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Effect of L-thyroxine on bone biomechanical and morphometric parameters in rats

Sedat Aydoğdu[1*†], Reyhan Rabia Kök[1†] and Kamil Beşoluk[1]

Abstract

Background Morphometric measurements and biomech anical tests are very crucial in determining the effects of drugs and chemicals on bones. L-thyroxine is often used instead of thyroid hormone in research conducted on experimental animals. The aim of the study is to examine the effects of L-thyroxine on the morphometric and mechanical properties of rat bones. A total of 16 healthy male *Wistar Albino* rats were used, including control and L-thyroxine groups. After the experimental process, the left/right humerus and femur were resected from all animals. Morphometric measurements were performed on the bones. Then, three indices of the bones were calculated using the measurement results. Three-point bending was performed to determine the effect of L-thyroxine on the mechanical properties of the bones.

Results Humerus maximum length and proximal width difference was determined. The maximum length and proximal width of the right humerus were lower in rats that received L-thyroxine. Differences were observed in the weight, diaphysis diameter and weight/length index of the right femur. These parameters were higher in the control group. No statistical difference was detected between L-thyroxine and control groups in mechanical properties. Maximum loading, stiffness, strength and elastic modulus in the humerus were observed to be higher in the control group. However, stiffness, strength and elastic modulus in the femur were determined to be higher in the L-thyroxine group. According to the correlation between the mechanical properties of bones and conventional indices, a high correlation was found between elastic modulus and weight/length index in the humerus. In the femur, a high negative correlation was observed between maximum loading and the weight/length index.

Conclusion This study showed that L-thyroxine had an effect on the morphometry of long bones in rats but did not affect their mechanical properties. L-thyroxine has been observed to mainly affect the bones in the right side of the body.

Keywords Femur, Humerus, L-thyroxine, Rat, Three-point bending

Introduction

In scientific studies, rats constitute the majority of experimental animals used in physiology, pharmacology and many medical fields due to their rapid reproduction, ease of care and feeding [1, 2]. Rat models provide information about bone metabolism and can also reveal the positive or negative effects of drugs. Fracture models of small animals are also widely used to define routine or pathological bone healing [3–6].

The thyroid is an endocrine gland composed of follicular cells located in front of the trachea, the shape of which varies depending on the animal species [7, 8]. In skeletal development, the hypothalamic-pituitary-thyroid axis plays a key role in the acquisition of bone mass. This axis is also responsible for regulating adult bone

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turnover. This is essential for optimum bone mineralization and strength [9, 10]. During growth and adulthood, thyroid hormones have widespread and complex effects on nearly all tissues, including bone tissue [11–13]. Thyroid hormones play a role in skeletal muscle contraction, metabolism, myogenesis, and muscle regeneration [14, 15]. L-thyroxine is used in experimental studies instead of thyroid hormone produced in the thyroid gland. L-thyroxine can be supplied in tablet, capsule, or liquid form for clinical applications [16, 17]. L-thyroxine is known as the tetraiodo derivative of p-hydroxyphenyl ether of tyrosine. It was observed that its use in rats caused a significant increase in absolute and relative heart weight, a significant decrease in absolute and relative thyroid gland weight [18, 19].

Bone tissue, which is one of the passive elements of the locomotor system, is composed of organic and inorganic substances. Organic substances give flexibility to the bone, while inorganic substances give hardness and durability to the bone [8, 20]. The appendicular skeleton consists of two parts: the forelimb bones and the hindlimb bones. In experimental studies, the humerus and femur are frequently preferred among the forelimb and hindlimb bones [3, 21–23].

Morphometric measurements performed on bones are frequently preferred in defining changes in bones. Morphometry is the totality of measurements performed to reveal differences in structures such as length, width, and thickness [24, 25]. Morphometric measurements on bones are generally performed with digital calipers. Additionally, morphometric measurements can be made on two-dimensional images such as photographs and x-rays [26–29]. In recent years, measurements have also been carried out on three-dimensional models [30–32]. Experimental studies in rats reveal changes in bones through morphometry. Audiogenic stress, Methenolone Enanthate Supplement (MES), trenbolone and testosterone are known to affect bone morphometry in rats [22, 23, 33–35].

The use of biomechanical tests in the field of engineering is frequently used to measure bone tissue strength. Tensile, torsional, compression, three-point bending, and four-point bending tests are widely used among biomechanical tests. The three-point bending test is generally preferred among biomechanical tests for rat bones. The three-point bending test involves applying a force to a bone by a mechanical testing device, which causes the bones to fracture [36–41]. As a result of the mechanical test performed, biomechanical parameters such as deformation, stiffness, elastic modulus, and strength are obtained [3, 6, 41–43].

The effects of various drugs, hormones, and bacterium on rat bones were examined using morphometric and biomechanical measurements. The effects of melatonin, bisphenol F and S, nesfatin-1 and sodium fluoride on rat bones were investigated by biomechanical methods [6, 37–39, 44]. In addition to examining the effects of drugs, surgical applications or treatment of fracture models are evaluated using biomechanical methods [43, 45].

