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Identification of mechanical and structural properties of dog bone tissue – methodological aspects

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Purpose: In this paper, the results of biomechanical studies conducted on an animal model are presented. The research shows the importance of detailed consideration of anatomical and functional differences relative to the human skeleton. The aim of this study was to assess the mechanical properties of cortical bone tissue, determine density values and compare different methods for measuring cancellous bone tissue density in terms of their practical application and limitations. *Methods*: In the presented research, the Young's modulus of canine cortical bone was determined using microindentation. The density of cancellous bone tissue in the proximal epiphysis of the canine femur was determined using several selected research methods. *Results*: A comparison of Young's modulus values for different bone areas showed that the highest values of the bone tissue's Young's modulus were obtained within the middle part of the bone shaft. It was observed that the distribution of density values is similar across the methods used, however, the range of the determined values differs slightly. *Conclusions*: The determination of values and assessment of the mechanical properties of cortical bone tissue, along with the comparison of different methods for measuring cancellous bone tissue density, allows for more accurate prediction of fracture risk and more effective planning of surgical interventions.

Key words: *dog bone tissue, micro-CT, Vickers hardness, density, ash density*

1. Introduction

Bone tissue, a highly complex biological material, is characterized by its heterogeneous structure, anisotropy and viscoelastic properties. Bone tissue is a hierarchically structured, multifunctional composite exhibiting pronounced heterogeneity, anisotropy and viscoelastic behaviour. The structure of the bone is composed of two primary compartments: cortical and trabecular bone, which exhibit significant disparities in their microarchitecture, thereby resulting in disparate mechanical responses to loading. A thorough understanding of the structure and mechanical parameters of bone tissue is crucial for comprehending its function in both physiological and pathological conditions.

Although the method for determining parameters such as hardness and density has been extensively described in the literature and applied to human [19] and rodent bone tissue [30], its use in dogs has been limited. While numerous mechanical and structural parameters have been reported for human and small-animal bones, corresponding data for veterinary species – particularly dogs – remain scarce. This limitation is largely due to the substantial morphological and geometric variability observed among approximately 400 recognized dog breeds,

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which complicates the establishment of uniform biomechanical reference values. At the same time, dogs are increasingly serving as an important research model in many fields. Therefore, the development and validation of precise and repeatable methods for assessing the structural and mechanical parameters of bone tissue in this species are extremely important. Evaluating the accuracy, repeatability, and mutual correlations of methods for measuring cancellous bone density will, in the future, allow for better development of recommendations for preclinical and clinical research.

Conducting biomechanical studies on an animal model requires detailed consideration of anatomical and functional differences compared to the human skeleton. The canine skeleton, consisting of about 321 bones, 90 of which are in the pelvic limbs [7], has specific morphological and biomechanical properties. The typical gait of a dog is a walk, during which at least two limbs maintain contact with the ground. This movement pattern generates unique force distributions that differ significantly from those observed in humans. While in bipedal humans the knee joint is among the most heavily loaded, the quadrupedal kinematic chain of a dog results in the shoulder and stifle being among the most loaded joints. Crucially, these canine joints also differ significantly in geometry from their human counterparts.

Previous studies on the kinematics of dog gait have included the analysis of flexion angles in the hip and knee joints [11], [23], ground reaction force values [3], and the distribution of loads acting on the pelvic limb bones [25]–[27]. These studies are essential due to the considerable anatomical and biomechanical diversity among approximately 400 dog breeds. As quadrupeds, dogs exhibit distinct load distribution, kinematic chain dynamics, and angular configurations of both fore- and hindlimbs, which fundamentally differentiate their joint loading patterns from those observed in humans. The results of these works clearly indicate significant species differences and the need for further research to deepen the knowledge of dog locomotion biomechanics and the material properties of bone tissue in the context of planning orthopedic treatment. These insights are crucial not only for advancing basic biomechanical understanding but also for supporting the design and optimization of veterinary implants and prosthetic devices.

The aim of this paper was a comprehensive assessment of the mechanical properties of cortical bone tissue and a comparison of different methods for measuring the density of cancellous bone tissue in terms of their practical application and limitations.

2. Materials and methods

2.1. Measurement of microhardness and determination of Young's modulus for cortical bone tissue of dogs

To assess the mechanical properties of cortical bone tissue, samples from the femoral diaphyses of large-breed dogs were used. Each bone was divided into three segments approximately 5 mm high: a proximal segment (L1), located near the proximal epiphysis of the femur; a central segment (L2), representing the middle part of the diaphysis; and a distal segment (L3), adjacent to the distal epiphysis. The segments were separated using a Struers Accutom-5® precision metallographic cutter, after which the samples were embedded in PMMA resin to stabilize their position during measurements. Due to the difficulties in obtaining biological material from dogs, the study was conducted using 4 long bones originating from 4 different individuals.

