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Evaluation of Femur Length in Rats Using Three Different Methods and the Reliability of the Anthropometric Measurement Method

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ABSTRACT

Objective: The skeletal system evaluated in rodent models plays a crucial role in various experimental studies, including bone development, fracture healing, biomechanical testing, osteoporosis, drug efficacy, and safety. This study aims to assess the applicability of the anthropometric method, which is widely used in humans, for measuring femur length in rats. Additionally, the reliability of the anthropometric method was compared with dissection and radiological methods. **Materials and Methods:** A total of 66 male Wistar Albino rats (14–16 weeks old) were used. The right femur length was measured using three different methods: anthropometric measurement, digital caliper measurement after dissection, and radiological measurement via Micro-CT. The obtained data were analyzed, and the agreement between methods was evaluated using the Bland-Altman test. **Results:** The mean femur length was measured as 37.57 mm anthropometrically, 33.55 mm using a digital caliper post-dissection, and 33.84 mm using Micro-CT. The intraclass correlation coefficient (ICC) values at a %90 confidence interval were as follows: 0.987 (0.979–0.992) between the dissection and radiological groups, 0.709 (0.564–0.812) between the anthropometric and dissection groups, and 0.713 (0.569–0.814) between the anthropometric and radiological groups. The anthropometric method showed a significant agreement with both dissection and radiological methods, with a difference of approximately 4 mm. **Conclusion:** The anthropometric method has been demonstrated to be a reliable, easy-to-use, non-invasive, cost-effective, and time-saving alternative for measuring femur length in rats. Since this method allows for repeated measurements on the same animal, it can reduce the number of animals used in studies, thereby promoting more ethical and efficient research in bone studies.

Keywords: Femur Length, Rat, Anthropometry, Dissection, Micro-CT.

Sıçanlarda Femur Uzunluğunun 3 Farklı Yöntem ile Değerlendirilmesi ve Antropometrik Ölçüm Yönteminin Güvenilirliği

ÖZ

Amaç: Kemirgen modellerinde değerlendirilen iskelet sistemi; kemik gelişimi, kırık iyileşmesi, biyomekanik testler, osteoporoz, ilaç etkinliği ve güvenliği gibi birçok deneyde önemli yer tutmaktadır. Bu çalışmada, sıçanlarda en çok değerlendirilen kemiklerden olan femur'un uzunluğunu ölçmek için insanlarda yaygın olarak kullanılan antropometrik yöntemin sıçanlardaki uygulanabilirliği, bunun yanında antropometrik yöntemin güvenilirliğinin diseksiyon ve radyolojik yöntemler ile karşılaştırılması amaçlanmıştır. **Gereç ve Yöntem:** 66 adet erkek Wistar Albino sıçan kullanılmıştır ve sağ femur uzunluğu antropometrik olarak, diseksiyon sonrası dijital kumpas ile ve Mikro-BT ile radyolojik olarak ölçülmüştür. Elde edilen veriler analiz edilmiş ve Bland-Altman testi ile yöntemler arasındaki uyum değerlendirilmiştir. **Bulgular:** Femur uzunluğu; antropometrik olarak 37.57 mm, diseksiyon sonrası dijital kumpas ile 33.55 mm, mikroCT ile 33.84 mm bulunmuştur. %90 güven aralığında ICC katsayıları; diseksiyon ve radyolojik grup arasında 0.987 (0.979-0.992), antropometrik ve diseksiyon grup arasında 0.709 (0.564-0.812), antropometrik ve radyolojik grup arasında 0.713 (0.569-0.814) şeklindedir. Antropometrik yöntem, diseksiyon sonrası ve radyolojik yöntemlerle arasında 4 mm'lik farkla önemli derecede uyumlu olarak bulunmuştur. **Sonuç:** Sonuç olarak sıçanlarda femur uzunluğunu ölçmek için antropometrik yöntem, güvenilir, kolay uygulanabilir, invaziv olmayan, maliyet ve zaman tasarrufu sağlayan alternatif bir yöntem olduğu ortaya koyulmuştur. Bu yöntem, kullanılan hayvan sayısını azaltıp tek bir hayvanın tekrar tekrar değerlendirilmesine olanak sağladığından kemik çalışmalarında daha etik ve daha verimli çalışmaların yapılmasına imkân sağlayabilir.

