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RESEARCH ARTICLE

Computed tomography reconstruction and morphometric analysis of the humerus and femur in New Zealand rabbits

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Yeni Zelanda tavşanlarında humerus ve femurun bilgisayarlı tomografi rekonstrüksiyonu ve morfometrik analizi

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Öz

Amaç: Bu çalışma, Yeni Zelanda tavşanının bilgisayarlı tomografi (BT) görüntülerini kullanarak humerus ve femurun üç boyutlu (3B) modellerini oluşturmak ve cinsiyetler ile sağ ve sol taraflar arasında farklılık olup olmadığını ortaya koymak amacıyla gerçekleştirilmiştir..

Gereç ve Yöntem: Çalışmada 14 haftalık (10 dişi, 10 erkek) Yeni Zelanda tavşanı kullanılmıştır. Önce hayvanların bilgisayarlı tomografi (BT) ile görüntüleri 0.5mm kalınlığında çekilerek DICOM formatında kaydedildi. Daha sonra görüntüler MIMICS 20.1 programı ile yeniden yapılandırılarak humerus ve femurun 3 boyutlu modelleri elde edildi. Elde edilen 3B model üzerinden ölçümler gerçekleştirilerek morfometrik veriler elde edildi. Daha sonra tavşanların disseksiyonu gerçekleştirilerek, humerus ve femur'un ağırlıkları ölçüldü.

Bulgular: Elde edilen verilere göre erkek tavşanlarda sağ humerus ve femurun sol taraftan hacim ve yüzey alanının büyük olduğu tespit edildi ($p<0.05$). İncelenen diğer morfometrik değerler arasında ise bir fark tespit edilmemiş ve tavşan kemiklerinin homotipik varyasyon göstermediği belirlenmiştir. Ayrıca cinsiyetler arası farklılıklarında önemsiz bulunması humerus ve femur kemiklerinin cinsiyet belirlemede kullanılamayacağı düşünüldü.

Öneri: Yapılan bu çalışma ile elde edilen humerus ve femur'a ait morfolojik verilerin, tavşan üzerine yapılcak çalışmalara ve deneysel modellemelere, klinik alanında kullanılan görüntüleme sistemleriyle elde edilen verilere temel teşkil ederek, hastalıkların tanısında hekime yardımcı olacağı düşünülmektedir.

Anahtar kelimeler: Bilgisayarlı tomografi, Femur, Humerus, Üç boyutlu rekonstrüksiyon, Yeni Zelanda tavşanı.

Abstract

Aim: This study was carried out to create three-dimensional (3D) models of the humerus and femur using computed tomography (CT) images of the New Zealand rabbit and to reveal whether there are differences between sexes and the right and left sides.

Materials and Methods: The study was applied on 14 month old (10 male and 10 female) New Zealand rabbits. First, 0.5 mm thick images of the animals were taken by computed tomography (CT) and saved in DICOM format. Then, 3D models of the humerus and femur were obtained by reconstructing the images with the MIMICS 20.1 program. Morphometric data were obtained from the obtained 3D model. After this procedure, the rabbits were dissected and the weights of the humerus and femur were measured.

Results: It was determined that the right humerus and femur volume and surface area in male rabbits were larger from the left side ($p<0.05$). There was no difference between the other morphometric values examined and rabbit bones did not show homotypic variation. In addition, it was thought that the humerus and femur bones could not be used to determine gender because the differences between the sexes were insignificant.

Conclusion: It was thought that this study would provide the basis for studies and experimental models on rabbits by revealing the morphological features of the humerus and femur and would help the physician in the diagnosis of diseases by forming the basis of the data obtained with the imaging systems used in the clinical field.

Keywords: Computed tomography, Femur, Humerus, Three dimensional reconstruction, New Zealand rabbit.

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Introduction

The development of new medical methods is usually applied to humans after testing on animals (Eken et al 2009). Rabbits have been used frequently in recent years due to their docility, ease of handling, low costs related to management and surgical facilities. Rabbits are preferred in studies on bone tissue because they are more useful than large animals and their bones are larger than small rodents to enable applications (Bottagisio et al 2019). Long bones of rabbits are preferred in various bone implant trials, especially in bone tissue products with mechanical stability in bone regeneration and models are created on this (Ghanmi et al 2018, Ilea et al 2019, Karimzadeh Bardeei et al 2021). The success of these models and the use of correct anatomical information in surgical and clinical applications have added a new dimension to the diagnosis of diseases (Dayan et al 2019, Eken et al 2009).