The motivation for the study is that information on the effects of L-thyroxine, which is frequently used in experimental studies, on bones is limited. The aim of this study was to investigate the effects of L-thyroxine on bones using morphometry and biomechanical methods. In addition to morphometric changes in the bones, bone mechanical properties were revealed by biomechanical testing.

Material and methods

Animals collection

In the study, 16 healthy male *Wistar Albino* rats, 10–12 weeks old, weighing 220–250 g, were used. At the Laboratory of the Department of Biochemistry, Faculty of Veterinary Medicine, Selçuk University, bones obtained from a study (titled Effects of Dill Application on Experimentally Hyperthyroid Rats, numbered BAP-SRPSU-21212063) were used in this study.

Rats were housed in polycarbonate cages at 55% humidity and 24 °C temperature, 12 h night and 12 h day. A total of 16 rats were housed for 28 days. The rats were divided into 2 different groups. Control group (n = 8) the feed and water needs of the animals were supplied ad libitum during the experiment. The L-thyroxine group (n = 8) was fed ad libitum, and 100 µg/kg L-thyroxine (Sigma, USA) per day was administered by gavage [46–48].

Extraction of biological material

All animals were given ad libitum feed for 28 days during the experimental period. After the experimental process, all animals were anesthetized (IP injection, Xylazine 5 mg/kg, Ketamine 95 mg/kg) and sacrificed by cervical dislocation, and then the upper and lower limbs of the animals were dissected. Left/right humerus (n = 32) and left/right femur (n = 32) resected from all animals. Subsequently, the skin and soft tissues (including muscles, tendons, and ligaments) surrounding the bones were carefully dissected [3, 39].

Morphometric measurement of bones

After removal of the skin and soft tissues, measurements were made on the humerus and femur using a 0.01 mm accuracy digital calliper. Morphometric measurements (Maximum length, proximal width, distal width, and diaphysis diameter) were made according to the reference

measurement points applied in previous studies on animals [21–23, 49–51].

Morphometric measurements performed on the humerus and femur are shown in Fig. 1. In measuring the diaphysis diameter of the humerus, the lower border of the tuberositas deltoidea was considered. Similarly, in measuring the diaphysis diameter of the femur, the lower border level of the trochanter tertius was considered.

Bone indices were calculated using the data obtained from morphometric measurements. Index 1, robusticity index, and bone weight/bone length index were calculated, the equations of which are given below [50, 52–56].

The robusticity index helps determine whether bones are denser and more robust. The lower the index of robusticity, the denser and more robust the bone [53, 54]. The bone weight/bone length index is a more straightforward index of bone density than the robusticity index used by Seedor [55]. Subsequently, the bones were wrapped in sterile gauze cloths moistened with saline and stored in a freezer (−24∘C) until the biomechanical test [38, 39, 44].

$$Index\ 1 = \frac{Diaphysis\ diameter}{Maximum\ length} \times 100$$

$$Robusticity\ index = \frac{Maximum\ length}{\sqrt[3]{Weight\ of\ bone}}$$

$$Weight/length\ index = \frac{Weight\ of\ bone}{Maximum\ length}$$

Biomechanical test

The bones were thawed at room temperature before the biomechanical test and subjected to a three-point bending using Shimadzu universal strength tester (Shimadzu Autograph AGS-X Series, Kyoto, Japan) [38, 39, 44]. The three-point bending test was performed at room temperature to evaluate the material mechanical properties of the cortical bone corresponding to the mid-diaphysis of the bone. When determining the span between the support points, the length and diameter values of the bones used in the study were taken into account. The span for the humerus was determined as 15 mm and for the femur as 20 mm. Force was applied to the bones from the craniocaudal. A preload of 8 Newton (N) was first applied. The load was applied at a rate of 5 mm/min until the bones fractured. Linear regression of the force/displacement graph using the software was obtained. Thus, the maximum load (F) applied to the bones was determined and recorded [3, 38, 39, 57, 58]. Three-point bending was performed on 6 bones from each group. Morphometric measurements and biomechanical tests performed on the humerus and femur are presented in Fig. 2.

Statistical analysis

The data obtained from morphometric measurements and biomechanical tests were evaluated in the statistical package program IBM SPSS Statistics Standard Concurrent User V 22 (IBM Corp., Armonk, New York, USA). The homogeneity of the variances was tested using the Levene test. Normal distribution of the data of numerical variables was evaluated with the Shapiro Wilk normality test. Comparisons between control and L-thyroxine groups were performed using Student's t-test for parametric variables and Mann–Whitney U test for non-parametric variables. $p < 0.05$ was considered statistically significant. The relationship between

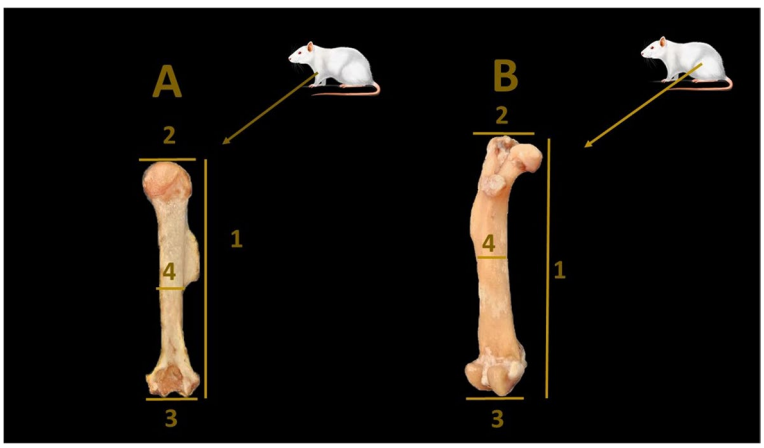

Fig. 1 Morphometric measurements of the humerus (**A**) and femur (**B**). Maximum length (1), proximal width (2), distal width (3), diaphysis diameter (4)

