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Interrelationships between morphometric, densitometric and mechanical properties of lumbar vertebrae in pigs

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Abstract: This study was performed to determine interrelationships between morphometric, densitometric and mechanical properties of the lumbar vertebrae in pigs. Six lumbar vertebrae (L_1-L_6) were isolated *post mortem* from healthy males (n = 6) of the Large Polish White breed at the age of 8 months. Computed tomography technique was used to determine total bone volume (B_{vol}), cross-sectional area (A) and trabecular bone mineral density (Td) of the vertebral body, mean volumetric bone mineral density (MvBMD), as well as calcium hydroxyapatite density in the trabecular (Td_{Ca-HA}) and cortical (Cb_{Ca-HA}) bones of the lumbar vertebrae. Using dual-energy x-ray absorptiometry (DEXA) method, bone mineral density (BMD) and bone mineral content (BMC) were determined. The compression test of the lumbar vertebrae was performed to derive mechanical parameters such as ultimate force (F_u), ultimate stress (σ_u), Young's modulus (E), stiffness (S) and work to the ultimate force point (W). Pearson's correlation coefficient was determined for all the investigated variables. Results obtained in this study showed positive correlations between bone weight, B_{vol}, A and BMC, while the same parameters were negatively correlated with densitometric parameters such as Td, Cb_{Ca-HA}, Td_{Ca-HA} and MvBMD. Positive correlations were found between Td, Td_{Ca-HA}, Cb_{Ca-HA} and MvBMD, while BMC was found to be negatively correlated with Td, Td_{Ca-HA}, Cb_{Ca-HA} E and σ_u. Mechanical parameters such as F_u, E, σ_u, S and W were found to be positively correlated. Furthermore, Cb_{Ca-HA} and BMD showed positive correlations with the values of F_u, σ_u, and W. In conclusion, due to relatively poor correlations of the morphometric and densitometric parameters with mechanical strength of the lumbar vertebrae, results of this study suggest that densitometric measurements should be followed by mechanical evaluation for precise determination of the axial skeleton properties in pigs. Considering the striking similarities existing between humans and pigs in terms of size and shape of spine, as well as physiology and anatomy of the gastrointestinal tract and skeletal system, the use of these animals in pre-clinical studies on bone metabolism regulation with pharmacological and dietary factors seems to be very advantageous when interpolating obtained results for humans.

Keywords: bone mineral density, quantitative computed tomography, lumbar spine, pig

INTRODUCTION

The skeletal system in vertebrates consists of two types of bone tissue – the trabecular and cortical bone compartments. Both these compartments are characterized by different structural characteristics, metabolic activity, modeling and remodeling capacity, as well as physiological functions in the organism. Cortical bone, possessing significantly higher bone mineral density than the trabecular, forms the shafts of long bones and skull bones and counteracts bending forces. Trabecular bone is present as the internal structure of the metaphyses and epiphyses of long bones and the pelvis, as well as vertebral bodies, and serves as the structure resistant to compressive stress [1]. Due to much more intensive metabolic turnover rate of the trabecular bone compartment, when compared to the cortical bone, the metabolic response of the trabecular bone to physiological, pharmacological and nutritional factors is faster than in the cortical bone compartment [2]. Annual turnover rates of the trabecular and cortical compartments were assessed at 20-25% and 1-3%, respectively [3]. The structural content of spinal vertebrae is formed of the cortical shell and cancellous core [4]. Due to the relatively poor appearance of cortical bone in the spine, the determination of morphological, densitometric and mechanical parameters of the vertebrae may be considered as a methodological approach for investigation of the trabecular bone compartment properties. In humans, vertebral fractures are common osteoporotic complications which lead to permanent spine deformities, back pain, physical functioning impairment, immobility, social isolation and depression in the sufferers [5, 6].