On each segment, microhardness measurements were carried out in four zones (A1, A2, B1, B2) indicated in the figure (Fig. 1), with six individual tests performed for each zone. A CSM MicroCombi Tester® was used, employing the Vickers microindentation method. A maximum load of 2000 mN with a contact force of 15 mN was used in each measurement, simultaneously recording the penetration depth and the applied force. The obtained data were used to determine the microhardness values and the longitudinal modulus of elasticity of the tested bone tissue.

2.2. Characterization of cancellous bone tissue density in the proximal femoral epiphysis of a dog

The main objective of the research was to determine the variation in the density of cancellous bone tissue in the proximal femoral epiphysis of large-breed dogs to obtain reference values for biomechanical models. Cylindrical samples with a diameter of 5 mm were prepared, collected from five locations: the femoral neck (S1), the upper part of the trochanter (S3), the lower part of the trochanter (S2), the medial side of the metaphysis (S4) and the lateral side of the metaphysis (S5).

The research procedure consisted of two complementary stages. In the first stage, the density of the samples was determined using the hydrostatic method.

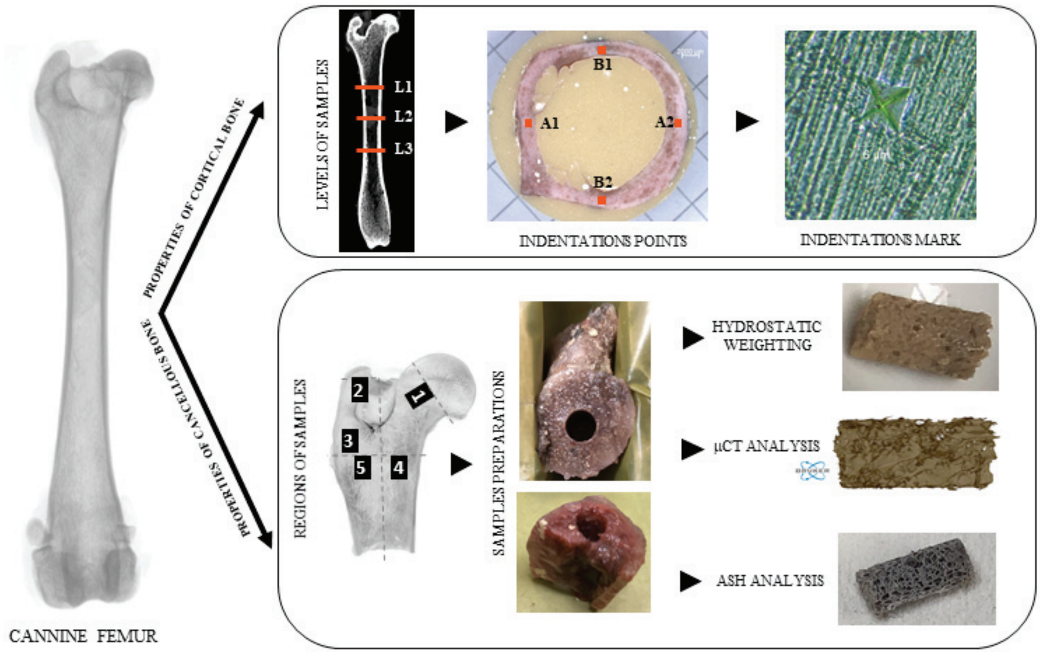

Fig. 1. Diagram of the experimental research procedure for microindentation measurement and identification of sites and regions for collecting cylindrical cancellous bone tissue samples for density measurement

Each sample was weighed on a RADWAG PS 1000/C/2 precision hydrostatic balance with a measurement accuracy of 0.001 g, and its volume was determined based on buoyancy in extraction gasoline (density 0.74 g/cm^3).

In the second stage, the samples were subjected to detailed analysis using a Bruker® SkyScan1172 micro-computed tomograph. The measurement was performed at a lamp voltage of 65 kV, a current of 153 µA, and a spatial resolution of 8.93 µm, using an aluminum filter. The reconstructed images were analyzed in CTAn® software, which allowed for the determination of bone mineral density (BMD), bone volume (BV) and total sample volume (TV).

The final stage was the assessment of sample mineralization using the calcination method. The samples were incinerated in a muffle furnace for 48 hours at a temperature of 500 °C, according to the [14], to remove organic substances. After calcination, the samples were weighed and their volume was determined based on micro-CT data. The values obtained in this way were used to calculate the ash density, a key indicator of the degree of mineralization of the tested cancellous bone tissue.