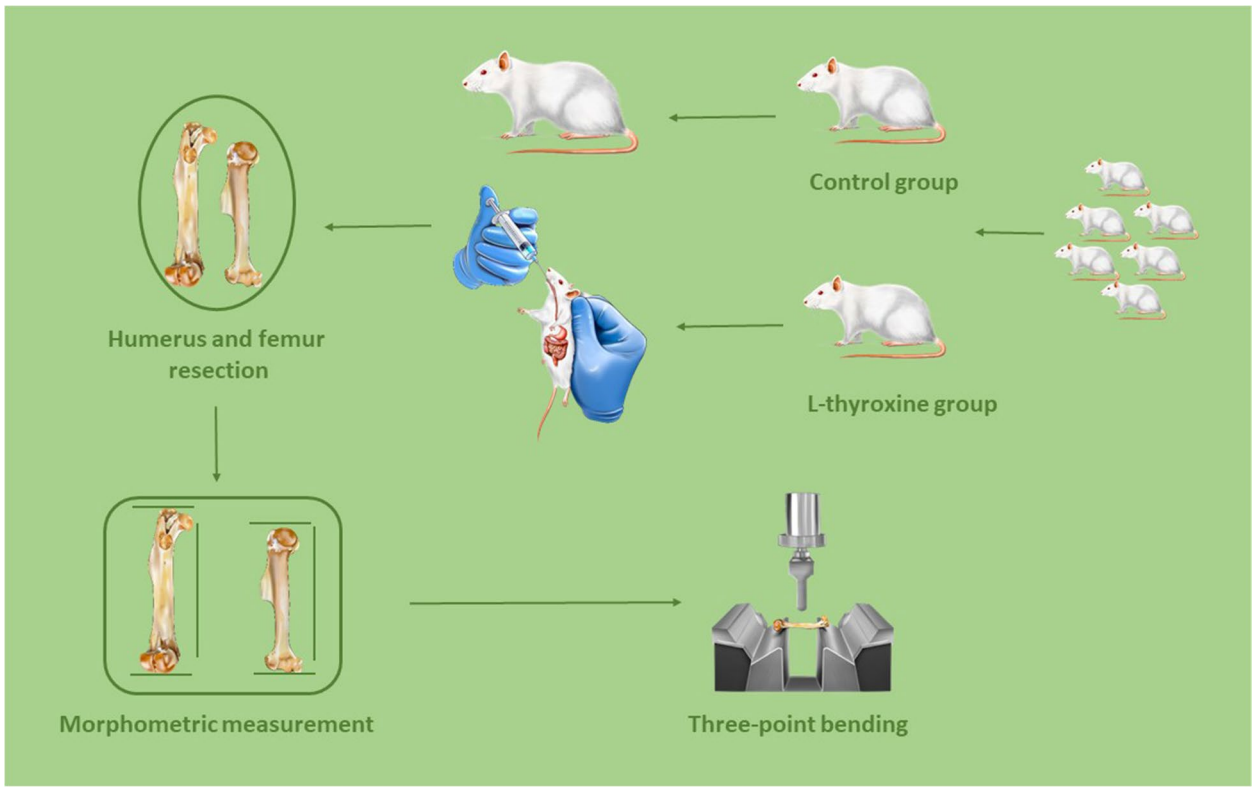

Fig. 2 Experimental setup for morphometric measurements and biomechanical tests

biomechanical parameters and indices was evaluated with Pearson's correlation coefficients.

Results

Morphometric measurement results

Morphometric measurements and calculated indices were performed to determine the effect of L-thyroxine on the humerus are presented in Table 1.

A statistical difference was found in maximum length and proximal width in the right humerus. It was observed that the bone maximum length and proximal width were higher in the control group. No difference was observed between the groups in other parameters and indices.

Morphometric measurements and calculated indices were performed to determine the effect of L-thyroxine on the femur are presented in Table 2.

A statistical difference was found in the weight and diaphysis diameter of the right femur. It was observed that the right femur weights and diaphysis diameter were higher in the control group. Additionally, a statistical difference was observed in the weight/length index in the right femur. The weight/length index of the right femur was higher in the control group.