With the technological developments, imaging methods have started to be used frequently in medicine in the diagnosis and treatment of diseases (Dayan et al 2019, Yılmaz et al 2020). Computed tomography (CT) is a medical imaging technique used in radiology to obtain detailed images of the body in a non-invasive way for diagnostic purposes, based on the transmission and absorption of x-rays by different tissues, and the scattering rays are taken by means of detectors, cross-sectional images are created and these images are evaluated with computerized systems (Dayan et al 2019, Van Eijnatten et al 2018). CT is increasingly used in animal research models, bone diseases, surgical procedures, and bone repair to observe and measure three-dimensional (3D) bone structures (Marchand et al 2011, Özkadif et al 2015). While in clinical operations, ordinary radiographs can be helpful for preoperative evaluation in most cases of simple radius curves, three-dimensional anatomical models based on computed tomography scan images are needed in patients with complex deformities to obtain accurate surgical planning (Athertya and Poonguzhali 2012, Karimzadeh Bardeei et al 2021). These models can be made with images obtained with tomography devices with various programs, and the bone models can be examined by rotating them in any direction or by adding the desired medical apparatus to the model virtually (Coutin et al 2013, Özkadif et al 2016).
In recent years, many studies have been done on the long bones of laboratory animals. In New Zealand rabbits, Pazvant and Kahvecioğlu (2009) examined the anterior and posterior extremities, Ajayi et al (2012) examined the limb and foot by dissection method, Pazzaglia et al (2015) made measurements with CT images of the right femur, El-Ghazali and El-behery (2018) studied the pelvic and hind limb bones using radiography and dissection method, Özkadif et al (2015) studied on CT images of the antebrachium. Dayan et al (2019) made three-dimensional modeling in guinea pigs femur and humerus tomography images, Özkadif et al (2016) worked on various imaging techniques of the femur in chinchilla, Yılmaz et al (2020) made three-dimensional modeling of the humerus, radius and ulna in the cat, Onwuama et al (2018) studied the pelvis and hind limb bones of grasscutter by dissection method. In recent years, it was seen that 3D modeling together with tomography images were continually used in the veterinary area.

In the literature review, there are not enough studies in which femur and humerus morphometric data of New Zealand rabbits were presented on 3D models by revealing the difference between female and male. The aim of this study was to perform 3D modeling of the femur and humerus on CT images of New Zealand rabbits and to obtain morphometric values on these models, to reveal the homotypic variation between the right and left bones, to analyze morphological differences between female and male rabbits, and to determine the relationship of these values with body weight.

Material and Methods

Ethical Statement

The study was approved by the Ethics Committee of the Faculty of Health Sciences of Karamanoglu Mehmetbey University (2021/03-22).

Materials

In the study, 20 healthy 14-month-old New Zealand rabbits (10 females, 10 males) given as cadavers to The Basic Sciences Laboratory of the Faculty of Health Sciences of Karamanoglu Mehmetbey University were used.

Methods

New Zealand rabbits were placed in the prone position and symmetrically on a CT device (Siemens, Somatom Sensation 64, Erlangen, Germany) to obtain CT images of the humerus and femur. 0.5 mm thick images were obtained and transferred to the hard disk in DICOM format. CT device technical parameters were: 130 kVp, 300 mA, 330 msec, 0.50 mm section thickness, 512 x 512 pixels (Özkadif et al 2015, Yilmaz and Demircioğlu 2021, Turgut et al 2023).

