INTERRELATIONSHIPS BETWEEN DENSITOMETRIC, MORPHOMETRIC, AND MECHANICAL PROPERTIES OF THE FEMUR IN MALE POLISH MERINO SHEEP

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Abstract

The aim of the study was to determine the interrelationships between densitometric, morphometric, and mechanical properties of the femur in 5 month old Polish Merino sheep. The study was performed on 24 healthy male lambs at the age of 5 months. All right femora were weighed and their length was measured. Using QCT technique, the determination of volumetric bone mineral density (vBMD) of the trabecular and cortical bone compartments was performed. Total bone volume and mean volumetric bone mineral density of whole femur were also determined. Geometrical properties such as cross-sectional area, second moment of inertia, mean relative wall thickness, and cortical index were calculated on the basis of computed tomography measurements. Mechanical properties of the femur in terms of maximum elastic strength (Wy) and ultimate strength (Wf) were determined using a three-point bending test. Furthermore, Pearson's correlation coefficient was determined for all the investigated variables of the femur. Obtained results have shown significant correlations between morphometric, densitometric, and mechanical parameters of the femur. Statistically significant correlations between vBMD of cortical bone and Wy ($r = 0.61$) and Wf ($r = 0.57$) indicate the highest value of this densitometric parameter for mechanical strength prediction of bones in vivo. Thus, computed tomography technique may serve in sheep as non-invasive and precise tool for in vivo monitoring of metabolic responses of skeleton, in terms of morphological and densitometric properties, to physiological, metabolic, nutritional, pharmacological, and toxicological factors influencing bone tissue metabolism.

Key words: sheep, femur, bone properties, quantitative computed tomography, densitometry.

Bone metabolic diseases affect both humans and animals. Osteoporosis is the most common metabolic bone disease in humans. In animals, osteoporosis may be found in all species; however, the highest incidence is observed in poultry, especially in laying hens (4, 23). The main clinical problem resulting from osteoporosis is fracture of several bones in axial and peripheral skeleton leading to severe pain and locomotory dysfunctions. Fractures are associated with decreased bone mass of skeleton determined by bone morphology and geometry, bone tissue deposition, microarchitectural organisation, and bone mineral density. In humans, fractures associated with osteoporosis are usually located at the proximal femur, lumbar vertebrae, and distal radius. Fractures of the hip and vertebrae pose the most serious health risk associated with high-cost hospital care and high morbidity and mortality rates (11). The rat is the most frequently used animal model for investigations on osteoporosis. It is relatively cost-effective model; however, some limitations complicate interpretation of the data and sample collection. There are noticeable differences between human bones and those of rodents concerning bone mineral metabolism, lack of lamellar bone, and limited capacity for bone remodelling process. Furthermore, sequential collection of body fluids in reasonable volumes for wide biochemical analyses, as well as bone sample collection and performance of surgical procedures are difficult due to small size of rats (14, 15). Considering that sheep are easy to handle and house, submissive, relatively inexpensive, available for cohort studies, ovulate spontaneously, and have hormone profiles similar to women, this model may serve for experimental studies on bone metabolic diseases. There are no limitations for serial sample collection of body fluids in sheep and bones are large enough to perform morphological, densitometric, and biomechanical analyses. Mineral metabolism in sheep is
similar to that observed in humans. Bone size, structure, and gross anatomy of the sheep skeleton generally represent the human. Histological studies have shown similar bone remodelling processes in human and sheep (16). Furthermore, physiological influences and effects of hormones on production of biochemical bone formation marker – osteocalcin in sheep and humans are comparable (9, 14). The femur in sheep consist of two types of bone tissue: compact (cortical) and cancellous (trabecular). Both these bone compartments are characterised by different structural characteristics, metabolic activity, modelling and remodelling capacity, as well as physiological functions. While cortical bone has higher bone mineral density than trabecular, forms the shafts of long bones and skull bones, and counteracts bending forces, the cancellous bone builds metaphyses and epiphyses of long bones, pelvis, and vertebral bodies, and serves as the structure resistant to compressive stress (2, 18). Furthermore, the cortical and trabecular bone compartments have differentiated metabolic turnover rate reaching annually 1%-3% and 20%-25%, respectively (5). Thus, investigation with the use of femur model serves as a methodological approach to study the effects of various factors influencing bone metabolism in both the trabecular and cortical bone compartments (6, 17).

To assess the diagnostic value for non-invasive evaluation of bone quality of the femur with the use of quantitative computed tomography technique, the determination of the interrelationships between densitometric, morphometric, and mechanical properties of this bone in Polish Merino sheep was performed in this study.