Table 1 Morphometric measurements were obtained from the humerus

		Control Mean ± Std	L-thyroxine Mean ± Std	p
Weight of bone (gr)	Left	0.44 ± 0.01	0.42 ± 0.03	$0.141^{€}$
	Right	0.44 ± 0.02	0.42 ± 0.04	$0.123^{£}$
Maximum length (mm)	Left	27.86 ± 0.53	27.29 ± 0.71	$0.089^{£}$
	Right	27.97 ± 0.59	27.28 ± 0.66	$0.045^{£*}$
Proximal width (mm)	Left	5.86 ± 0.12	5.81 ± 0.14	$0.458^{£}$
	Right	5.84 ± 0.13	5.54 ± 0.28	$0.021^{€*}$
Distal width (mm)	Left	7.23 ± 0.25	7.17 ± 0.27	$0.650^{£}$
	Right	7.24 ± 0.20	7.17 ± 0.22	$0.533^{£}$
Diaphysis diameter (mm)	Left	2.60 ± 0.09	2.58 ± 0.07	$0.604^{£}$
	Right	2.60 ± 0.76	2.60 ± 0.18	$0.986^{£}$
Index 1	Left	9.33 ± 0.31	9.45 ± 0.32	$0.454^{£}$
	Right	9.32 ± 0.38	9.54 ± 0.62	$0.392^{£}$
Robusticity index	Left	3.66 ± 0.08	3.64 ± 0.07	$0.721^{£}$
	Right	3.69 ± 0.05	3.65 ± 0.06	$0.466^{£}$
Weight/length index	Left	15.87 ± 0.55	15.38 ± 0.83	$0.198^{£}$
	Right	15.85 ± 0.53	15.36 ± 1.04	$0.345^{€}$

Numerical variables are given as mean ± standard deviation. £ Student's t-test, € Mann–Whitney U test, * $p < 0.05$. In the index equations, the weight is taken as milligrams

Table 2 Morphometric measurements were obtained from the femur

		Control	L-thyroxine	p
		Mean ± Std	Mean ± Std	
Weight of bone (gr)	Left	1.00 ± 0.07	0.93 ± 0.07	0.069£
	Right	1.00 ± 0.05	0.93 ± 0.06	0.019£*
Maximum length (mm)	Left	37.00 ± 0.39	36.52 ± 0.84	0.159£
	Right	37.06 ± 0.45	36.49 ± 0.85	0.116£
Proximal width (mm)	Left	8.88 ± 0.24	8.66 ± 0.33	0.157£
	Right	8.64 ± 0.25	8.62 ± 0.28	0.890£
Distal width (mm)	Left	7.10 ± 0.20	6.97 ± 0.21	0.255£
	Right	7.05 ± 0.25	7.03 ± 0.18	0.851£
Diaphysis diameter (mm)	Left	4.63 ± 0.21	4.45 ± 0.17	0.097£
	Right	4.63 ± 0.19	4.38 ± 0.16	0.013£*
Index 1	Left	12.50 ± 0.59	12.21 ± 0.60	0.336£
	Right	12.50 ± 0.54	12.01 ± 0.59	0.106£
Robusticity index	Left	3.70 ± 0.08	3.74 ± 0.06	0.326£
	Right	3.71 ± 0.06	3.74 ± 0.05	0.529€
Weight/length index	Left	27.06 ± 1.73	25.58 ± 1.50	0.088£
	Right	27.04 ± 1.16	25.49 ± 1.21	0.021£*

Numerical variables are given as mean ± standard deviation. £ Student's t-test, € Mann–Whitney U test, * $p < 0.05$. In the index equations, the weight is taken as milligrams.

Biomechanical test results

The mechanical properties of the bones were determined using the three-point bending test. The results obtained from the humerus are presented in Table 3.

According to the three-point bending test results performed on the humerus, no statistical differences were observed in F, deformation, stiffness, strength, and elastic modulus. Additionally, no difference was found between the diameters of the humerus. The results obtained from the femur are presented in Table 4.

There was no statistical difference observed between the biomechanical parameters and diameters obtained from the femur. The correlation between traditional indexes (Index 1, robusticity index, weight/length index) calculated using morphometric measurements and biomechanical parameters (F, deformation, stiffness, strength, elastic modulus) was examined. The correlation obtained from the measurements of the humerus is presented in Table 5.

According to the results obtained from the humerus, a high correlation was found between elastic modulus and weight/length index. The correlation obtained from the femur measurements is presented in Table 6.

According to the results obtained from the femur, a high negative correlation was observed between the F applied to the bone and the weight/length index. No correlation was detected between other values.

Discussion

The hypothalamic-pituitary-thyroid axis plays an essential role in skeletal development and the acquisition of bone mineralization, strength, and mass [11–13]. Thyroid hormones are also effective in skeletal muscle contraction, myogenesis and muscle regeneration [14, 15]. L-thyroxine is often used instead of thyroid hormone in experimental studies [16, 17]. It is known to cause a significant increase in absolute and relative heart weight and a significant decrease in absolute and relative thyroid gland weight in rats [19]. However, data on how L-thyroxine affects bone morphometry and mechanical properties are limited.