The mechanical properties of bones depend on both the mechanical quality and the amount of bone material, and on the architectural disposition of bone material [7]. Non-invasive methods of measurement of bone mineral density (BMD) are crucial for *in vivo* diagnosis of bone mass in humans and experimental animals, as well as for monitoring effectiveness of treatment of metabolic bone diseases. Bone mineral density determined in humans with use of the dual-energy x-ray absorptiometry (DEXA) method was reported as a good

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predictor for vertebral fracture risk [8, 9]. In experimental animals, data on correlations between BMD and mechanical strength of the spine are strictly limited. Furthermore, theoretical considerations limit the use of bone mineral density alone as a surrogate for vertebral strength prediction. Even though BMD is an integral value of material content, it cannot simply reflect the potentially important effects of geometric and architectural differences and densitometric inhomogeneities on vertebral strength [10].

Numerous similarities between pigs and humans, such as nutrient digestion and digestive system anatomy and physiology, have been reported [11]. Moreover, it was shown that pig's growth plate in bones resembles human growth plate in terms of cellular numbers in the different zones, cell kinetics and patterns of closure more closely than those of rodents [12]. Thus, the use of pigs for the investigation of common interrelationships between skeletal system and biologically-active substances administered via the gastrointestinal tract and potentially influencing bone tissue seems to be very advantageous when interpolating obtained results for humans.

The aim of this study was to determine interrelationships between morphometric, densitometric and mechanical properties of the lumbar vertebrae in pigs. To achieve this, six lumbar vertebrae (L_1-L_6) were isolated from pigs and evaluated with the use of computed tomography technique, dual-energy x-ray absorptiometry and compressive test. Pearson's correlation coefficient was determined for all the investigated variables.

MATERIALS AND METHODS

The experimental procedures used throughout this study were approved by the Ethics Committee on Animal Experimentation of the Agricultural University in Lublin, Poland.

Experimental design and sampling procedure. The study was performed on 6 healthy male pigs of the Large Polish White breed. The animals were kept under standard rearing conditions with *ad libitum* access to fresh water and commercial diet feed. Animals were sacrificed at the age of 8 months of life to isolate six lumbar vertebrae (L_{1} - L_6). After isolation procedure, the lumbar vertebrae were cleaned of remaining soft tissues, each bone was weighed, wrapped in gauze soaked in isotonic saline and stored at -25ºC for further analyses.

Determination of bone morphometric, densitometric and mechanical properties. The lumbar vertebrae were thawed for 4 h at room temperature before assessment of bone morphological properties, bone mineral density and biomechanical testing. Bone mineral density (BMD) and bone mineral content (BMC) were determined for each lumbar vertebra separately (L_1-L_6) in the dorsoventral direction using dual-energy x-ray absorptiometry (DEXA) method and Norland XR-46 apparatus, supplied with Research Scan software (Norland, Fort Atkinson, WI, USA). Quantitative computed tomography (QCT) method and Somatom Emotion - Siemens apparatus (Siemens, Erlangen, Germany), supplied with Somaris/5 VB10B software were used for volumetric bone mineral density (vBMD) determination of the trabecular bone of each lumbar vertebra. The volumetric bone mineral density was measured for the trabecular bone of the vertebral body using 2 mm thick, cross-sectional QCT scans, placed at 50% of each vertebral body length, and the cross-sectional area (A) of the vertebral body at this point was measured automatically. Using Osteo CT application package (Software Version B10/2004A), the determination of calcium hydroxyapatite (Ca-HA) density for both the trabecular (Tb_{Ca-HA}) and cortical bone (Cb_{Ca-HA}) was performed for each vertebral body. The lumbar vertebrae were scanned together with the water- and bone-equivalent calibration phantom; Ca-HA measuring scans were 10-mm thick (Figure 1). Using Volume Evaluation application package, the total bone volume (B_{vol}) of each vertebra was determined. For these calculations, the volume-of-interest (VOI) was defined by limiting the minimum and maximum density for the investigated bone at 60 and 3,000 Hounsfield units (HU), respectively. Using this application, the mean volumetric bone mineral density (MvBMD) for each lumbar vertebra was measured. Biomechanical evaluation of the lumbar vertebrae required removal of the posterior and transversal processes. The mechanical parameters of each vertebral body were measured by compression test at a load rate of 20 mm/min, using the Zwick/Roell Z010 machine (Zwick/Roell, Ulm, Germany). The mechanical parameters, such as ultimate force (F_u), ultimate stress (σ_u), Young's modulus (E), stiffness (S) and work to the ultimate force point (W), were derived as reported previously [13].