Material and Methods

Experimental design and sampling procedure. The experimental procedures used throughout this study were approved by the Local Ethics Committee on Animal Experimentation. The study was performed on 24 healthy male Polish Merino lambs sired by the same ram and born physiologically to ewes. The lambs and ewes were kept indoors in pens under standard rearing conditions with free access to drinking water. Ewes were fed a standard diet recommended for this physiological stage. From the 22nd d of life, the lambs were fed a commercial concentrate and hay ad libitum. The weaning of the lambs was performed at the age of 10 weeks of their life. The lambs were sacrificed at the age of 5 months of life, the lambs were fed a commercial diet recommended for this physiological stage. From the 22nd d of life, the lambs were fed a commercial concentrate and hay ad libitum. The weaning of the lambs was performed at the age of 10 weeks of their life. The lambs were sacrificed at the age of 5 months of life and the right femur was isolated for analyses. All bones were weighed and the length was measured. Bone samples were kept frozen at −25°C until further analyses. Using Quantitative computed tomography (QCT) technique and SOMATOM EMOTION SIEMENS apparatus (Siemens, Germany) equipped with Somaris/5 VB10B software (version B10/2004A), the determination of volumetric bone mineral density (vBMD) of the femur was performed. Volumetric BMD was measured for both the trabecular and cortical bone with the use of 2-mm thick, cross-sectional, metaphyseal and mid-diaphyseal QCT scans, respectively. The trabecular bone mineral density (Td) was determined on the scan placed at 15% of total femur length, measuring from the distal bone extremity, proximal to the distal growth plate. The cortical bone mineral density (Cd) was measured on the scan of diaphysis at 50% of femur length. The total bone volume (Bvol) and mean volumetric bone mineral density (MvBMD) of whole femur were determined using Volume Evaluation application package. For measurements, 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, respectively. Geometrical properties such as cross-sectional area (A), second moment of inertia (Ix), mean relative wall thickness (MRWT), and cortical index (CI) were calculated on the basis of measurements of horizontal and vertical diameters of mid-diaphyseal cross-section of the femur (3, 7, 8). The mechanical properties of the femur in terms of maximum elastic strength (Wy) and ultimate strength (Wf) were determined in INSTRON 3367 apparatus (Instron, USA) using a three-point bending test. The distance between bone supports was set at 40% of femur length, and the measuring head loaded bone samples with a constant speed of 50 mm/min.

Statistical analysis. Statistical analysis was performed using Statistica software (version 6.0). Pearson’s correlation coefficient (r) was determined for all the investigated variables of the femur and P<0.05 was considered as statistically significant.

Results

The values of Pearson’s correlation coefficient between all the investigated parameters of the femur are shown in Table 1. Bone weight was positively correlated with such parameters as: bone length, Td, Cd, Bvol, A, and Ix (P<0.05). Bone length was positively correlated with all of these parameters, as well as MvBMD and Wy (P<0.05). Trabecular bone mineral density was positively correlated with Cd, MvBMD, Bvol, A, and Ix (P<0.05). Cortical BMD was positively correlated with all of these parameters, as well as Wy and Wf (P<0.05). Cross-sectional area of the femur was positively correlated with Ix (r=0.80; P<0.05). Furthermore, these parameters were found to be positively correlated with total bone volume of the femur (P<0.05). Positive correlations were also found between geometrical parameters such as MRWT and CI (r=0.99; P<0.05) and between Wy and Wf representing mechanical parameters of the femur (r=0.81; P<0.05). Negative correlations of MRWT and CI with bone weight, Bvol, and Ix were also stated (P<0.05).

Discussion

Considering that sheep is the animal model commonly used to study regulatory processes of systemic growth and development with the use of physiological, pharmacological, metabolic, and nutritional factors, both in prenatal and postnatal life, very limited data are available on the usefulness of computed tomography technique for in vivo assessment of quality of the skeletal system (13, 19, 20, 22).
Table 1
The values of Pearson’s correlation coefficient between all the investigated parameters of the femur in ram lambs at the age of 5 months