2.3. Data evaluation and statistical analysis

Statistical analysis for individual parameter groups was conducted using various statistical tests:

- Statistical analysis of microhardness values of bone tissue within the three levels (L1, L2, L3) was performed using ANOVA with Tukey's post hoc test;
- Next, statistical analysis of the value $E_{IT} = \sqrt{\pi^2 S}\sqrt{Ap}$, the Young's moduli of bone tissue within the three levels (L1, L2, L3), was conducted using the Kruskal–Wallis test;
- Statistical analysis of both Young's moduli and microhardness values of bone tissue within the four regions (A1, A2, B1, B2) was performed using repeated measures analysis of variance with Tukey's post-hoc test;
- Analysis of statistical differences for the determined densities was performed using the Kruskal–Wallis test with Dunn's post hoc test ($p = 0.05$).

3. Results

3.1. A determination of microhardness and Young's modulus of dog cortical bone tissue

Microindentation studies of dog cortical bone tissue allowed for the determination of microhardness (*HV*) and Young's modulus (E_{IT}) values for individual levels and regions. Based on the obtained results (Fig. 2A), it

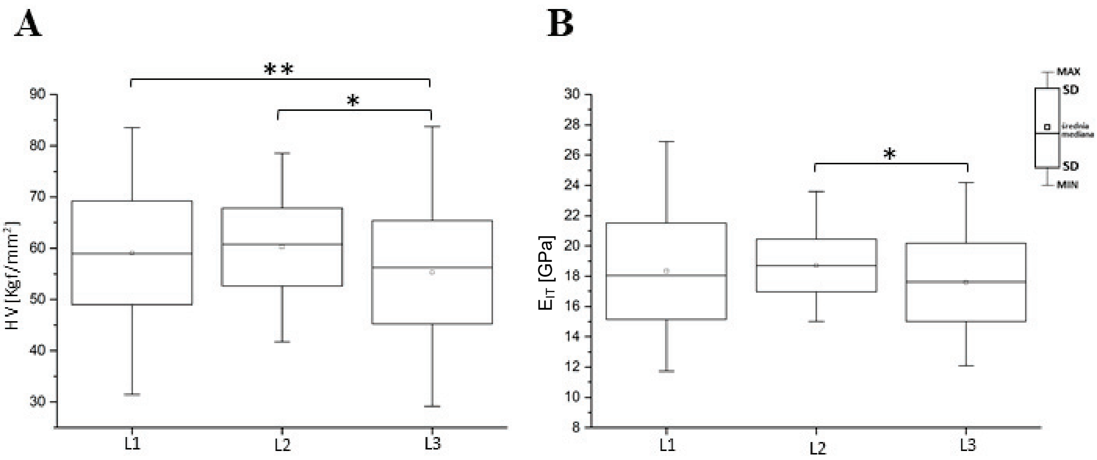

Fig. 2. Comparison of elastic modulus (A) and microhardness (B) values
for samples from three levels of the diaphysis; * $p < 0.1$, ** $p < 0.05$