Statistical analysis. Statistical analysis was performed using Statistica software (version 6.0). Pearson's correlation coefficient was determined between all the investigated variables. The level of statistical significance was set at *P*-value < 0.05.

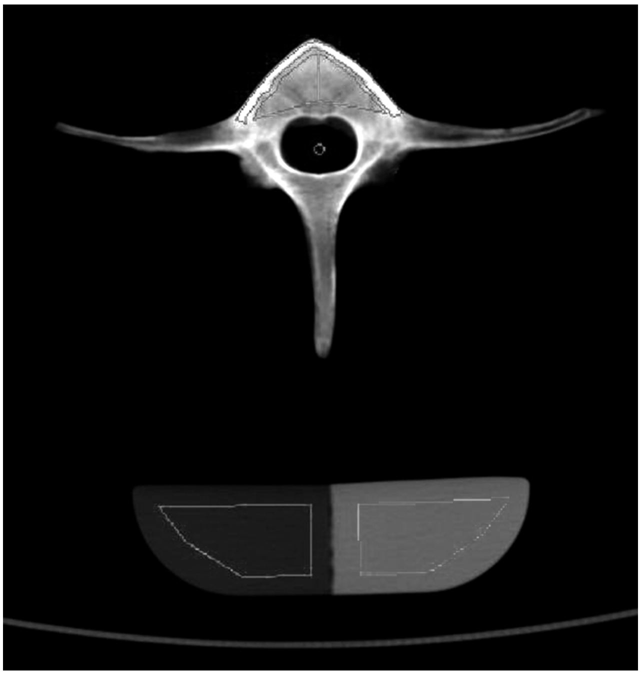

Figure 1 Measurement of calcium hydroxyapatite density in the trabecular (Tb_{Ca-HA}) and cortical bone (Cb_{Ca-HA}) in the lumbar vertebra of pig using Osteo CT application package. The values of Tb_{Ca-HA} and Cb_{Ca-HA} were measured within automatically defined regions of interest (ROI) for the trabecular and cortical bone in the centre and in the margins of the vertebral body, respectively. The bone sample was scanned together with the water- and bone-equivalent calibration phantom consisting of the water-equivalent part (on the left) and bone-equivalent part (on the right). The measuring scan was 10 mm thick and placed in the middle of each investigated vertebra.

Table 1 Values of Pearson's correlation coefficient between all investigated parameters of lumbar vertebrae in pigs