<table>
<thead>
<tr>
<th>Investigated parameter</th>
<th>Bone weight</th>
<th>Bone length</th>
<th>Td</th>
<th>Cd</th>
<th>MvBMD</th>
<th>Bvol</th>
<th>A</th>
<th>Ix</th>
<th>MRWT</th>
<th>CI</th>
<th>Wy</th>
<th>Wf</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone weight</td>
<td>x</td>
<td>0.834*</td>
<td>0.496*</td>
<td>0.436*</td>
<td>0.089</td>
<td>0.963*</td>
<td>0.756*</td>
<td>0.879*</td>
<td>−0.559*</td>
<td>−0.560*</td>
<td>0.249</td>
<td>0.083</td>
</tr>
<tr>
<td>Bone length</td>
<td>0.834*</td>
<td>x</td>
<td>0.638*</td>
<td>0.650*</td>
<td>0.461*</td>
<td>0.828*</td>
<td>0.770*</td>
<td>0.724*</td>
<td>−0.263</td>
<td>−0.261</td>
<td>0.454*</td>
<td>0.282</td>
</tr>
<tr>
<td>Td</td>
<td>0.496*</td>
<td>0.638*</td>
<td>x</td>
<td>0.592*</td>
<td>0.734*</td>
<td>0.531*</td>
<td>0.526*</td>
<td>0.508*</td>
<td>−0.044</td>
<td>−0.055</td>
<td>0.356</td>
<td>0.139</td>
</tr>
<tr>
<td>Cd</td>
<td>0.436*</td>
<td>0.650*</td>
<td>0.592*</td>
<td>x</td>
<td>0.465*</td>
<td>0.482*</td>
<td>0.573*</td>
<td>0.456*</td>
<td>0.057</td>
<td>0.067</td>
<td>0.608*</td>
<td>0.572*</td>
</tr>
<tr>
<td>MvBMD</td>
<td>0.089</td>
<td>0.461*</td>
<td>0.734*</td>
<td>0.465*</td>
<td>x</td>
<td>0.174</td>
<td>0.369</td>
<td>0.181</td>
<td>0.243</td>
<td>0.240</td>
<td>0.167</td>
<td>0.068</td>
</tr>
<tr>
<td>Bvol</td>
<td>0.963*</td>
<td>0.828*</td>
<td>0.531*</td>
<td>0.482*</td>
<td>0.174</td>
<td>x</td>
<td>0.748*</td>
<td>0.946*</td>
<td>−0.635*</td>
<td>−0.636*</td>
<td>0.226</td>
<td>0.100</td>
</tr>
<tr>
<td>A</td>
<td>0.756*</td>
<td>0.770*</td>
<td>0.526*</td>
<td>0.573*</td>
<td>0.369</td>
<td>x</td>
<td>0.748*</td>
<td>0.801*</td>
<td>−0.073</td>
<td>−0.075</td>
<td>0.400</td>
<td>0.223</td>
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<tr>
<td>Ix</td>
<td>0.879*</td>
<td>0.724*</td>
<td>0.508*</td>
<td>0.456*</td>
<td>0.181</td>
<td>0.946*</td>
<td>0.801*</td>
<td>x</td>
<td>−0.580*</td>
<td>−0.581*</td>
<td>0.230</td>
<td>0.112</td>
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<tr>
<td>MRWT</td>
<td>−0.559*</td>
<td>−0.263</td>
<td>−0.044</td>
<td>0.057</td>
<td>0.243</td>
<td>−0.635*</td>
<td>−0.073</td>
<td>−0.580*</td>
<td>x</td>
<td>0.999*</td>
<td>0.158</td>
<td>0.056</td>
</tr>
<tr>
<td>CI</td>
<td>−0.560*</td>
<td>−0.261</td>
<td>−0.055</td>
<td>0.067</td>
<td>0.240</td>
<td>−0.636*</td>
<td>−0.075</td>
<td>−0.581*</td>
<td>0.999*</td>
<td>x</td>
<td>0.160</td>
<td>0.068</td>
</tr>
<tr>
<td>Wy</td>
<td>0.249</td>
<td>0.454*</td>
<td>0.356</td>
<td>0.608*</td>
<td>0.167</td>
<td>0.226</td>
<td>0.400</td>
<td>0.230</td>
<td>0.158</td>
<td>0.160</td>
<td>x</td>
<td>0.813*</td>
</tr>
<tr>
<td>Wf</td>
<td>0.083</td>
<td>0.282</td>
<td>0.139</td>
<td>0.572*</td>
<td>0.068</td>
<td>0.100</td>
<td>0.223</td>
<td>0.112</td>
<td>0.056</td>
<td>0.068</td>
<td>x</td>
<td>0.813*</td>
</tr>
</tbody>
</table>