Anahtar kelimeler: Femur Uzunluğu, Sıçan, Antropometri, Diseksiyon, Mikro-BT.

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INTRODUCTION

Bone is a complex tissue that adapts to both environmental influences and internal physiological factors of the body. Osteoclasts facilitate the resorption of old bone, followed by the formation of new bone by osteoblasts, ensuring continuous remodeling throughout life. Osteoclast and osteoblast activity occur sequentially, maintaining anatomical and structural integrity while supporting skeletal renewal (Gürgül et al., 2007, Cvetkovic et al., 2013). The skeletal system, with its composition and organization determined by the mechanical properties of bone, bone geometry, mineral density, and structure, is a mechanically optimized system that adapts to functional demands. Hydroxyapatite, which provides most of the strength and stiffness of the skeleton, also allows for the use of radiological techniques to assess bone mass and structure (Bagi et al., 2011).

Rodent models play a significant role in preclinical studies investigating fracture healing, biomechanical testing, and osteoporosis. These models provide valuable predictive insights into drug efficacy and safety in humans and enable the examination of the physiological process of bone healing. Due to their surgical feasibility and standardization, experiments on rat bones are of great importance (Prodinger et al., 2018, Cao et al., 2022, Prodinger, Bürklein, et al., 2018). Both the femur and tibia are suitable bones for experimental studies in rats. The femur, with its thick muscular structure, prevents the formation of non-standard fractures and allows for the use of appropriate implants. Additionally, it is frequently preferred because it enables comparisons across different studies (Prodinger et al., 2018, Prodinger, Bürklein, et al., 2018).

The femur has been extensively evaluated in various rat studies focusing on age- and sex-specific bone characteristics, bone histomorphometry, resistance testing, biomechanical testing (Cvetkovic et al., 2013, Prodinger et al., 2018, Castillo et al., 2022, Silveira et al., 2020), hip implants (Paish et al., 2020), diabetes and vitamin K2 (Mahmoud et al., 2023), trabecular and cortical bone microarchitecture (Williams et al., 2019), bone mineral density (Silveira et al., 2020), osteoporosis (Pandit et al., 2020, Sato et al., 2018), different drug effects (Matuszewska et al., 2021), and the impact of swimming on bone (Freitas et al., 2025). Morphometric measurement methods have included digital calipers or radiological techniques such as dual-energy X-ray absorptiometry, bidirectional radiographic imaging, and micro-computed tomography (Micro-CT). These methods generally require the sacrifice of the animal, followed by careful dissection of the femur and removal of surrounding muscle tissue. In experiments requiring periodic observation, such as the evaluation of skeletal growth at 6, 9, and 12 weeks, a large number of rats may be needed (Sato et al., 2018, Y. Sato et al., 2024).

Anthropometry refers to the systematic direct measurement of physical characteristics such as body weight and height in humans. It is a simple, portable, non-invasive, and cost-effective measurement method widely used in clinical practice due to its numerous benefits. A review of the literature reveals no studies in which the femur of rats has been assessed anthropometrically (Rumbo-Rodríguez et al., 2021, Padilla et al., 2021). Implementing anthropometric measurements in rats could offer several advantages, including reducing the number of animals required and enabling continuous assessment of a single animal in longitudinal studies, such as fracture healing or bone growth.