Investigated parameter	Bone weight	B_{vol}	Area	Td	MvBMD	Td_{Ca-HA}	Cb_{Ca-HA}	BMD	BMC	Fu	YM	Stress	Stiffness	Work
Bone weight	x	0.48*	0.50*	-0.38*	-0.03	-0.28	-0.62*	-0.46*	0.71*	-0.14	-0.09	-0.25	0.05	-0.18
B_{vol}	0.48*	x	0.63*	-0.50*	-0.53*	-0.45*	-0.48*	-0.26	0.74*	-0.25	-0.31	-0.38*	-0.13	-0.18
Area	0.50*	0.63*	x	-0.41*	-0.40*	-0.50*	-0.48*	-0.16	0.42*	0.13	0.06	-0.11	0.12	-0.13
Td	-0.38*	-0.50*	-0.41*	x	0.62*	0.88*	0.43*	0.39*	-0.59*	0.19	0.13	0.28	0.05	0.16
MvBMD	-0.03	-0.53*	-0.40*	0.62*	x	0.60*	0.46*	0.32	-0.32	0.09	0.14	0.30	0.02	0.01
Td_{Ca-HA}	-0.28	-0.45*	-0.50*	0.88*	0.60*	x	0.31	0.22	-0.40*	-0.03	-0.08	0.08	-0.09	-0.01
Cb_{Ca-HA}	-0.62*	-0.48*	-0.48*	0.43*	0.46*	0.31	x	0.80*	-0.49*	0.25	0.25	0.51*	-0.09	0.44*
BMD	-0.46*	-0.26	-0.16	0.39*	0.32	0.22	0.80*	x	-0.29	0.36*	0.31	0.55*	0.02	0.52*
BMC	0.71*	0.74*	0.42*	-0.59*	-0.32	-0.40*	-0.49*	-0.29	x	-0.31	-0.35*	-0.37*	-0.08	-0.19
Fu	-0.14	-0.25	0.13	0.19	0.09	-0.03	0.25	0.36*	-0.31	x	0.88*	0.62*	0.67*	0.72*
YM	-0.09	-0.31	0.06	0.13	0.14	-0.08	0.25	0.31	-0.35*	0.88*	x	0.60*	0.67*	0.67*
Stress	-0.25	-0.38*	-0.11	0.28	0.30	0.08	0.51*	0.55*	-0.37*	0.62*	0.60*	x	0.39*	0.54*
Stiffness	0.05	-0.13	0.12	0.05	0.02	-0.09	-0.09	0.02	-0.08	0.67*	0.67*	0.39*	x	0.28
Work	-0.18	-0.18	-0.13	0.16	0.01	-0.01	0.44*	0.52*	-0.19	0.72*	0.67*	0.54*	0.28	x

*$P < 0.05$.

RESULTS

The values of Pearson's correlation coefficient between all the investigated parameters of the lumbar spine in the pigs are shown in Table 1. Positive correlation was found between all the investigated parameters describing bone morphology, namely bone weight, total bone volume (B_{vol}) and cross-sectional area (A) of the vertebral body ($P < 0.05$). Bone weight was negatively correlated with trabecular bone mineral density (Td), calcium hydroxyapatite density of the cortical bone (Cb_{Ca-HA}) and bone mineral density (BMD; $P < 0.05$). Total bone volume of the lumbar vertebrae and A were found to be negatively correlated with Td, mean volumetric bone mineral density (MvBMD), calcium hydroxyapatite density in the trabecular bone (Td_{Ca-HA}) and Cb_{Ca-HA} ($P < 0.05$). The results obtained in this study also showed positive correlations between Td and MvBMD, Td_{Ca-HA}, Cb_{Ca-HA}, as well as BMD ($P < 0.05$). Mean volumetric bone mineral density was positively correlated with both the calcium hydroxyapatite density of the trabecular and cortical bones ($P < 0.05$). Bone mineral density measured for the lumbar vertebrae showed positive correlations with Cb_{Ca-HA}, ultimate force (F_u, Figure 2A), ultimate stress (σ_u, Figure 2B) and work to the ultimate force point (W, Figure 2C; $P < 0.05$). The analysis of bone mineral content in the L_1-L_6 vertebrae showed positive correlation of this parameter with bone weight, B_{vol} and A, while Td, Td_{Ca-HA}, Cb_{Ca-HA}, Young's modulus (E) and σ_u were found to be negatively correlated with BMC ($P < 0.05$). Positive and statistically significant correlations were found between all the mechanical parameters of the lumbar vertebrae, except for the correlation between stiffness and work to the ultimate force point. Furthermore, Cb_{Ca-HA} was found to be positively correlated with σ_u (Figure 3A) and W (Figure 3B), while σ_u and B_{vol} were negatively correlated ($P < 0.05$).