The images were then imported into MIMICS 20.1 (The Materialize Group, Leuven, Belgium). 3D modeling of the humerus and femur of female and male rabbits was performed on the transferred images (Figure 1-2), and volume and surface areas were obtained from these models. Osteometric measurements were performed on 3D models. In the morphometric measurements, the measurement points specified in the relevant literature were used on the humerus and femur (Von den Driesch 1976). Following these procedures, both the left and right humeral and

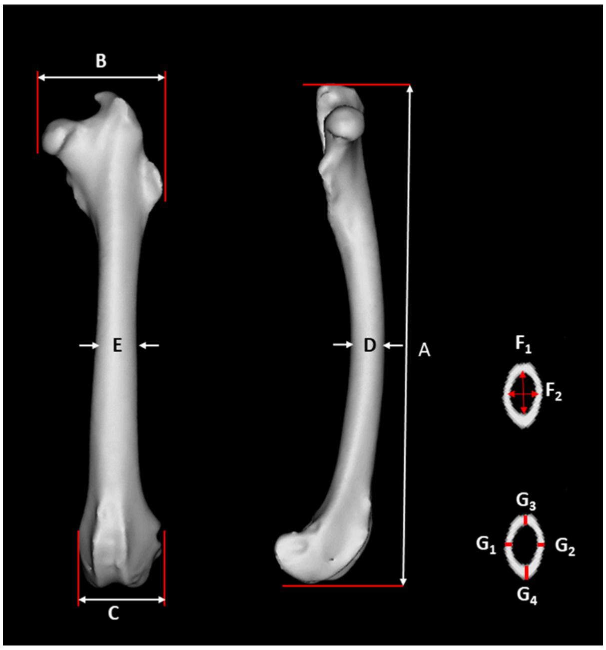

Figure 1. PMeasuring points on 3D model of humerus; A- maximum length of the humerus; B- proximal width of the humerus; C-distal width of the humerus; D- diaphysis diameter of the humerus (cranio-caudal); E- diaphysis diameter of the humerus (medio-lateral); F- endosteal diameter of the humerus (F1: cranio-caudal F2: medio-lateral); G- periosteal diameter of the femur (G1: medial, G2: lateral, G3: cranial, G4: caudal).

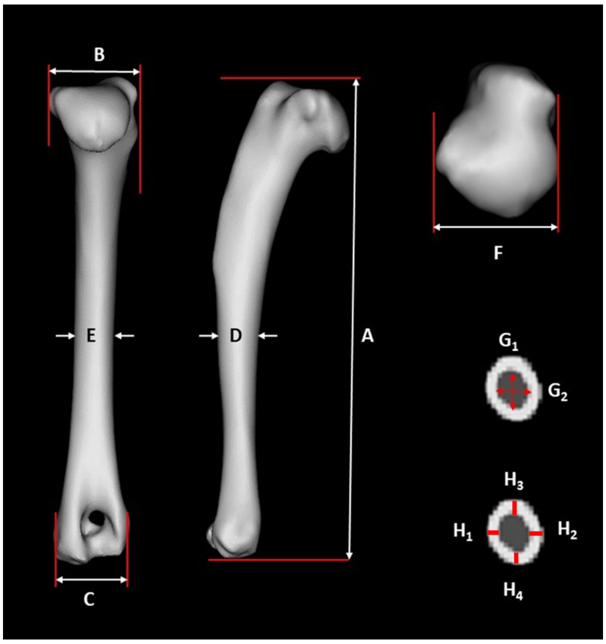

Figure 2. Measuring points on 3D model of femur; A- maximum length of the femur; B- proximal width of the femur; C- distal width of the femur; D- diaphysis diameter of the femur (cranio-caudal); E- diaphysis diameter of the femur (medio-lateral); F- diameter of the caput ossis femoris; G- endosteal diameter of the femur (G1: cranio-caudal G2: medio-lateral); H- periosteal diameter of the femur (H1: medial, H2: lateral, H3: cranial, H4: caudal).

femoral joints were disarticulated, then the soft tissues were dissected and the weights of the bones were measured. Relative weights were obtained by dividing the relative bone weight by body weight. The descriptions and abbreviations of the osteometric parameters were presented in Table 1.

Statistical Analysis

In the study, the analysis of the morphometric data obtained from the bones was performed using the SPSS software version 21.0. The harmony of the variables to the normal distribution (histogram and probability graphs) and analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk tests) were examined and determined that the data were parametric. Paired sample t test was used for statistical comparisons of right and left values of bones, and Independent Samples t test was used for comparisons of female and male rabbits. The correlations were also tested through Pearson Correlation coefficient calculations and were separately performed for each sex. Data are expressed as means ± standard error (SE). $P < 0.05$ was accepted statistically significant.