Table 3 Diameters and biomechanical parameters obtained from the humerus

	Control (n:6)	L-thyroxine (n:6)	p
	Mean ± Std	Mean ± Std	
$ExtD_{ML}$(mm)	2.60 ± 0.11	2.59 ± 0.06	0.810€
$ExtD_{CrCd}$(mm)	3.19 ± 0.07	3.22 ± 0.05	0.437£
$IntD_{ML}$ (mm)	1.43 ± 0.06	1.50 ± 0.07	0.078£
$IntD_{CrCd}$(mm)	1.78 ± 0.21	1.84 ± 0.06	1.000€
F (N)	68.59 ± 8.78	63.90 ± 11.61	0.448£
Deformation(mm)	0.61 ± 0.19	0.67 ± 0.09	0.531£
Stiffness (N/mm)	262.17 ± 115.73	199.23 ± 71.11	0.283£
Strength (MPa)	74.28 ± 16.43	64.69 ± 15.34	0.321£
Elastic Modulus(MPa)	894.24 ± 431.48	650.81 ± 221.05	0.247£

ExD_{ML}; medio-lateral external diamater; ExD_{CrCd}; cranio-caudal external diamater; $IntD_{ML}$; medio-lateral internal diamater; $IntD_{CrCd}$; cranio-caudal internal; F; force. £ Student's t-test, € Mann–Whitney U test, * $p < 0.05$

Table 4 Diameters and biomechanical parameters obtained from the femur

	Control(n:6)	L-thyroxine (n:6)	p
	Mean ± Std	Mean ± Std	
$ExtD_{ML}$(mm)	4.53 ± 0.13	4.44 ± 0.14	0.247£
$ExtD_{CrCd}$(mm)	3.36 ± 0.09	3.35 ± 0.09	0.781£
$IntD_{ML}$ (mm)	2.76 ± 0.08	2.80 ± 0.05	0.233£
$IntD_{CrCd}$(mm)	1.95 ± 0.14	1.88 ± 0.04	0.423€
F (N)	122.21 ± 10.57	121.76 ± 15.54	0.955£
Deformation(mm)	0.96 ± 0.19	0.98 ± 0.27	0.894£
Stiffness (N/mm)	334.33 ± 143.24	342.23 ± 168.64	0.932£
Strength (MPa)	85.77 ± 16.96	86.30 ± 23.33	0.965£
Elastic Modulus(MPa)	807.60 ± 334.55	846.34 ± 429.88	0.631€

ExD_{ML} medio-lateral external diamater; *ExD_{CrCd}* cranio-caudal external diamater; *$IntD_{ML}$* medio-lateral internal diamater; *$IntD_{CrCd}$* cranio-caudal internal; *F* force

£ Student's t-test, € Mann–Whitney U test, * $p < 0.05$

Table 5 Correlation between indexes and the biomechanical parameters on humerus

	Index 1		Robusticity index		Weight/length index	
	r	p	r	p	r	p
F	−0.105	0.745	−0.101	0.755	0.146	0.651
Deformation	0.116	0.720	−0.090	0.780	−0.415	0.179
Stiffness	0.009	0.978	−0.147	0.649	0.559	0.59
Strength	−0.009	0.978	−0.147	0.649	0.472	0.122
Elastic Modulus	0.078	0.810	−0.223	0.485	0.580	0.048*

r, correlation coefficient. * $p < 0.05$

Table 6 Correlation between indexes and the biomechanical parameters on femur

	Index 1		Robusticity index		Weight/length index	
	r	p	r	p	r	p
F	0.130	0.688	0.257	0.420	−0.608	0.036*
Deformation	−0.077	0.811	−0.060	0.852	−0.115	0.722
Stiffness	0.077	0.813	0.094	0.772	0.016	0.961
Strength	0.165	0.607	0.066	0.838	−0.050	0.877
Elastic Modulus	0.068	0.833	0.076	0.814	0.030	0.926

r, correlation coefficient. * $p < 0.05$

Studies have shown that drugs, experimental models, chemicals and supplements affect bone morphometry and mechanical properties in rats [22, 23, 33–35, 37–39].

It has been determined that long bone development is negatively affected in rats exposed to audiogenic stress. Similarly, it was observed that the length of the humerus and femur decreased significantly in rats that were administered MES together with exercise [33, 35]. In this study, it was observed that the maximum length of the right humerus decreased in rats given L-thyroxine. In another study using Methenolone Enanthate, it was reported that it positively affected the femur length in adolescent female rats [34]. In the study using male rats, it was determined that L-thyroxine had no effect on femur length. It is thought that this difference is due to the experimental model, drugs used, and the age and gender of the rats in the studies.

Trenbolone and testosterone have been reported to negatively affect the development of the humerus and femur in rats. It has been reported that the humerus and femur lengths in rats administered both anabolic androgenic steroids were shorter than in healthy rats [22, 23]. It has been established that L-thyroxine has a negative impact on specific morphometric parameters in rats. L-thyroxine was observed to negatively affect the maximum length of the right humerus. In addition, it was found that the proximal width of the right humerus and the weight of bone, diaphysis diameter and weight/length index of the right femur were lower.

Various biomechanical tests are used to determine the mechanical properties of bones. One of the tests generally preferred in experimental studies conducted on rats is the three-point bending test. In this test, the maximum force applied to the bone, deformation, stiffness, strength and elastic modulus are determined. In general, bone is not a very ductile material. Therefore, the ability to sustain postyield deformation is very low [3, 38, 57]. In the present study, similarly, while the bones in both groups had elastic strain region, the ability of the bones to yield plastic strain region (post-yield displacement) was very low.

