thoracic vertebra, and the lumbar vertebra is more sensitive to age-related changes than the thoracic vertebra in humans. In addition, the study of Dominik *et al.* reported that the tBMD of the lumbar vertebra is lower than that of the thoracic and cervical vertebrae [29]. Therefore, it is assumed that the lower tBMD of the lumbar vertebra compared with the thoracic vertebra in this study may reflect the difference in trabecular architecture between the thoracic and lumbar vertebrae in humans.

In the present study, there was no QCT-estimated significant difference between the left and right ilium. Previously, studies in human medicine had found the correlation between skeletal sites to be too poor to allow a clinically useful prediction of BMD at one site from measurement at another site [21]. The prediction of BMD at a given skeletal site from measurement at another site has been controversial.

The mean tBMD values of all the groups were significantly different at the level of the entire thoracic vertebra and lumbar vertebra excluding the ilium. In a published study by Lauten *et al.* using DEXA to evaluate BMD in cats, the mean BMD (g/cm^2) for approximately the first 10 months of age was significantly lower than the mean BMD (g/cm^2) between 2 and 5 years of age [17]. Although direct comparison with this study could not be made, this agreed with our results showing that the mean tBMD values of group 1 at the level of the thoracic vertebra and lumbar vertebra were significantly lower than those of group 2. A previous study reported that changes in body composition were evident during growth and development in young cats, particularly, during the first 6 month of age [23]. Our result showing lower tBMD values in group 1 than group 2 can be addressed

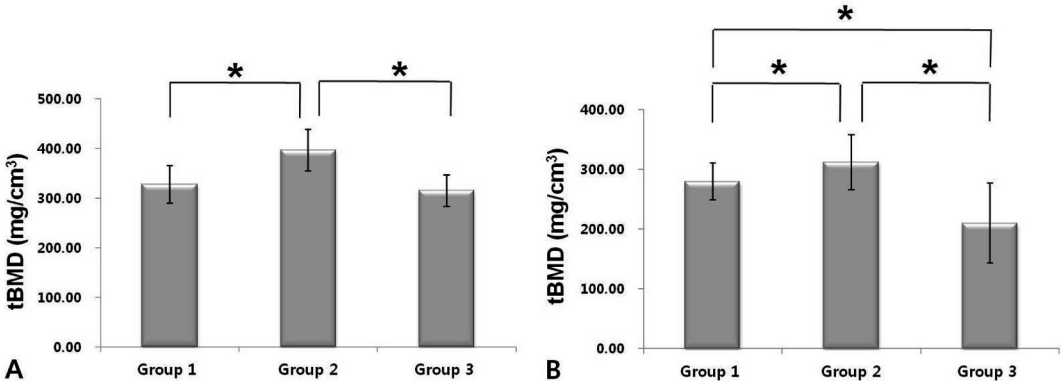

Fig. 6. Age-related changes in trabecular bone mineral density (tBMD) at the level of the T spine (A) and L spine (B). The mean tBMD value of group 2 was significantly higher than those of group 1 and group 3 at the level of both the thoracic and lumbar vertebrae (P<0.001). The mean tBMD value of group 3 was significantly lower than those of group 1 and group 2. *P<0.05.

based on a study showing that bone mineral content (BMC) increases up to about 6 months of age. In human medicine, normal aging is associated with bone marrow adiposity [27]. One study reported that a reduction in BMD was caused by a decrease in BMC and increase in fat content in the vertebra with aging [18]. Therefore, the lower mean tBMD in group 3 than group 2 is likely caused by the relatively high bone marrow fat/bone mineral content ratio. Several studies have suggested that an increase in marrow fat would cause an apparent decrease in tBMD [14, 16, 30]. The inverse relationship between bone marrow fat and tBMD is still controversial. Although bone marrow fat does not directly replace BMC, it has an etiological link to the reduction of BMD because increasing amounts of bone marrow fat affect bone turnover through the inhibition of osteoblast function and survival and the promotion of osteoclast differentiation and activation [7]. In particular, considering the remarkable reduction in the mean tBMD value for the lumbar vertebra in group 3, the mean tBMD value at the level of the lumbar vertebra may be an important parameter in relation to age for evaluating skeletal status.

The results of this study are likely to provide information for mean tBMD values at various anatomic sites. However, these values are not intended to be used as a sole diagnostic instrument, but should contribute to forming an overall clinical impression for cats in which these measurements are performed and should be an improvement over current clinical and research settings. In a further study, a comparison of BMD measurements obtained by use of other modalities such as chemical-physical analysis and DEXA in the feline skeleton with those obtained by QCT will be performed, and the accuracy of tBMD measurement using QCT will be assessed. Furthermore, densitometric normative reference values in relation to age and anatomic site should be established. Finally, quantification of densitometric differences for tBMD between healthy cats and cats affected bone disorders should be performed.

In the present study, tBMDs of normal cats were estimated using QCT, and age- related changes in tBMD and anatomic variation were identified. This study suggests normative tBMD values for the normal cat in accordance with aging, and these values can be applied to diagnosis of systemic diseases that affect tBMD and to monitor therapeutic response of bone disorders.

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