Table 1. Osteometric parameters and measuring points

Osteometric Parameters	Measuring Points
Maximum length of the bones	Distance between the proximal and the distal end
Proximal width of the bones	Greatest breadth of the proximal end
Distal width of the bones	Greatest breadth of the distal end
Medio-lateral endosteal diameter the bones	Medullary cavity diameter of the bones from medial to lateral on smallest breadth of diaphysis
Cranio-caudal endosteal diameter of the bones	Medullary cavity diameter of the bones from cranial to caudal on smallest breadth of diaphysis
Diaphysis diameter of the bones (cranio-caudal)	Smallest breadth of diaphysis from cranial to caudal
Diaphysis diameter of the bones (medio-lateral)	Smallest breadth of diaphysis from medial to lateral
Lateral periosteal diameter of the bones	Lateral cortex thickness at the smallest diaphyseal breadth of the bones
Medial periosteal diameter of the bones	Medial cortex thickness at the smallest diaphyseal breadth of the bones
Cranial periosteal diameter of the bones	Cranial cortex thickness at the smallest diaphyseal breadth of the bones
Caudal periosteal diameter of the bones	Caudal cortex thickness at the smallest diaphyseal breadth of the bones
Diameter of the caput ossis femoris	Greatest depth of the caput ossis femoris

Results

In this study, the humerus and femur of New Zealand rabbits were evaluated in terms of homotypic variation and sexual dimorphism between right and left bones of rabbits by measuring volume, surface area, diameter and length. As a result of the measurements, the mean weights of female and male New Zealand rabbits used were found to be 3264 ± 15 g and 2662 ± 86 g, respectively. Although the weight of the female New Zealand rabbits used in the study was higher than the male rabbits, the relative bone weight of the males was found to be greater than the female rabbits (Table 2). Although the average weight of the femur in female rabbits is heavier than that of male rabbits, no statistical difference was found between the humeral consequences (P<0.05).

Morphometric data of the humerus were given in Table 3. The humeral volume was found to be 2280.39±107.71 mm3 on the left, 2312.59±93.79 mm3 on the right, and the surface area was 2870.99±106.84 mm2 on the left and 2763.49±176.15 mm^2 on the right in females. These values were 2148.22±108.86 mm3 and 2715.82±158.58 mm^3, 2154.92±150.92 mm^2 and 2892.29±144.71 mm^2 in males, respectively. According to the results obtained, no difference was found when the volume and surface areas of the right and left humerus were compared in female rabbits (P>0.05). It was determined that the volume and surface area of the right humerus in male rabbits were greater than the left side and it was statistically significant (P<0.05).

The periosteal diameter of the humerus and femur were given in Figure 3 and Figure 4. There was no statistical difference between the right and left bones in males and females in lateral, medial, cranial and caudal periosteal diameter measurements. In addition, when the male and female humerus and femur bones were compared, no difference was found (P>0.05).

Morphometric data of the femur are given in Table 4. The femur volumes were found to be 3787.52±112.12 mm^3 on the left, 3793.81±107.22 mm^3 on the right, and the surface area was 4899.11±112.92 mm^2 on the left and 4897.83±99.46 mm^2 on the right, in females. These values were 3333.35±44.11 mm^3 and 4367.80±291.81 mm^3, 3861.44±265.22 mm^2 and 4923.99±126.08 mm^2 in males, respectively. When the volume and surface areas of the right and left femurs were compared in female rabbits, no statistical difference was found (P>0.05). In male rabbits, the volume and surface area of the right femur was larger than the left and were statistically significant (P<0.05). As a result of the analysis of the osteometric parameters of the humerus and femur, there was no statistical difference between the right and left lateral bones. Similarly, no difference was found between female and male rabbits (P>0.05).