In the study examining melatonin's protective role against Bisphenol F and S, a difference was detected between the melatonin-treated groups and the control group. According to the mechanical tests results, significant differences were observed in deformation and elastic modulus. Three-point bending tests were performed on the tibia in ovariectomized rats treated with sodium fluoride. Stiffness and strength were found to be low in rats treated with sodium fluoride. It was determined that *spirulina platensis* and different doses of sodium fluoride changed the mechanical properties of bones in healthy rats. Tibiae and ulna were used to determine the mechanical properties of the bones [6, 37, 39, 44]. In the present study, no difference was observed in the bone mechanical

properties of L-thyroxine. This difference is thought to be due to variations in experimental models, gender, and the type of bones used in mechanical tests in the studies.

The effect of nesfatin-1, one of the adipokines, on the mechanical properties of rat humerus was investigated. A difference was found in maximum loading between rats receiving nesfatin-1 after ovariectomy and the control group. It has been reported that the maximum loading applied to rats receiving Nesfatin-1 was higher [38]. In this study, no statistical difference was observed between the L-thyroxine and control groups in terms of maximum loading. This difference is thought to be due to the therapeutic use of Nesfatin-1 and its effect on the healing process.

It was observed that the maximum loading and deformation obtained from the three-point bending test performed on the humerus and femur were consistent with the literature in terms of the age and gender of the rats used in the study [3, 38, 39]. It was understood that the average maximum loading value increased in studies using male and older age rats (older than 10–12 weeks) [3, 39]. It is thought that the effects of age and gender factors should be taken into consideration in addition to experimental application in the response of bone tissue.

This investigation has some limitations. Studies conducted on laboratory animals using L-thyroxine have primarily focused on the effects on organs and biochemical parameters [19, 59, 60]. The effects of thyroid hormones on bones are known. Morphometry and biomechanics are frequently preferred methods in bone studies [9, 10]. L-thyroxine is frequently used instead of thyroid hormone in experimental studies. In studies using L-thyroxine, the focus on organs other than bone tissue and the consideration of different parameters create limitations in the discussion of existing study data.

Conclusion

The current study, the effect of L-thyroxine, which is frequently used in experimental studies, on the morphometric and mechanical properties of bones was investigated. It was observed that L-thyroxine affected the morphometric properties of the humerus and femur of long bones, but had no effect on their mechanical properties. It has been determined that it has an effect, especially on the bones on the right side of the body. It has been observed that it negatively affects the maximum length and proximal width of the humerus and the weight of bone, diaphysis diameter and weight/length index of the femur. The results obtained reveal that the effects of L-thyroxine on bones should be taken into consideration in experimental studies with L-thyroxine. It is thought that these findings will contribute to the literature by correlating the results obtained from different disciplines.

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Authors'contributions
K.B. and S.A. conceptualized the study. S.A. and R.R.K. collected the study materials and performed the measurements. S.A prepared figures. S.A. and R.R.K. wrote the original draft of the manuscript. All authors have read and agreed to the published version of the manuscript.

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Data availability
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
All experimental procedures were carried out according to the ethical guidelines for using animal samples permitted by Experimental Animal Production and Research Center Ethics Committee of the Veterinary Faculty of Selçuk University, Turkey (Approval number: 2023/164). Every procedure was carried out in accordance with the relevant laws and standards. The authors obtained informed consent from the owners to use animals in the study.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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References

1. Peterson J, Guyer M, Felsenfeld A, Old S, Mockrin S. Genome sequence of the Brown Norway rat yields insights into mammalian evolution. Nat. 2004;428(6982):493–521.
2. Harkness JE, Turner PV, vandeWoude S, Wheler CL. Harkness and Wagner's biology and medicine of rabbits and rodents. 2013: John Wiley & Sons.
3. Prodinger PM, Foehr P Burklein D, Bissinger O, pilge H, Kreutzer K, Von Eisenhart-Rothe R. Whole bone testing in small animals: systematic characterization of the mechanical properties of different rodent bones available for rat fracture models. Eur. J. Med. Res. 2018;23:1–11
4. Vashishth D. Small animal bone biomechanics. Elsevier 2008. p. 794–797.
5. Bissinger O, Kreutzer K, Gotz C, Hapfelmeier A, Pautke C, Vogt S, Gabriele W, Wolff KD, Tischer T, Prodinger PM. A biomechanical, micro-computertomographic and histological analysis of the influence of diclofenac and prednisolone on fracture healing in vivo. BMC Musculoskelet Disord. 2016;17:1–11.
6. de Cassia Alves Nunes R, Chiba FY, Pereira AG, Pereira RF, de Lima Coutinho Mattera MS, Ervolino E, Louzada MJQ, Buzalaf MAR, Silva CA, Sumida DH. Effect of sodium fluoride on bone biomechanical and histomorphometric parameters and on insulin signaling and insulin sensitivity in ovariectomized rats. Biol. Trace Elem. Res. 2016;173:144–53.
7. Saglam F, Cakır B. Clinical Approach To Thyroid Diseases In Primary Health Care. Ank Med J. 2012;12(3):136–9.
8. Konig HE, Liebich HG, Cerveny C. Veterinary anatomy of domestic mammals textbook and colour atlas 2004; Schattauer: Stuttgart.
9. Gogakos AI, Bassett JD, Williams GR. Thyroid and bone. Arch Biochem Biophys. 2010;503(1):129–36.