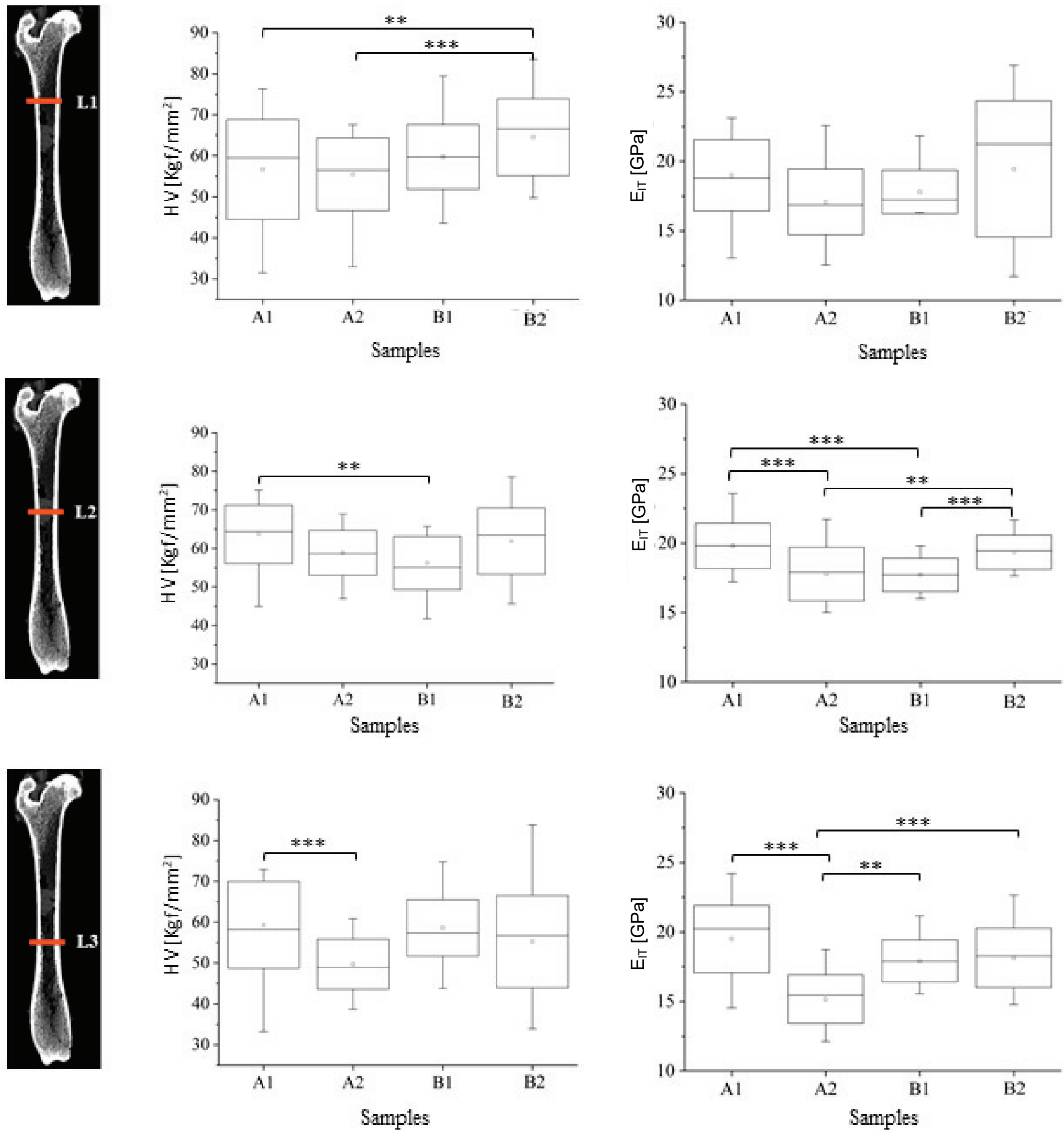

Fig. 3. Comparative chart of microhardness and elastic modulus values for the analyzed regions
for samples from three levels of the diaphysis; * $p < 0.1$, ** $p < 0.05$, ***$p < 0.01$

can be observed that the average values of the Young's modulus of bone tissue within the three levels have similar values (E_{IT} L1 = 18.33 GPa, E_{IT} L2 = 18.71 GPa, E_{IT} L3 = 17.59 GPa). Statistical analysis of the Young's modulus of bone tissue within the three levels (L1, L2, L3) showed that groups L2 and L3 differ statistically from each other at a significance level of ($p < 0.1$).

The highest microhardness values (Fig. 2B) were obtained for the central part of the diaphysis (level L2), where the average value was $HV = 60.25$ Kgf/mm^2. At level L1, the average HV value was only by 1% lower than at level L2 and amounted to 59.09 Kgf/mm^2, and the values were characterized by greater variability, as evidenced by the large standard deviation in this group.

The average microhardness value at level L3 was by 6.4% lower than at L1 and by 8.2% lower than at level L2, amounting to 55.3 Kgf/mm^2. Similar to level L1, the obtained values were characterized by a large standard deviation. The differences in the average micro-hardness values are statistically significant for groups L2 and L3 ($p < 0.1$) and for groups L1 and L3 ($p < 0.05$).

The values of both Young's modulus and hardness obtained in the studies are characterized by a large variation within the regions (A1, A2, B2, B1) where the measurements were conducted. A comparison of Young's modulus values for individual bone levels (Fig. 3) showed that the highest values of Young's modulus of bone tissue within the same level were obtained: for level L1 in region B2 (19.44 GPa), at level L2 in region A2 (19.80 GPa), and at level L3 in region A2 (19.48 GPa). At level L1, the results are characterized by the largest standard deviation. The differences in Young's modulus values between regions for levels L2 and L3 are statistically significant. At level L2, between regions A1(L2) and A2(L2) $p < 0.01$, between regions A1(L2) and B2(L2) $p < 0.05$, between regions A2(L2) and B1(L2) $p < 0.01$, between regions B1(L2) and B2(L2) $p < 0.01$. At level L3, statistically significant differences occurred between regions A1(L3) and A2(L3) $p < 0.01$, between regions A1(L3) and B1(L3) $p < 0.05$, between regions A1(L3) and B2(L3) $p < 0.01$.