Table 2: The body and organ weights of the animals used in the study (Mean±SE)GenBank Accession numbers

Parameters	Female	Male
Body weight (g)	3264±15	2662±86
Humerus weight (g)		
Left	3.88±0.16	3.58±0.09
Right	3.91±0.17	3.53±0.10
Femur weight (g)		
Left	7.04±0.28	6.15±0.17
Right	7.06±0.27	6.15±0.19
Relative weight		
Humerus	0.12±0.004	0.22±0.007
Femur	0.13±0.005	0.23±0.009

In the analysis of the values between the osteometric measurements of the humerus and femur by gender, it was determined that there was a strong positive correlation between the weight and volume values in female and male rabbits (P<0.01). In addition, a positive correlation was found between right and left side bone volumes (P<0.05). For female rabbits, a positive correlation was shown between left and right maximum length of the humerus and left and right maximum length of the femur (r=0.970, 0.899; P=0.000), proximal width of humerus(r=0.648, 0.755; P=0.043, 0.017), left diaphysis diameters of the femur (r=0.808, 0.869; P=0.05, 0.01), right diaphysis diameters of the femur (r=0.781, 0.844; P=0.008, 0.002), medio-lateral endosteal diameter of the humerus (r=0.640, 0.675; P=0.046, 0.032). In female rabbits, positive correlation was determined between left and right femur volume and left maximum length of femur (r=0.774, 0.767; P=0.009, 0.010), right side maximum length of femur (r=0.786, 0.794; P=0.007, 0.006), left proximal width of femur (r=0.637, 0.638; P=0.047, 0.048), diaphysis diameters of humerus (r=0.637, 0.808; P=0.047, 0.005), diaphysis diameters of femur (r=0.843, 0.846; P=0.002, 0.002). For male rabbits, there was positive correlation between the right and left maximum height of the humerus and the maximum length of the right (r=0.962, 0.887; P=0.000, 0.001) and left (r=0.971, 0.962; p=0.000, 0.000) femur, the proximal width of the right (r=0.682, 0.636; P=0.030, 0.048) and left humerus (r=0.680, 0.645; P=0.030, 0.045). There was also positive correlation between the diaphysis and endosteal diameters of the humerus and the femur in male rabbit (P<0.01). In addition, there was a positive correlation between the right and left maximum height of the femur and the proximal diameter of the right (r=0.655, 0.728; P=0.040, 0.017) and left humerus (r=0.656, 0.724; P=0.039, 0.018), endosteal diameters of the humerus (r=0.686, 0.657; P=0.028, 0.039) in male rabbit (P<0.05). While there was a strong positive correlation between the proximal diameters of the femur and its distal diameters (P<0.01), there is a negative correlation between the medio-lateral diaphysis diameter (P<0.05).

Table 3. The morphometric data of the humerus in female and male New Zealand rabbits (Mean±SE)

Parameters	Female			Male		
	Left	Right	P*	Left	Right	P*
Humerus volume (mm³)	2280.39±107.71	2312.59±93.79	0.822	2148.22±108.86	2715.82±158.58	0.028*
Surface area (mm²)	2870.99±106.84	2763.49±176.15	0.519	2154.92±150.92	2892.29±144.71	0.001**
Maximum length of the humerus (mm)	67.75±0.87	67.56±0.99	0.497	67.42±0.73	67.33±0.72	0.679**
Proximal width of the humerus (mm)	13.53±0.15	13.44±0.18	0.468	12.64±0.31	12.59±0.33	0.269
Distal width of the humerus (mm)	10.73±0.12	10.69±0.15	0.704	10.61±0.15	10.66±0.17	0.555
Medio-lateral endosteal diameter of the humerus(mm)	3.27±0,11	3,41±0,09	0.094	3.29±0.09	3.48±0.13	0.311
Cranio-caudal endosteal diameter of the humerus(mm)	4.26±0,21	4.43±0,07	0.466	4.21±0.18	4.26±0.16	0.755
Diaphysis diameter of the humerus (cranio-caudal) (mm)	6.48±0.13	6.50±0.13	0.684	6.30±0.13	6.35±0.14	0.343
Diaphysis diameter of the humerus (medio-lateral) (mm)	5.89±0.12	5.9210±0.12	0.655	5.77±0.11	5.77±0.09	0.956

Paired sample t test was used for pairwise comparison (*p<0.05, **p<0.01).