Several studies have evaluated the morphometry of long bones in rats, with digital calipers and Micro-CT being the primary measurement techniques. However, no studies have been identified that utilize anthropometric measurements. In this study, femur length was assessed using anthropometric methods, digital calipers, and Micro-CT, and the results were compared to evaluate the accuracy and reliability of anthropometric measurements in rats. Additionally, based on the human anthropometric assessment method, reference points for femur length measurement in rats were established.

MATERIALS AND METHODS

Study type

It involved 66 male Wistar albino rats (14–16 weeks old) housed at our university's animal research center. All procedures involving animals were conducted in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals and adhered to international guidelines on animal research ethics. Using G*Power software (effect size: 0.40, confidence level: 0.90, power: 0.80), the minimum number of rats required was calculated as 65. The study was conducted with 70 rats.

Surgical procedure and experimental design

This study was conducted using 70 male Wistar Albino rats (14–16 weeks old) at the Balıkesir University Experimental Animals Production, Care, and Research Center. Under anesthesia (80 mg/kg ketamine and 8 mg/kg xylazine, intraperitoneally), the right knee joint was shaved and covered with a sterile drape. An arthrotomy was then performed using a medial parapatellar approach to the right knee. A closed fracture was created at the midshaft of the femur using the three-point bending technique. After the fracture was induced, the same individual confirmed via palpation whether the fracture was at the diaphyseal level and whether there was any comminution, ensuring the creation of a standardized fracture pattern. The fracture was then fixated with a 1.0 mm Kirschner wire, which was retrogradely advanced from the intercondylar area of the femur to the trochanteric region and anchored at the greater trochanter. The correct positioning of the wire was confirmed by tactile feedback, ensuring precise delivery of the fixation. At

the knee region, the wire was trimmed at the condyle to prevent restriction of joint movement. Finally, the patella was repositioned, and the medial parapatellar incision was sutured. Following the surgery, rats were allowed unrestricted movement, and no immobilization of their lower limbs was applied. The animals were housed in standard laboratory cages (4 rats per cage) under controlled conditions, including a temperature of 22±2°C, relative humidity of 50–60%, and a 12-hour light/dark cycle. They had free access to standard pellet feed and water throughout the study.

Sacrification was performed at either the 2nd or 4th week via cervical dislocation under high-dose anesthesia, ensuring minimal pain and distress. prior to cervical dislocation, while the animals were still under anesthesia, body length, body weight, and anthropometric femur length measurements were planned to be recorded. Following sacrification, the entire femur, including the fracture site with callus tissue, was carefully dissected from the hip and knee joint. Subsequently, femur length was first measured using a digital caliper, followed by an additional measurement using Micro-CT imaging. All surgical procedures were performed by the same author. Additionally, anthropometric length measurement, post-excision femur length measurement, and femur length measurement using Micro-CT were planned to be conducted by different authors, who were blinded to each other's data.

Femoral length measurement

Measurements were made in three different ways:

1-Anthropometric femur length measurement (Anthropometric group): In humans, femur length is anthropometrically measured as the distance between the trochanter major and the condylus lateralis (Shantanu et al., 2023). Similarly, in rats, the same anatomical reference points were used to measure the right femur length with a digital caliper (Mitutoyo Corporation, CD-15D, Japan) with a precision of 0.001 mm.

2- Femur length measurement using a digital caliper (Dissection group): The right femur was carefully dissected from the hip and knee joints. Femur length was measured using a digital caliper with a precision of 0.001 mm (Mitutoyo Corporation, CD-15D, Japan). The measurement was taken parallel to the corpus femoris, from the trochanter major to the distal surface of the condylus lateralis (Williams et al., 2019, Freitas et al., 2025, Lezón et al., 2008, Foster, 2019, Jäger et al., 2005).