DISCUSSION

Biomechanical studies on experimental animals have shown that during standing and walking, the spine in quadrupeds may be subjected to all kinds of loads. As the result of the control of posture and movements, the muscles induce significant axial compression of the spine [14]. Considering the lumbar spine in both humans and quadrupeds, the facet joints play a key role in transforming axial torsion moments to the pelvis, making loading conditions very similar for quadrupeds and humans [15, 16]. These data are strongly supported by the results of analysis of bone architecture, which is closely related to its mechanical function, and arranged in such a way as to optimally bear the physiological loads. According to Wolff's law, the process of functional adaptation of bone includes resorption of unloaded bone, while mechanical loading stimulates bone formation. Furthermore, bone tissue adapts to heavy loads rather than to minor ones, and only regular loads such as during locomotion. Thus, the architecture of the trabecular bone in the vertebrae provides evidence of loading conditions of the spine [14]. The results of comparative studies in humans and quadrupeds have shown that the trabecular bone architecture is oriented from one endplate to another, proving regular axial compression as fundamental in the spine in both cases [14, 17].

Although there are striking similarities between the human species and quadrupeds in terms of spine structure and

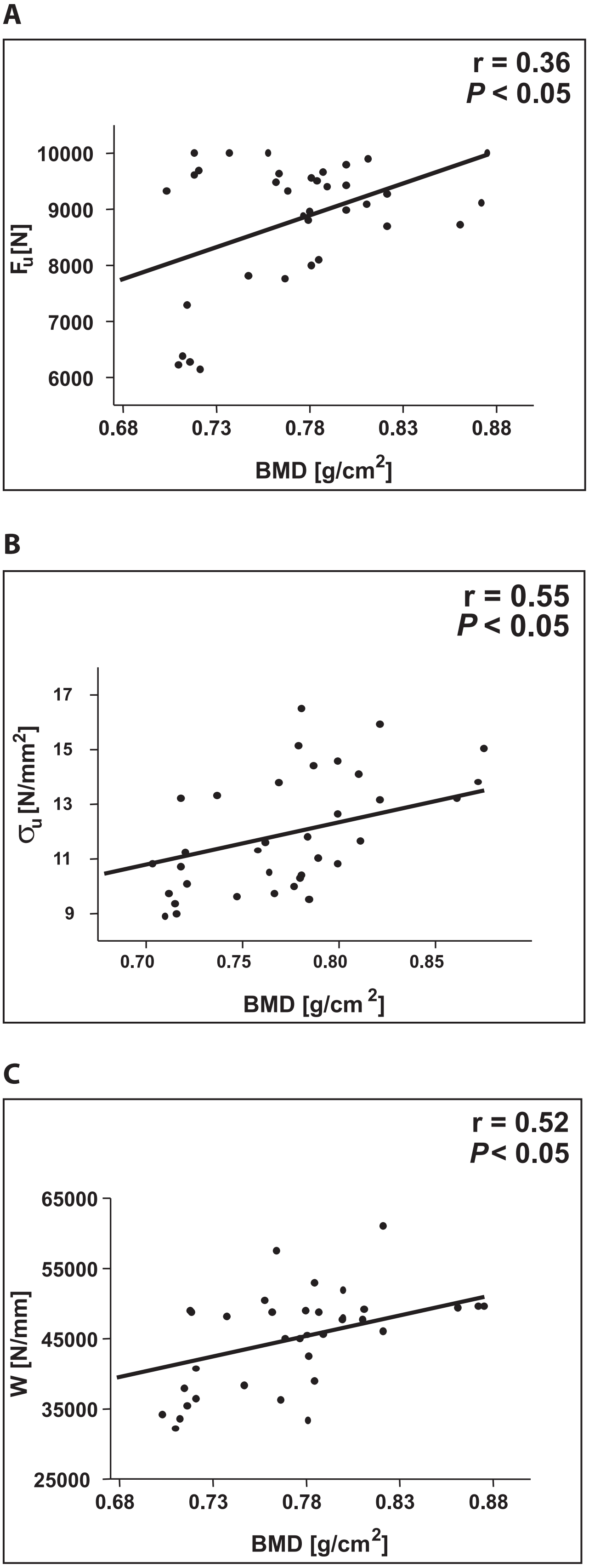

Figure 2 Correlations between ultimate force (Fu), ultimate stress (σu), work to the ultimate force point (W) and bone mineral density (BMD) of the lumbar vertebrae in pigs.
r – Pearson's correlation coefficient.
P – statistical significance.