On the other hand, the highest microhardness values of bone tissue (Fig. 3) were observed at level L1 in region B2 $HV = 64.51$ Kgf/mm^2, at level L2 in region A2 $HV = 63.63$ Kgf/mm^2, and at level L3 in region A2 $HV = 59.31$ Kgf/mm^2. For microhardness values at level L1, statistically significant differences occurred between regions A1(L1) and B2(L1) $p < 0.01$, between regions A2(L1) and B2(L1) $p < 0.05$. At level L2, statistically significant differences were obtained for A2(L2) and B1(L2), $p < 0.05$, while at level L3, the differences in microhardness values are statistically significant for A1(L3) and A2(L3), $p < 0.01$.

3.2. Determination of the density of dog cancellous bone tissue

The highest average density value determined by the hydrostatic method (Fig. 4A) was obtained in sample S2 and amounted to 1.3 g/cm^3. The lowest hydrostatic density value was observed in sample S1 and was 1.07 g/cm^3. The differences in hydrostatic density of the tested samples were statistically significant between regions S1 and S2 ($p < 0.05$) and S2 and S5 ($p < 0.1$).

Analysis of the results of the apparent density (Fig. 4B) showed that the highest value of 1.17 g/cm^3 was obtained in sample S2, while the lowest was in sample S1 and was equal to 0.84 g/cm^3. The differences in apparent density of the tested samples were statistically significant between regions S1 and S2 ($p < 0.1$).

The determined bone mineral density (BMD) values (Fig. 4C) are similar in both distribution and values to the determined hydrostatic density. The highest BMD value occurred in sample S2 (BMD = 1.13 g/cm^3), while the lowest was in sample S5 (BMD = 0.91 g/cm^3). The differences in BMD of the tested samples were statistically significant between regions S1 and S2 ($p < 0.1$), S2 and S4 ($p < 0.05$), and S2 and S5 ($p < 0.01$).

The ash density of the incinerated samples (Fig. 4D) in relation to the volume of the samples before incineration showed that the highest values were in sample S2 (ash density = 0.52 mg/mm^3), and the lowest were in sample S5 (ash density = 0.26 mg/mm^3). The differences in ash density values of the incinerated samples were statistically significant between regions S1 and S2 ($p = 0.0207$), S2 and S4 ($p = 0.0036$), and S2 and S5 ($p = 0.0007$). In Figure 5, the spatial structure of selected samples tested using micro-computed tomography is shown.

3.3. Comparison of methods

Each of the methods used has a specific profile of advantages and limitations, and their precision depends on the characteristics of the preparation, sample preparation procedures, bone type, and measurement conditions. In Table 1, the key aspects of each method are presented.

In the case of micro-computed tomography, a significant advantage is its non-invasiveness and the acquisition of detailed spatial data, which makes this method the gold standard in structural studies of cancellous