3-Femur length measurement using micro-ct Imaging (Radiological group): Following sacrification, the carefully extracted right femurs were preserved in a 10% formaldehyde solution until they were transported for Micro-CT analysis. Micro-CT scans were performed by positioning the femurs in a plastic holder and scanning them at a dose of 50 kVp and 45 mA, with a step angle of 0.75 degrees and an exposure time of 40 ms per projection, using a U-CT (MILabs MicroCT-OI) scanner. The scanned sections were reconstructed with voxel sizes of 60 micrometers. The images were then transferred in DICOM format to the RadiAnt software, where the distance measurement tool was selected. To measure femur length, the trochanter major and condylus lateralis were marked, and the distance between these two points was measured in millimeters (mm) (Wong et al., 2022, Hernández-Becerra et al., 2020) (Figure 1).

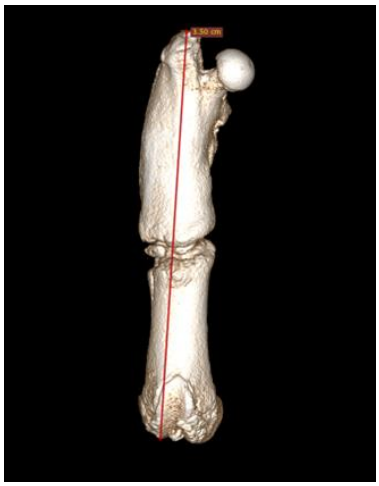

Figure 1. Radiological measurement of femur length.

Body length measurement

Body length measurements were taken while the animals were under anesthesia, immediately before cervical dislocation. During the measurement, the rats were placed in a fully stretched supine position (Soon et al., 2006).

- Nose-to-tail length: The distance from the tip of the nose to the tip of the tail was measured (Kim et al., 2012).

- Nose-to-anus length: The distance from the tip of the nose to the anus was measured using a non-stretchable measuring tape (Novelli et al., 2007).

Body weight and lee index measurement

The body weight of the rats was measured using a precision scale with 1-gram sensitivity immediately before sacrification (Pazvant & Kahvecioğlu, 2009). Subsequently, the Lee index, which is recognized as an obesity index in rats, was calculated. A Lee index value above 300 was considered indicative of obesity. The index was determined using the following formula (Lee, 1929):

- Lee index= [body weight1/3 (g)/naso-anal length (cm)] × 1000 (Lee, 1929).

Statistical analysis

The mean and standard deviations of the data obtained from the study were calculated using one-way analysis of variance (ANOVA). Since femur length was assessed using three different methods—anthropometric, post-dissection, and radiological measurements—these were categorized into three groups, and ANOVA was used to analyze differences

between groups, followed by a post-hoc LSD correction. The Intraclass Correlation Coefficient (ICC), which evaluates the consistency and agreement of quantitative measurements, was calculated among the groups (Y. Sato et al., 2024). The agreement among the three groups was further assessed using the Bland-Altman test. Statistical analyses were performed using IBM SPSS version 30 and MedCalc® version 20.111 (MedCalc Software Ltd, Ostend, Belgium). Related with the results of the power analysis, The *p*-value < 0.10 was considered statistically significant.

Ethical approval

This study was approved by the local animal ethics committee on 28.03.2024 (approval number3-6/2024).

RESULTS

On the day the experimental fractures were induced, three rats were excluded due to comminution.

Additionally, one rat died the following day, bringing the total number of exclusions to four. As a result, the study was conducted with 66 rats.

The average femur length measurement values were found to be 37.57 mm for Anthropometric Femur Length Measurement (anthropometric group), 33.55 mm for Femur Length Measurement Using a Digital Caliper following dissection (dissection group), and 33.84 mm for Femur Length Measurement Using Micro-CT Imaging (radiological group) (Table 1).

Table 1. Descriptive statistical values of variables.