Discussion

Rabbit was preferred in many modeling and bone studies. It was seen that the humerus and femur, which were long bones in the extremity, were preferred in morphometric studies (Karimzadeh Bardeei et al 2021, Selçuk and Tıpırdamaz 2020). Three-dimensional modeling and CT images were frequently used in the diagnosis of diseases and in the development of treatment methods (Dayan et al 2019). The focus of this study was to investigate the distribution of volume fractions and sex differences in the femur and humerus of New Zealand rabbits, where data was limited in the literature.

Ajayi et al (2012) found that the trochanter major was higher in the femur than the caput femoris in their study of ten adult New Zealand rabbits without specifying gender differences. They stated that the large trochanter major allowed for stronger contraction of the gluteal muscles and stronger abduction movement. In the presented study, it was observed that the trochanter major was higher than the caput femoris in both genders.

In a study of fifteen male and fifteen female adult New Zealand rabbits, Pazvant and Kahvecioğlu (2009) found no statistical difference between the right and left values in the maximum length, proximal width, distal width and diaphysis diameter values measured in the humerus and femur of both males and females. Considering the right and left values in female and male, they stated that the difference between the sexes was not statistically significant. Ajayi et al (2012) found no statistical difference between the right and left sides of the femur length, diaphysis diameter, distal width, and proximal width values. The current study was similar to both studies in terms of length and diameter measurements. The measurements of the right and left bones did not differ, possibly due to the use of healthy animals in the studies.

In a study in which no sexual dimorphism was performed by El-Ghazali and El-behery (2018) on ten healthy adult New Zealand rabbits, the length of the femur was 90.40±7.43 mm. In the present study, the maximum length of the femur in male rabbits was 87.17±0.71 mm on the left side and 87.16±0.72 mm on the right side. In female rabbits, these values were found to be 87.53±0.90 mm and 87.50±0.84 mm, respectively.

In the study, in which male New Zealand rabbits were divided into three groups and length measurements were made on tomography images of the right femur by Pazzaglia et al (2015), the weight of 6-month-old rabbits was 3.5-4.2 kg, the right femur length was 102.66±1.51 mm, the weight of 18-month-old rabbits was 3.5-4.5 kg, the right femur length was 102.16±0.71 mm, the weight of aged (average 7.5 years old) rabbit was 3–3.7 kg and femur length was 103.36±1.02 mm. It also reported that the length of the right femur did not differ between groups. In the present study, the mean weight of 14-month-old female rabbits was 3.264±0.015 kg, male rabbit weight was 2.662±0.086, right femur length was 87.50±0.84 mm in females and 87.16±0.72 mm in males. It was thought that the discrepancy between the two studies may be attributed to differences in the age and weight of the animals used.

Dayan et al (2019) found no difference in the length and diameter measurements of the humerus and femur in their modeling study on CT images of twelve male guinea pigs. However, they reported that the left humeral volume

Table 4: The morphometric data of the femurs in female and male New Zealand rabbits (Mean±SE)

Parameters	Female			Male		
	Left	Right	P	Left	Right	P
Femur volume (mm^3)	3787.52±112.12	3793.81±107.22	0.905	3333.35±44.11	4367.80±291.81	0.009*
Surface area (mm^2)	4899.11±112.92	4897.83±99.46	0.975	3861.44±265.22	4923.99±126.08	0.006*
Maximum length of the femur (mm)	87.53±0.90	87.50±0.84	0.774	87.17±0.71	87.16±0.72	0.770
Proximal width of the femur (mm)	17.24±0.15	16.32±1.00	0.382	17.19±0.18	17.30±0.17	0.383
Distal width of the femur (mm)	13.41±0.18	13.45±0.18	0.630	13.01±0.15	13.02±0.18	0.943
Diameter of the caput ossis femoris (mm)	6,97±0,08	7,11±0,09	0,081	7,16±0,11	7,16±0,15	0.948
Medio-lateral endosteal diameter of the femur(mm)	4,30±0,12	4,56±0,26	0,384	4,13±0,12	3,92±0,14	0,148
Cranio-caudal endosteal diameter of the femur(mm)	4,59±0,09	4,65±0,26	0,858	4,76±0,13	4,93±0,21	0,279
Diaphysis diameter of the femur (cranio-caudal) (mm)	6.54±0.19	6.55±0.18	0.679	6.27±0.12	6.24±0.11	0.539
Diaphysis diameter of the femur (medio-lateral) (mm)	7.65±0.11	7.71±0.09	0.540	7.81±0.15	7.78±0.12	0.671