10. Williams GR. Actions of thyroid hormones in bone. Endokrynol Pol. 2009;60(5):380–8.
11. Graham RW. Thyroid hormone actions in cartilage and bone. Eur Thyroid J. 2013;2:3–13.
12. Bassett JD, Williams GR. Role of thyroid hormones in skeletal development and bone maintenance. Endocr Rev. 2016;37(2):135–87.
13. Williams GR, Bassett JD. Thyroid diseases and bone health. J Endocrinol Invest. 2018;41(1):99–109.
14. Machado M, Bachini F, Itaborahy A. Thyroid hormones and skeletal muscle beyond thermogenesis. J Sci Sport Exerc. 2024;6(4):315–23.
15. Salvatore D, Simonides WS, Dentice M, Zavacki AM, Larsen PR. Thyroid hormones and skeletal muscle—new insights and potential implications. Nat Rev Endocrinol. 2014;10(4):206–14.
16. Laurent I, Tang S, Astere M, Wang KR, Deng, S, Xiao L,LI QF. Liquid L-thyroxine versus tablet L-thyroxine in patients on L-thyroxine replacement or suppressive therapy: a meta-analysis. Endocr. 2018;61:28–35.
17. Vita R, Fallahi P, Antonelli A, Benvenga S. The administration of L-thyroxine as soft gel capsule or liquid solution. Expert Opin Drug Deliv. 2014;11(7):1103–11.
18. Harington CR, Barger G. Chemistry of thyroxine: constitution and synthesis of thyroxine. Biochem J. 1927;21(1):169.
19. Soukup T, Zacharova G, Smerdu V, Jirmanova I. Body, heart, thyroid gland and skeletal muscle weight changes in rats with altered thyroid status. Physiol Res. 2001;50(6):619–26.
20. Dursun N. Veteriner Anatomi I. 12 ed. Ankara Medisan Yayınevi 2008.
21. Arslan MS, Besoluk K. The efects of alone and combined treatment of Tarantula cubensis alcoholic extract and Methenolone Enanthate on two long bones of young Rats. Rev Cient Fac Cienc Vet. 2023;33(2):1–7.
22. Sarı A, Lok S. The effects of trenbolone supplementation on the extremity bones in running rats. TJSE. 2019;21(2):366–71.
23. Kilci A, Lok S. Morphometric effects of testosterone supplementation on certain extremity bones in young swim-trained rats. TJSE. 2018;20(3):140–4.
24. Zelditch ML, Swiderski DL, Sheets HD. Geometric morphometrics for biologists: a primer. Academic Press 2012.
25. Ocakoglu G. Comparison of two sample tests in statistical shape analysis. 2011; Ph.D. Thesis; Bursa Uludag University (Turkey).
26. Senol E, Gundemir O, Duro S, Szara T, Demiraslan Y, Karadag H. A pilot study: Can calcaneus radiographic image be used to determine sex and breed in cats? Vet med sci. 2022;8(5):1855–61.
27. Gundemir O, Szara T, Pazvant G, Erdikmen DO, Duro S, Perez W. Radiogrametric analysis of the thoracic limb phalanges in Arabian horses and thoroughbred horses. Anim. 2021;11(8):2205.
28. Toryan E, Szara T, Gundemir O. Linear measurements and shape analysis in the calcaneus of selected dog breeds. Anat Histol Embryol. 2024;53(4): e13078.
29. Szara T, Gundemir O, Gunay E, Gun G, Avanus K, Pazvant G. Sex determination in domestic rock pigeons (Columba livia) using radiographic morphometry. Acta Zool. 2024;105(1):38–45.
30. Yilmaz O, Kocyigit A, Kirbas Dogan G, Kanik B. Three-Dimensional Morphometric Analysis of the Metatarsal and Phalangeal Bones in Van Cats. Anat Histol Embryol. 2025;54(1): e70006.
31. Kocyigit A, Demircioglu I, Comparison of craniometric measurements of New Zealand rabbit (Oryctolagus cuniculus L.) using three-dimensional scanner with digital calliper measurements: A methodological study. Anat. Histol. Embryol. 2024;53(2):e13023.
32. Demircioglu I, Koçyigit A, Demiraslan Y, Yilmaz B, Ince NG, Aydogdu S, Dayan MO. Digit bones (acropodium) of gazella (Gazella subgutturosa); three-dimensional modelling and morphometry. Pak Vet J. 2021;41(4):481–6.
33. Ozdemir M, Lok S. The effects of methenolone enanthate supplement with exercise on rats' bones. TJSE. 2019;21(2):276–80.
34. Bozkurt I, Pepe K, Ozdemir M, Ozdemir O, Coskun A. Morphometric evaluation of the effect of methenolone enanthate on femoral development in adolescent rats. Sci Res Essays. 2011;6(7):1634–8.
35. Doyle W, Kelley C, Siegel M. The effects of audiogenic stress on the growth of long bones in the laboratory rat (Rattus norvegicus). Growth. 1977;41(3):183–9.
36. Turner C. Biomechanics of bone: determinants of skeletal fragility and bone quality. Osteoporos Int. 2002;13:97–104.