	Mean	Standard Deviation	Minimum	Maximum
Body weight (g)	281.12	37.14	200	367
Nose-to-anus length (cm)	22.03	0.94	18.8	23.5
Nose-to-tail length (cm)	40.99	1.62	36.1	43.1
Lee index ($g^{1/3}$/cm)	2.96	0.08	2.62	3.17
Anthropometric femur length (mm)	37.57	1.17	34.0	41.6
Dissection Group (mm)	33.55	1.27	30.5	36.6
Radiologic Group (mm)	33.84	1.28	30.3	36.4

Table 2 indicates that there is a statistically significant difference between the anthropometric group and both the dissection group and the radiological group ($p<0.10$), whereas no significant difference was obtained between the dissection group and the radiological group ($p>0.10$).

Table 2. Mean differences between groups.

Groups		Mean difference	p
Anthropometric group	Dissection group	4.022	0.000
	Radiological group	4.153	0.000
Dissection group	Anthropometric group	-4.022	0.000
	Radiological group	0.130	0.549
Radiological group	Anthropometric group	-4.153	0.000
	Dissection group	-0.130	0.549

The reliability of repeated measurements was evaluated using intraobserver correlation coefficient (ICC) values. The analysis revealed that the correlation between the dissection group and the radiological group was within the range of 0.81 to 1.00, indicating near-perfect reliability. In contrast, the correlation between the anthropometric group and the other two groups ranged from 0.61 to 0.80, suggesting a significant level of reliability. Furthermore, the p-value for all comparisons was calculated as 0.0001, indicating that the tested relationships were not due to random chance but were statistically highly significant. Specifically, the ICC value for the dissection and radiological groups was 0.987 (90% CI: 0.979–0.992, p=0.0001), while the correlation between the anthropometric and dissection groups was 0.709 (90% CI: 0.564–0.812, p=0.0001). Similarly, the ICC value between the anthropometric and radiological groups was 0.713 (90% CI: 0.569–0.814, p=0.0001). As can be seen from Table 3, these results indicate that while all groups demonstrate a strong correlation, the highest level of agreement was observed between the dissection and radiological groups, whereas the anthropometric group exhibited a relatively lower but still significant correlation with the other two groups.

Table 3. Intraclass Correlation Coefficient (ICC) analysis for repeated measurement reliability.

	ICC (90% Confidence interval)	p
Dissection Gr. / Radiological Gr.	0.987 (0.979-0.992)	0.0001
Anthropometric Gr. / Dissection Gr.	0.709 (0.564-0.812)	0.0001
Anthropometric Gr. / Radiological Gr.	0.713 (0.569-0.814)	0.0001

The agreement between the measurement methods, as shown in Figure 2, indicates that the blue line represents the mean difference between the two methods, while the upper and lower lines represent the confidence interval. The data points are randomly distributed around the mean line, and except for a few outliers, they fall within the confidence interval, suggesting a good agreement between the methods. The mean difference between the dissection group and the radiological group is close to zero, whereas the mean differences between the anthropometric and dissection groups and the anthropometric and radiological groups are 4.0 mm and 4.2 mm, respectively. Therefore, it can be concluded that the anthropometric method exhibits a systematic bias, consistently yielding higher values compared to both the dissection group and the radiological group.