Paired sample t test was used for pairwise comparison (*p<0.01).

was larger than the right side and they did not detect any difference between the femoral volumes. In a study conducted on twelve adult chinchillas, Özkadif et al (2016) determined that there was no difference between female and male femur volumes. Onwuama et al (2018) found that the femur lengths were greater than females in their study of twelve adult grasscutters. In the present study, no difference was found the length measurements made in New Zealand rabbits, but it was found that the right humerus and femur volume in male rabbits was larger than on the left side, and there was no statistical difference when the data obtained in terms of gender were compared.

Conclusion

As a result, with the CT system, which is a reliable system, the desired region in live animals can be examined and information can be obtained, especially about rigid tissues. In the presented study, morphometric measurements were carried out by performing 3D modeling on tomography images obtained from the femur and humerus of female and male New Zealand rabbits. In the measurements made in the study, it was determined that the rabbit bones did not show homotypic variation when the right and left values were compared. In addition, it was thought that humerus

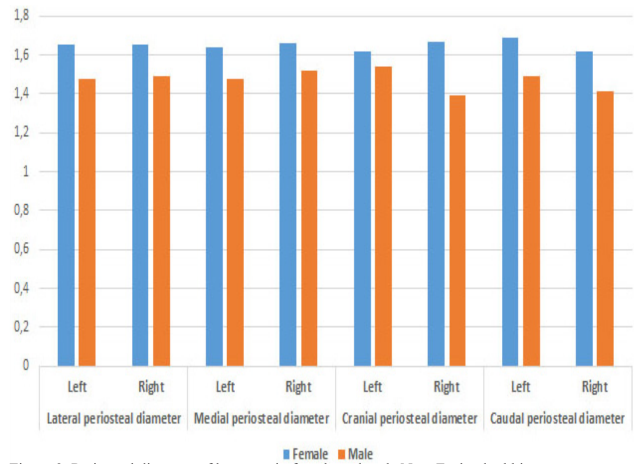

Figure 3. Periosteal diameter of humerus in female and male New Zealand rabbits

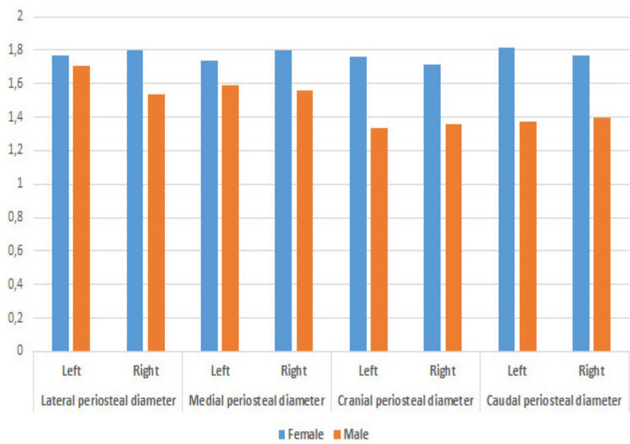

Figure 4.Periosteal diameter of femur in female and male New Zealand rabbits

and femur bones could not be used to determine gender due to the lack of significant differences in osteometric measurements between genders. The study presents a detailed analysis of the morphological characteristics of the humerus and femur. The data obtained from this study could use as a basis for future research and experimental models on rabbits and could assist physicians in diagnosing diseases using imaging systems commonly used in clinical settings.

Conflict of Interest

The authors did not report any conflict of interest or financial support.

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Author Contributions

Motivation/Concept: MLS; Design: MLS; Control/Supervision: MLS; Data Collection and/or Processing: MLS; Analysis and/or Interpretation: MLS; Literature Review: MLS; Writing the Article: MLS; Critical Review: MLS

Ethical Approval

The study was approved by the Ethics Committee of the Faculty of Health Sciences of Karamanoğlu Mehmetbey University (2021/03-22).