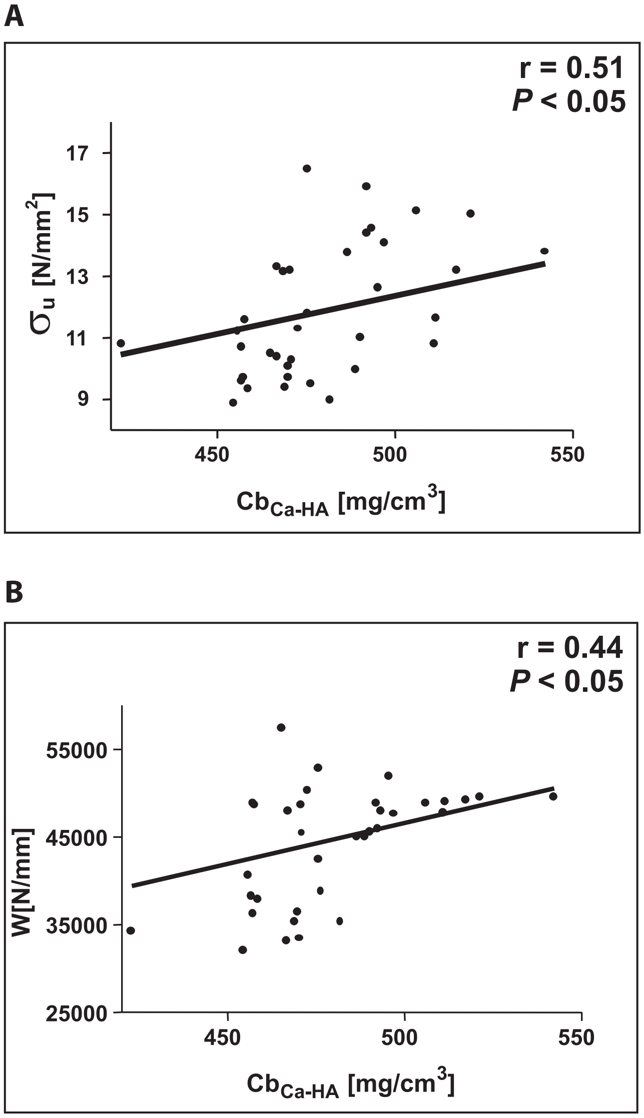

Figure 3 Correlations between ultimate stress (σu), work to the ultimate force point (W) and calcium hydroxyapatite density of the cortical bone (CbCa-HA) of the lumbar vertebrae in pigs.
r – Pearson's correlation coefficient.
P – statistical significance.

physiology, the existence of the most important differences must be mentioned here to avoid potential pointless interpretations of the experimental results. As reported by Smit (2002), the most significant difference between humans and animals concerns the trabecular bone density. Significantly higher trabecular bone density in the vertebral body of quadrupeds strongly indicates higher axial compression stress of the spine and higher mechanical strength of the bones in animals than in humans. It has been shown in experimental studies that the mechanical strength of the lumbar vertebrae in goats and humans is similar, even the value of the cross-sectional area of the lumbar vertebrae is significantly lower in goats [14]. The other differences result from the anatomical structure of the vertebrae in humans and quadrupeds. The articular surfaces have an angle of more than 60º to the frontal plane in humans, while in quadrupeds that angle is less than 30º; however, the stiffness of the lumbar segments to axial torsion is similar in both cases [18-20].