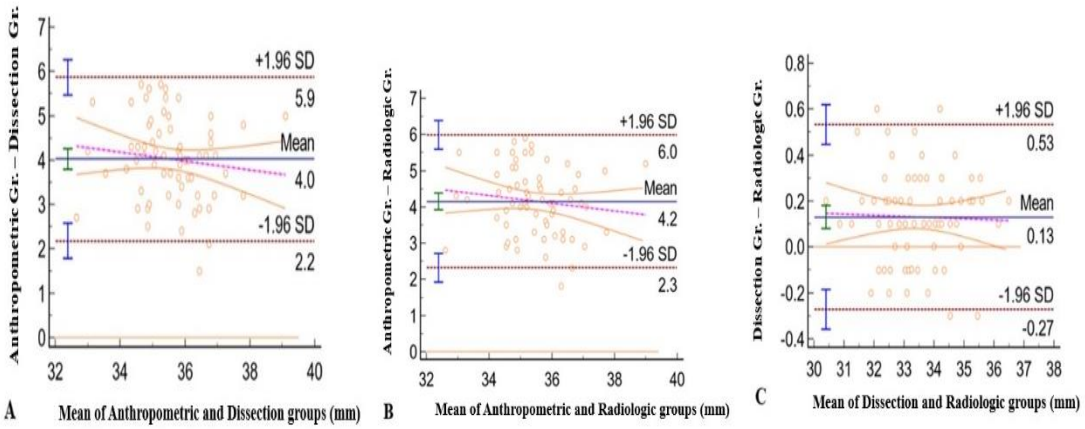

Figure 2. The agreement between the measurement methods

DISCUSSION

This study demonstrated that the anthropometric measurement method, widely used in humans, can also be applied to rats, allowing repeated femur evaluations during or at the end of an experiment without the need for animal sacrifice. Compared to dissection-based digital caliper measurements and radiological Micro-CT imaging, the anthropometric method consistently showed a higher femur length measurement, with a mean difference of approximately 4 mm. Despite this difference, the strong agreement observed between the three methods suggests that the anthropometric method is a reliable alternative that is non-invasive, easy to apply, and cost-effective. This makes it a promising approach for future experimental studies, particularly those requiring longitudinal skeletal assessments.

A review of the literature revealed limited studies directly comparing radiographic measurements and digital caliper measurements. Järvinen et al. (1998) examined the accuracy of femur length measurements obtained via dual-energy X-ray absorptiometry (DXA) in comparison to digital caliper measurements, reporting a measurement error of less than 1%, thereby confirming the accuracy of DXA-based femur length assessments (Järvinen et al., 1998). Similarly, in the present study, femur length measurements obtained using a digital caliper after dissection were found to be interchangeable with those obtained via Micro-CT imaging, supporting previous findings.

Rodent models play a crucial role in preclinical research, particularly in studies investigating fracture healing, metabolic bone diseases, and drug effects on bone morphology. Appendicular bones, such as the femur, tibia, and humerus, are frequently used due to their accessibility, well-characterized structure, and cost-effectiveness (Cao et al., 2022, Castillo et al., 2022). Numerous studies have employed these bones to evaluate morphological, histological, and biomechanical changes (Williams et al., 2019, Freitas et al., 2025, Lezón et al., 2008, Foster, 2019, Jäger et al., 2005, Wong et al., 2022, Hernández-Becerra et al., 2020, Ponyrko et al., 2021).

The femur is one of the most commonly studied bones in rat models due to its thick muscular structure, resistance to non-standard fractures, and suitability for various biomechanical tests. For instance, Mahmoud et al. (2023) investigated the impact of vitamin K2 supplementation on diabetic and non-diabetic rats, analyzing femur length after sacrificing the animals (Mahmoud et al., 2023). Similarly, Ponyrko et al. (2021) conducted a longitudinal study on hyperglycemic rats, in which femurs were sacrificed and analyzed at different time points (days 2, 30, 60, 90, 120, 150, and 180) for morphometric and histological evaluation (Ponyrko et al., 2021).

In studies examining the effects of antiepileptic drugs on bone metabolism, findings remain limited and inconclusive. Nowińska et al. (2012) explored the impact of vigabatrin, an antiepileptic drug, on skeletal development in 4-week-old rats. However, as all animals were sacrificed at the same time, the study did not allow for a week-by-week assessment of femur growth (Nowińska et al., 2012). The inability to conduct repeated in vivo evaluations is a major limitation in such research, further supporting the need for alternative non-invasive methods like anthropometric assessments.

Bone growth is a complex process influenced by hormonal regulation, genetic predisposition, and nutritional status. Consequently, bones are often used as biomarkers to assess endocrine function, metabolic status, and dietary effects. Kim et al. (2020) studied the impact of a botanical extract (H. japonicus) on bone growth by sacrificing all animals after 4 weeks and performing morphometric analyses of the femur and tibia using Micro-CT (Kim et al., 2020). Although the study provided valuable insights, it was limited to a short-term (4-week) analysis, preventing the evaluation of long-term skeletal effects.