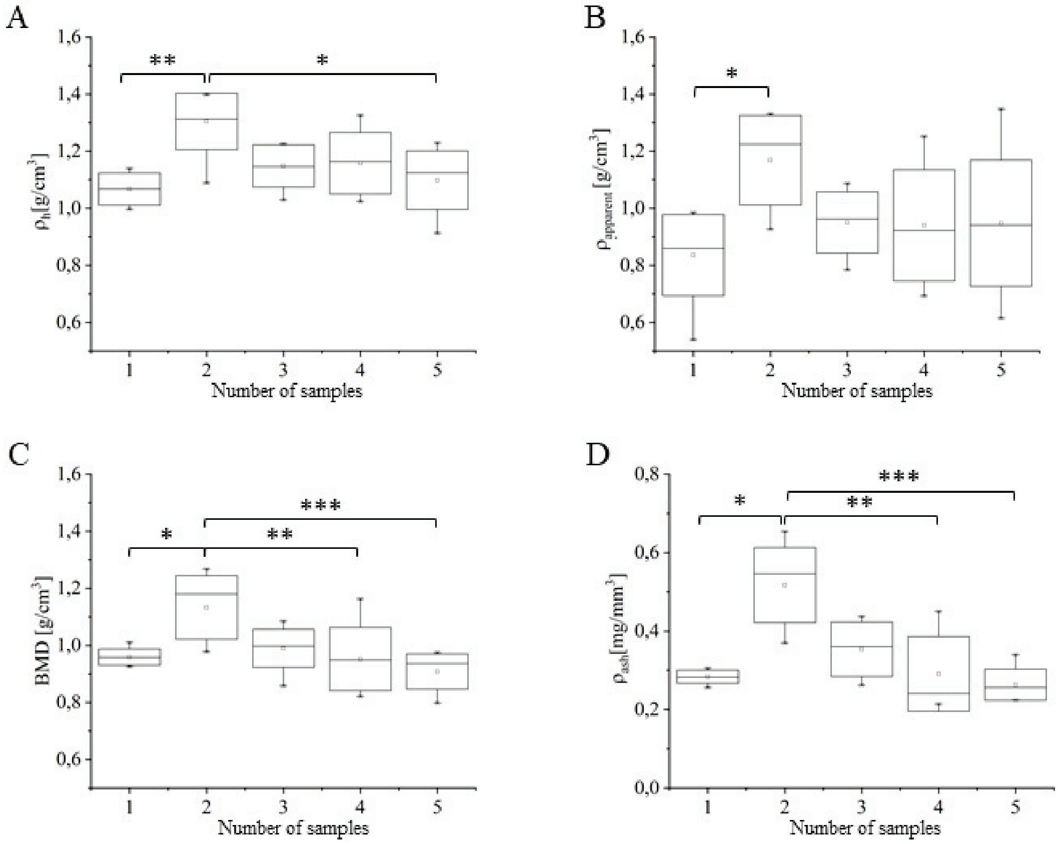

Fig. 4. Density values of dog cancellous bone tissue samples determined by selected methods: hydrostatic method (A), apparent density (B), BMD (C), ash density (D); * $p < 0.1$, ** $p < 0.05$, *** $p < 0.01$

bone [15]. However, the high cost and the need for specialized equipment, as well as potential susceptibility to image artifacts, can negatively affect the reliability of the data. The hydrostatic method, despite its simplicity and low cost, is susceptible to disturbances from air trapped in pores and the accuracy of sample surface preparation [31]. Apparent density allows for a quick comparison of samples with similar shapes, but its sensitivity to errors in volume measurement (especially with irregularly shaped bone fragments) limits the precision of the results' interpretation. BMD as a diagnostic parameter offers a high level of comparability and interpretability, especially in clinical settings, but it does not reflect the overall structural density, focusing on mineralization [10]. Ash density is considered the reference method for mineralization analyses, providing true values of mineral content, which is particularly important for calibrating other techniques. However, it requires the permanent destruction of the sample and is susceptible to material loss during incineration, as well as the influence of thermal factors on the potential change in the mineral's crystal structure [33]. Incineration provides information about the mineral fraction but most strongly affects the disturbance of the sample's structure.

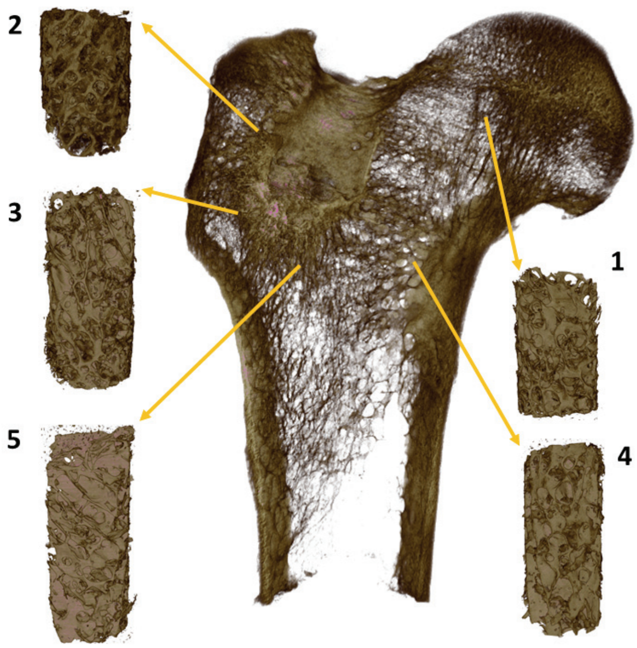

Fig. 5. Spatial structure of exemplary samples of cancellous bone tissue

The time required to perform the measurement presented in the table (Table 1) was estimated based on our own experience and literature data [15], [32]. It was