* P<0.05.
Discussion

Considering that sheep is the animal model commonly used to study regulatory processes of systemic growth and development with the use of physiological, pharmacological, metabolic, and nutritional factors, both in prenatal and postnatal life, very limited data are available on the usefulness of computed tomography technique for *in vivo* assessment of quality of the skeletal system (13, 19, 20, 22). QCT and dual-energy X-ray absorptiometry (DEXA) are used as the most common methods for non-invasive determination of bone mineral density of skeleton in humans and animals. The most important differences between these methods result from applied technological solutions. In both methods, X-rays crossing throughout examined area (volume) of interest undergo physical attenuation that is proportional to the density of the medium. While DEXA measures areal (two-dimensional and size dependent) BMD expressed in g/cm², QCT enables determination of real volumetric (three-dimensional and size independent) BMD in grams per cubic centimetre. In contrast to QCT where any selected anatomical structure is analysed independently, DEXA measures density of all anatomical structures located on X-ray trajectory. Thus, the measurements of BMD with DEXA method may be loaded by errors resulting from the presence of fat, muscles, and pathological changes on bone surface. The advantage of QCT over DEXA is providing vBMD separately in trabecular and cortical bone and being free of the inaccuracies caused by extrasosseous calcification and hyperostosis. While QCT method enables measuring of BMD of several bones in skeleton using all possible scan trajectories, DEXA is utilised mainly for measurements in posteroanterior or lateral direction. Depending on resolution in currently available scanners, volumetric BMD may be measured on QCT scans possessing thickness from few micrometers up to 1 centimetre (1, 10, 21).

The results of this study present the interrelationships between morphometric, densitometric, and mechanical properties of the femur obtained from 5 month old ram lambs. Considering morphological traits of the femur, positive correlation of bone weight and length was stated. These parameters were found to be positively correlated with morphological parameters, such as total bone volume, cross-sectional area, and second moment of inertia that were estimated with the use of computed tomography. Furthermore, mean relative wall thickness and cortical index were negatively correlated with bone weight, total bone volume, and second moment of inertia. The analysis of densitometric parameters of the femur has shown positive correlations between vBMD of the trabecular and cortical bone, as well as mean volumetric bone mineral density estimated for whole bone. However, only Td and Cd were positively correlated with total bone volume, cross-sectional area, and second moment of inertia. Thus, these parameters seem to correspond much better than MvBMD with the morphological characteristic of the femur. In the presented study, the investigation of mechanical properties of the femur has shown that maximum elastic strength was positively correlated with bone length and ultimate strength. However, both: the maximum elastic strength and ultimate strength of the femur were found to be correlated with cortical bone mineral density estimated at the midshaft. The obtained results show important contribution of bone mineralisation to mechanical properties of the femur in sheep; however, decisive role in resistance to bending forces of this bone is played by cortical bone compartment. Thus, Cd measurement of the femur seems to be an important predictor of bone mechanical properties in sheep. The observed lack of interrelationships between trabecular bone mineral density and mean volumetric bone mineral density of the whole bone with maximum elastic strength and ultimate strength is not surprising when one considers the main role of cancellous bone structure in resistance to compressive stress (2, 5). Results of densitometric analyses in this study are in contrast with data obtained from growing turkeys, in which Td, Cd, and MvBMD of the tibia were significantly correlated with the maximum elastic strength and ultimate strength. In both studies, the length of long bones was correlated with mechanical endurance. Moreover, MRWT and CI were found to be negatively correlated with bone length, total bone volume, and second moment of inertia both in turkeys and sheep. Noteworthy is the fact that the data in studies on turkeys were collected from four age-differentiated groups consisting of 4, 8, 12, and 20 week old birds (12). The results of our study, showing lack of interrelationships between geometrical parameters and mechanical endurance of bones are in contrast also with investigations on rats, which have shown positive correlations between cross-sectional area and moment of inertia with stiffness and strength of the femur. Furthermore, in the study on male and female rats between 18 and 44 weeks of life, MRWT was found to be positively correlated with stiffness and strength of the femur (8). However, the differences in the results obtained in the studies on rats, turkeys, and sheep may be the effect of discrepancy between physiological processes of skeletal modelling and remodelling conditioned by species-specific features, age, and sex of the experimental animals.

Considering the observed correlations among morphometric, densitometric, and mechanical parameters in this study, computed tomography may be proposed as a valuable technique for determination of volumetric BMD and morphological properties of bones in the skeleton of sheep *in vivo*. Among morphological traits of bones, this technique enables determination of bone volume, bone length, and geometrical parameters both *in vivo* and *ex vivo*. Measurement of cortical bone density in the midshaft of the femur indicates the highest value of this densitometric parameter for prediction of mechanical properties of bones *in vivo*. Thus, computed tomography technique may serve in sheep as non-invasive and precise tool for observations of skeletal system properties during different stages of development in animals. Furthermore, the assessment of skeletal properties with this technique may serve for effective breeding selection of animals aimed on optimal skeletal...
quality achievement, as well as for monitoring of metabolic responses of bone tissue in experimental studies on physiological, metabolic, nutritional, pharmacological, and toxicological factors influencing the skeletal system.

References