37. Rezaee T, Bouxsein ML, Karim L. Increasing fluoride content deteriorates rat bone mechanical properties. Bone. 2020;136: 115369.
38. Skic A, Puzio I, Tymicki G, Kołodziej P, Pawłowska-Olszewska M, Skic K, Beer-Lech K, Bienko M, Gołacki K. Effect of nesfatin-1 on rat humerus mechanical properties under quasi-static and impact loading conditions. Mater. 2022;15(1):333.
39. Yavas FT, Kilimci FS, Akkoc AN, Sahiner HS, Yilmaz OB. Melatonin's protective role against Bisphenol F and S-induced skeletal damage: A morphometric and histological study in rat. Anat Anz. 2024;256: 152314.
40. Wang F, Zheng L, Theopold J, Schleifenbaum S, Heyde C-E, Osterhoff G. Methods for bone quality assessment in human bone tissue: a systematic review. J Orthop Surg Res. 2022;17(1):174.
41. Ma C, Du T, Niu X, Fan Y. Biomechanics and mechanobiology of the bone matrix. Bone Res. 2022;10(1):59.
42. Niu Y, Du T, Liu Y. Biomechanical characteristics and analysis approaches of bone and bone substitute materials. J Funct Biomater. 2023;14(4):212.
43. Comelekoglu U, Bagis S, Yalin S, Ogenler O, Yildiz A, Sahin NO, Oguz I, Hatungil R. Biomechanical evaluation in osteoporosis: ovariectomized rat model. Clin Rheumatol. 2007;26:380–4.
44. Suzer B, Seyidoglu N, Tufekci K, Inan S. The relationship between Spirulina platensis and selected biomechanical indicators of tibiae in rats. Vet Med. 2020;01:18–24.
45. Uslu M, Yılmaz B, Mraja HM, Dascı MF, Sarac EY, Kucukyıldırım BO, Gulec MA, Yuksel S. The effects of cephalexin on fracture healing in a rat femur fracture model. JDRS. 2023;34(2):413–24.
46. Idris M, Idris O, Sabahelkhier M. The effects of induced hyperthyroidism on plasma FSH and LH concentrations in female of Wistar rats. Res J Recent Sci. 2012;1:55–65.
47. Yang J, Yi N, Zhang J, He W, He D, Wu W, Xu S, Li F, Fan G, Zhu X. Generation and characterization of a hypothyroidism rat model with truncated thyroid stimulating hormone receptor. Sci Rep. 2018;8(1):4004.
48. Liman BC, Liman N, Tekeli Y, Ergen E, Kavraal UK. Investigations on tissue lipid peroxidation, glutathione and antioxidant enzyme levels in the hippocampus of adult rats in experimental hypo- and hyperthyroidism induced by Propyl Thiouracil (PTU) and L-Thyoxine. BAUN Health Sci J. 2022;11(Supplement 1):22–8.
49. Driesch A. A guide to the measurement of animal bones from archaeological sites. Peabody museum 1976.
50. Pazvant G, Kahvecioglu K. Studies on homotypic variations of forelimb and hindlimb long bones of guinea pigs. J Ist Faculty Med. 2013;39(1):20–32.
51. Dayan MO, Besoluk K, Eken E, Aydogdu S, Turgut N. Three-dimensional modelling of the femur and humerus in adult male guinea pigs (guinea pig) with computed tomography and some biometric measurement values. Folia Morphol. 2019;78(3):588–94.
52. Riesenfeld A. Sexual dimorphism of skeletal robusticity in several mammalian orders. Cells Tissues Organs. 1978;102(4):392–8.
53. Riesenfeld A. Endocrine control of skeletal robusticity. Cells Tissues Organs. 1975;91(4):481–99.
54. Riesenfeld A. Metatarsal robusticity in bipedal rats. Am J Phys Anthropol. 1972;36(2):229–33.
55. Seedor JG, Quartuccio HA, Thompson DD. The bisphosphonate alendronate (MK-217) inhibits bone loss due to ovariectomy in rats. JBMR. 1991;6(4):339–46.
56. Monteagudo M, Hernandez E, Seco C, Gonzalez-Riola J, Revilla M, Villa L, Rico H. Comparison of the bone robusticity index and bone weight/bone length index with the results of bone densitometry and bone histomorphometry in experimental studies. Cells Tissues Organs. 1997;160(3):195–9.
57. Turner CH, Burr DB. Basic biomechanical measurements of bone: a tutorial. Bone. 1993;14(4):595–608.
58. Sharir A, Barak MM, Shahar R. Whole bone mechanics and mechanical testing. TVJ. 2008;177(1):8–17.
59. Mogulkoc R, Baltaci AK, Oztekin E, Sivrikaya A, Aydin L. Effects of hyperthyroidism induced by L-thyroxin administration on lipid peroxidation in various rat tissues. Acta Biol Hung. 2006;57(2):157–63.
60. Mogulkoc R, Baltaci AK, Oztekin E, Aydin L, Sivrikaya A. Melatonin prevents oxidant damage in various tissues of rats with hyperthyroidism. Life Sci. 2006;79(3):311–5.

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