Similarly, Maditz et al. (2015) investigated the impact of diet on bone growth in polycystic kidney disease (PKD) rats. Femur and tibia lengths were measured only at the end of the experiment (12 weeks post-intervention) using a digital caliper following dissection (Maditz et al., 2015). This end-point-only approach failed to assess gradual changes in bone growth, highlighting the need for alternative methods that allow repeated, non-invasive measurements over time.

In human research, long-term studies on longitudinal skeletal growth from infancy to adolescence are often limited by the extended duration required and financial constraints. As an alternative, animal models provide a valuable opportunity to study bone growth over shorter periods. Soon et al. (2006) utilized PIXImus (dual-energy X-ray absorptiometry) to evaluate skeletal development in rats aged 4–17 weeks. While the method demonstrated reliable results, it presented limitations such as manual adjustments, difficulty differentiating bone from soft tissue, and region-of-interest (ROI) selection issues (Soon et al., 2006). Given these limitations, anthropometric assessments may offer a simpler, cost-effective, and reproducible alternative for monitoring longitudinal skeletal development.

Studies examining the impact of physical activity on bone quality further highlight the limitations of endpoint-based evaluations. Freitas et al. (2025) conducted an 8-month study on the effects of swimming on bone structure in rats but only measured bone parameters at the end of the experiment using Micro-CT, making it impossible to assess progressive skeletal changes (Freitas et al., 2025). Similarly, Sato et al. (2018) evaluated juvenile osteoporosis treatments, measuring femur length at 6, 9, and 12 weeks via Micro-CT under anesthesia, but still required sacrificing animals for assessment (Sato et al., 2018). Dancause et al. (2012) investigated stress-related effects on offspring development, where femur length was measured only at postnatal day 80 following animal sacrifice (Dancause et al., 2012). Ekong et al. (2012) analyzed the impact of C. chalk exposure on bone morphometry and mineralization, but since femur dissection was performed at day 29, long-term skeletal effects remained unexamined (Ekong et al., 2012). These findings emphasize the need for methodologies that allow continuous in vivo bone assessment, such as anthropometric measurements.

When evaluating femur length during an experiment, radiological methods (e.g., Micro-CT) are commonly used. However, these techniques are expensive, require specialized equipment, and involve extensive image processing. Some experiments rely on dissection-based digital caliper measurements, which increase the number of animals required and introduce variability due to individual differences, even when experimental conditions are controlled (Y. Sato et al., 2024).

In contrast, anthropometric methods provide a non-invasive, repeatable, and cost-effective alternative. Additionally, non-invasive skeletal characterization techniques have broad applications in medicine, pharmacology, veterinary science, biological research, and forensic anthropology (Bagi et al., 2011).

Study Limitations and Strengths

This study has certain limitations. Although the sample size was adequate, variations in bone growth rates and structural differences between sexes and strains were not assessed. Additionally, the use of a closed fracture model may have influenced measurement results. Lastly, due to the small size of rat bones, anthropometric measurements may have a minor margin of error. Future studies should expand on these findings with repeated trials and larger sample sizes to validate measurement accuracy.

CONCLUSION

This study presents the anthropometric method as a viable alternative to radiological and post-dissection techniques for femur length measurement. Its non-

invasive nature allows repeated assessments within the same animal, reducing the number of animals required. This enhances experimental efficiency while promoting ethical research practices, making it a valuable tool for longitudinal skeletal studies in animal models.

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Conflict of Interest
The author declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

Author Contributions
Plan, design: AEA**; Material, methods and data collection:** AEA, AB, ASO, ÖE**; Data analysis and comments:** SS, ÖB, KA**; Writing and corrections:** AEA, AB, ASO, ÖE, TG.

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