The results of this study show the interrelationships between morphometric, densitometric and mechanical properties of the lumbar vertebrae in pigs. Positive correlations were stated for all the investigated morphometric parameters, such as bone weight, total bone volume and cross-sectional area of the vertebral body. The densitometric parameters determination also showed positive correlations between Td, MvBMD, Td_{Ca-HA}, Cb_{Ca-HA} and BMD in the majority of cases. Neither the region of interest defined for determination of bone mineral density of the trabecular or cortical bone compartments, or both combined, nor the method – volumetric or areal determination of bone mineral density, had significant influence on the obtained results, thus proving the comparable value of quantitative computed tomography and dual-energy x-ray absorptiometry for BMD measurement in the lumbar spine of healthy pigs. These results are in accordance with the outcome of densitometric studies in humans, where significant correlations between BMD determined with the use of QCT and DEXA were reported [3, 21, 22]. On the other hand, bone mineral content of the lumbar vertebrae was negatively correlated with Td, Td_{Ca-HA} and Cb_{Ca-HA}; however, the interrelationships between these parameters may be explained by bone mineral density inhomogeneities within the structure of the investigated bones, similar to those observed in spine of humans and other species of animals [10, 14, 23]. Results of the current study also show positive correlations between mechanical parameters. The highest values of the Pearson's correlation coefficient of the ultimate force and Young's modulus with all other mechanical parameters were found. These findings clearly indicate that both the F_u and E may be considered as very precise parameters characterizing the bone mechanical properties of the axial skeleton derived as a result of the compression test. It is worth noting the fact that ultimate force is determined as an independent parameter from the sample size, while Young's modulus is derived after adjusting for sample size [13]. The results of this study also show that parameters such as bone weight, B_{vol} and A are negatively correlated with the parameters describing bone mineral density, both areal and volumetric. However, bone weight, B_{vol} and A were found to be positively correlated with BMC, suggesting the possible use of these parameters to predict BMC in the axial skeleton of pigs. Except for BMD and Cd_{Ca-HA}, the obtained results also show relatively poor relationships between densitometric and mechanical parameters of the lumbar vertebrae, making difficult any prediction of axial skeleton strength simply on the basis of spine denensitometry. As opposed to BMC, the highest value for prediction of the mechanical properties of the lumbar vertebrae showed BMD that was positively correlated with the F_u, σ_u and W. Similarly to BMD, positive correlations between Cb_{Ca-HA} and the mechanical parameters of the lumbar spine were observed. On the basis of the results obtained in this study, one can conclude that determination of bone morphological properties and bone mineral density with the use of non-invasive techniques has reasonable value *per se* for evaluation of the axial skeleton of experimental animals. However, poor correlations of the morphometric and densitometric parameters with mechanical properties of the lumbar vertebrae of the investigated pigs limit consideration of the DEXA and computed tomography-derived measurements as ideal methods to predict compressive strength of the axial skeleton. These observations are in contrast to recent studies on turkeys, where clear positive correlations between morphometric, densitometric and mechanical properties were reported and computed tomography technique postulated as a useful tool for predicting *in vivo* mechanical properties of bones. However, the previous studies were performed on the appendicular skeleton of turkeys, and tibia served as the model for morphometric, densitometric and mechanical considerations. Furthermore, in contrast to this study which included 8-months-old pigs only, the previous experiment was performed on developing animals at 4 age-differentiated groups [24]. As opposed to the current study on pigs, investigations on humans comparing DEXA and QCT have shown similar correlations of bone mineral density measured with the use of both these techniques with the mechanical strength of the vertebrae. However, these studies concern the relationships between bone mineral density and mechanical properties of the thoracic and lumbar vertebrae in humans across a wide range of ages [25-27].

In conclusion, this study shows that determination of the morphometric, densitometric and mechanical parameters of the lumbar vertebrae in pigs provides a wide range of data characterizing bone properties of the axial skeleton. Relatively poor correlations of the morphometric and densitometric parameters with mechanical strength of the axial skeleton in pigs suggest that densitometric measurements with the use of non-invasive methods, such as DEXA and computed tomography, should be followed by mechanical evaluation of the lumbar vertebrae. Due to the striking similarities existing between humans and pigs in terms of size and shape of spine, as well as physiology and anatomy of gastrointestinal tract and skeletal system, use of these animals in pre-clinical studies on bone metabolism regulation with pharmacological and dietary factors seems to be very advantageous when interpolating obtained results for humans.

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