Table 1. Comparison of methods for measuring cancellous bone tissue density

Feature	Hydrostatic method	μCT (BMD)	Apparent density	Ash density
Measured parameter	Bulk density	Bone mineral density	Apparent density	Density of the mineral fraction
Measurement principle	Archimedes' principle; sample mass in relation to its total volume.	Attenuation of X-rays; calibration against phantoms of known density.	Ratio of the sample's mass to its total volume.	Mass of residue after mineralization at 500 °C in relation to volume.
Possible sources of error	Air bubbles adhering to the sample, inaccuracy of liquid density.	Artifacts, calibration errors, image noise.	Inaccuracy of the scale, errors in volume determination from μCT (segmentation).	Incomplete combustion of the organic fraction, loss of mineral mass, sample contamination.
Practical aspects and limitations	Low equipment requirements and costs, but high sensitivity to manual errors and sample hydration state.	Non-destructive method with high precision, limited by high cost, equipment requirements, and time-consuming analysis.	Conceptually simple, but combines errors from two techniques and depends on access to advanced equipment (μCT).	Provides a reference assessment of mineralization at low equipment costs, but is destructive and time-consuming.
Time	short (<2 h)	medium (2–24 h)	medium	long (>2 days)

assumed that if the time did not exceed 2 hours, it was categorized as "short"; between 2 and 24 hours as "medium"; and exceeding 2 days as "long".

4. Discussion

Studies on mechanical properties described in the literature show that dog bone tissue differs in terms of the values of measured parameters, even within the same species [13]. For example, research conducted by Pressel et. al. [24] on canine cancellous bone tissue showed that the average density, measured by the ultrasonic method within the femoral head, was 1.4 g/cm^3. Other studies [17], conducted on the distal part of the canine femur, allowed for the determination of the apparent density of cancellous bone tissue, which was 0.44 g/cm^3. In comparison, the results presented in this paper allowed for the determination of cancellous bone tissue density values in the femoral neck and head using various methods, which ranged from 0.84 to 1.07 g/cm^3. Density values in the proximal part of the diaphysis ranged from 0.91 to 1.1 g/cm^3.

The presented results showed that the choice of measurement method affects the obtained values. It was observed that the distribution of density values is similar in the hydrostatic method and in the analysis using a micro-computed tomograph (μCT), but the range of values differs slightly. The values of the determined apparent density show greater differences between samples. Thanks to the use of several methods, it was possible to obtain reliable density values, which can be used as input data for a numerical model.

Studies conducted by Acito et al. [1] on bone tissue samples from Beagle dogs showed that the Young's modulus of cancellous bone determined in a compression test was 530 MPa. Additionally, in these studies, the Young's modulus values of the femoral cortical bone were measured in a four-point bending test and ranged from 14.5 to 19.5 GPa depending on the location. In our own microhardness studies, Young's modulus values for cortical bone tissue samples from different levels were obtained in the range of 17.59–18.71 GPa, which is consistent with the literature data.

The observed variability in properties can also be considered in a broader evolutionary context. Studies by Chirchir [4] have shown that domestic dogs have a significantly lower density of trabecular bone compared to their wild ancestors, wolves. This decrease in bone density is interpreted as one of the byproducts of the domestication process, associated with changes in diet, locomotion strategies, and the overall mechanical loading of the skeleton. The lower trabecular bone density in domestic dogs may have implications for their susceptibility to certain orthopedic conditions and highlights how selection processes have influenced the fundamental biological properties of their skeletal system.

Local variability of properties is a reflection of the fundamental principle of bone biology (Wolff's law), according to which bone tissue adapts its morphology to the function of load-bearing. Studies of the density distribution of subchondral bone, for example in the talus of dogs, prove that its density map is highly correlated with the long-term distribution of loads in the joint, which allows this method to be used for noninvasive examination of joint biomechanics [6]. The need to consider material and structural heterogeneity is a universal challenge in modeling mineralized tissues. Similar problems are encountered in dental biomechanics, where it has been shown that precise models must take into account property gradients to accurately predict stress distribution [29].

A key indicator of bone tissue quality is its degree of mineralization. Ash density analysis is the gold standard for its assessment and, as confirmed by Kang et al. [14], is strongly correlated with mechanical parameters. The influence of metabolic state on bone quality is undeniable, and trabecular tissue is a sensitive indicator of disorders. The work of Shipov et al. [28] has shown that chronic renal failure in dogs leads to significant changes in the structural and mechanical properties of bone. Similarly, dogs with hyper-adrenocorticism have been found to have significantly lower bone mineral density (BMD), particularly in trabecular bone [18].

The clinical picture is further shaped by the effectiveness of treatments and the use of pharmaceutical preparations. Studies conducted by Nowak et al. [20], [21] clearly show how selective retinoid receptor agonists (tazarotene and bexarotene) modify the mechanical properties of bone. This illustrates how the patient's metabolic state, the action of drugs, and potential coexisting conditions are all linked, jointly determining the final quality and strength of the bone tissue.

It is important to distinguish between methods for assessing the mechanical properties of bone. Destructive tests, such as four-point bending [20], [21], determine global, macroscopic structural parameters, such as strength and stiffness. In contrast, imaging techniques, e.g., micro-computed tomography (μCT) [21], provide detailed data on the micro-architecture of the tissue, including density or trabecular structure. Such a multiscale approach is fundamental, as macroscopic mechanical properties result directly from microstructural features.

Despite the growing awareness of the importance of BMD, the early diagnosis of osteoporosis remains a clinical challenge, both in human medicine, where about 9 million osteoporotic fractures are diagnosed annually and in veterinary medicine [5]. In veterinary medicine, there is a lack of standardized BMD tests, so there is a search for rapid and precise assessment techniques, such as osteodensitometry software in qCT studies [34] or mobile applications for X-ray analysis [5].

Ash density is a direct and precise indicator of the mineral content in trabecular bone tissue. The lower values compared to hydrostatic methods result from the elimination of the organic fraction and the porosity of the tissue. This parameter can be an important indicator of bone quality in veterinary and orthopedic research. Ash density is a valuable, repeatable, and minerally specific parameter in assessing the quality of trabecular bone tissue in dogs. Its high correlation with biomechanical properties makes it an important tool in both scientific and clinical research.

The finite element method is a commonly used, albeit simplified, way of analyzing the biomechanical interactions between an implant and bone tissue [2], [12]. Computer modeling allows for the analysis of the cooperation of elements in systems with complex geometry and different mechanical properties, which allows for a better understanding of the processes occurring in the research model [8]. As shown by Kostenko et al. [16], the combination of precise imaging techniques μCT with finite element analysis allows for more accurate prediction of fracture risk and more effective planning of surgical interventions. A thorough knowledge of the anatomy of dogs, as well as the forces acting in individual joints, allows for more accurate implant design. The clinical implications of the research conducted are twofold: they provide reliable input data for numerical models and are in line with the trend of personalized medicine. Detailed knowledge of the material properties of bone is essential for matching implants, which minimizes the risk of complications [9]. The importance of the thickness and density of cortical bone for the stability of implants is well documented, for example in the context of orthodontic mini-implants, where these parameters correlate with the force needed to damage them [22].

5. Conclusions

Based on the conducted research and the analysis of the results, a significant variation in the mechanical and structural properties of the dog's femoral bone tissue was demonstrated, which depends on both the anatomical location and the measurement method used. The obtained density and Young's modulus values, although consistent with literature data, confirm that the heterogeneity within a single bone necessitates the precise definition of the areas being studied. The use of complementary research techniques, including the hydrostatic method and micro-computed tomography, proved to be crucial for obtaining reliable and comprehensive data characterizing the bone tissue. These data constitute a valuable and verified input base for advanced numerical models, including finite element method (FEM) analyses.

The mechanical properties of bone are significantly influenced the loads resulting from the function of the musculoskeletal system, and systemic factors, such as metabolic state and coexisting diseases, which has a direct impact on the clinical assessment of the patient and the risk of treatment failure. Consequently, the results of the research conducted have practical significance for veterinary orthopedics, contributing to the development of personalized treatment planning. A precise material characterization of bone allows for better matching of surgical implants and improvement of methods for predicting fracture risk, which is in line with the development of modern therapeutic strategies.

The paper also draws attention to the advantages and limitations resulting from the measurement methods used. Sources of disturbance reduce the accuracy of the measurement. Different density assessment techniques provide non-uniform results due to the different ways of defining the sample volume and different detection ranges, therefore it is important to introduce a normalization protocol for all methods and materials. This will allow for direct comparison of values and limitation of systematic deviations. Standardized, comparable results will allow, among other things, for the standardization of comparative studies between research centers, and even for a better assessment of fracture risk based on biomechanical models. Further research in this area should include an economic and time analysis in a broader context of preclinical and clinical research, to support decision making regarding the choice of the optimal method depending on the purpose of the study and available resources.

In summary, this research makes a significant contribution to the understanding of the mechanical and structural properties of dog bone. It has been shown that the combination of different measurement techniques is crucial for a comprehensive assessment, and the data obtained emphasize the importance of anatomical location and metabolic state. These results have direct application in improving numerical models and support the development of individualized orthopedic treatment strategies in veterinary medicine.

Abbreviations

The following abbreviations are used in this manuscript:

μCT Micro-computed tomography
BMD Bone mineral density
FEM Finite element method

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