



RESEARCH ARTICLE

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Analysis of Morphometric and Mineral Structure Changes in Skull Bones of Male and Female Rats at Different Periods of Life*

The study was conducted to determine the rates of morphometric and mineral changes in the skull (flat) bones of female and male rats during the pre-adolescent, adolescent-adulthood, young adult, and older adult stages, and to investigate whether gender has an effect on these changes.

In this study, 40 Wistar Albino rats (20 male, 20 female) were used. Four groups were formed with 5 female 5 male animals in each group. X-ray fluorescence (XRF) measurements were performed to determine bone mineral levels. Metric measurements of the skull were taken using a digital caliper, based on 15 measurement points, while 5 osteometric measurement points were used for the mandible. IBM SPSS Statistics Version 22.0 software was used for statistical analysis.

The Ca/P ratio was statistically significant between groups and sexes ($p < 0.001$). The Zn ratio was higher in females than males in all groups, but it was observed to decrease with age from group 1 to group 4. Significant differences were found between sexes in Mg, Al, Si, P, Cl, K, Ca, and Zn in rat skulls ($p < 0.001$). In some skull measurements (LD, BL, CBL, GBOC, BS, OZB, GBN, PB), female rats in Group 2 (between adolescence and adulthood) were found to have higher values.

In conclusion, changes in morphometric measurements and mineral composition of the skull bones of rats of both sexes were revealed depending on adolescence and adulthood. It was determined that age and sex factors had an influence on these.

Key Words: Skull, rat, morphometry, XRF, minerals

Erkek ve Dişi Ratlarda Kafatası Kemiklerindeki Morfometrik ve Mineral Yapı Değişikliklerinin Yaşamın Farklı Periyotlarındaki Analizi

Çalışma, dişi ve erkek ratların ergenlik öncesi, ergenlik- erişkinlik arası, genç erişkinlik dönemi ve ileri (yaşlı) erişkinlik dönemlerinde kafatası (yassı) kemiklerindeki morfometrik ve mineral değişim oranlarını ve bunlara cinsiyetin bir etkisinin olup olmadığını ortaya koymak amacıyla yapılmıştır.

Bu çalışmada 40 Wistar Albino rat (20 erkek, 20 dişi) kullanıldı. Çalışmamızda ergenlik öncesi, ergenlik-erişkinlik arası, genç erişkin ve yaşlı erişkin olarak 4 grup oluşturuldu ve her grupta 5 dişi 5 erkek hayvan kullanıldı. Kemik mineral düzeyleri için X-ray floresan (XRF) ölçümleri yapıldı. Kafatasının metrik ölçümleri ise dijital kumpas ile; 15 adet ölçüm noktası, mandibula için ise 5 osteometrik ölçüm noktası baz alınarak yapıldı. İstatistiksel analizinde IBM SPSS Statistics Versiyon 22.0 paket programı kullanıldı.

Ca/P oranı yaşamın her periyodunda dişilerde erkeklerden daha yüksekti. Ca/P oranı en yüksek erkeklerde 1. Grupta, dişilerde ise 2. Grupta görüldü. Ca/P oranı gruplar ve cinsiyetler arasında istatistiksel olarak anlamlıydı ($p < 0.001$). Zn oranı her grupta da dişilerde erkekler göre daha yüksekti, fakat 1. Gruptan 4. Gruba kadar yaş ilerledikçe oranın düştüğü gözlemlendi. Sıçan kafatasında cinsiyetler arasında Mg, Al, Si, P, Cl, K, Ca, ve Zn ($p < 0.001$) arasında anlamlı farklar bulundu. Kafatası bazı ölçümlerinde (LD, BL, CBL, GBOC, BS, OZB, GBN, PB) 2. grupta (ergenlik- erişkinlik arası) dişi ratların daha yüksek değerlere sahip olduğu görüldü.

Sonuç olarak, rat kafatası kemiklerindeki morfometrik ölçümler ve mineral yapı değişiklikleri ergenlik ve yetişkinlik dönemleri temel alınarak her iki cinsiyette ortaya konuldu. Kafatası kemiklerindeki morfometrik ve mineral düzey farklılıklarının yaş periyotlarının ve cinsiyet faktörünün etkili olduğu belirlendi.

Anahtar Kelimeler: Kafatası, rat, morfometri, XRF, mineraller

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Introduction

The Wistar Albino rat is a species of white-furred rat belonging to the Muridae family of the Rodentia order. It is the most common laboratory animal used in scientific studies. Therefore, it has an important place in medical and biological scientific research (1). Bones are composed of organic (30-40%) and inorganic (60-70%) substances. Inorganic substances provide hardness and strength to the bone and constitute the majority of bone tissue. Organic substances, on the other hand, provide elasticity to the bone. With age, the amount of organic matter in the bone decreases, while the amount

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of inorganic matter increases (2). Organic matter consists mainly of proteins (type I collagen). Calcium and phosphorus form calcium hydroxyapatite crystals and constitute the majority of the inorganic matter. Magnesium, sodium, potassium, and fluoride are also components of bone (3, 4). Calcium is responsible for stimulating bone formation and preventing osteoporosis (5). Bone structure and microarchitecture are fundamental elements for evaluating bone strength and the mechanical properties of bone. Bone contains 26 elements in varying proportions (6). However, the minerals in bone structure are found in different proportions in each bone of the skeletal system (7, 8). These proportions can vary depending on age, sex, skeletal area, and animal species (6, 7, 9). Mineralized tissues such as bones, teeth, and horns are primary storage sites in animals. These tissues contain major elements such as calcium (Ca), phosphorus (P), magnesium (Mg), and sulfur (S), as well as trace elements such as iron (Fe), zinc (Zn), and manganese (Mn). Much of the relevant research has focused particularly on essential elements such as Ca, P, and Mg, as they play a critical role in bone metabolism (10).

X-ray fluorescence (XRF) is an analytical technique used to determine the chemical composition of various materials. These materials can be in solid, liquid, powder, filtered, or other forms. XRF analysis is also quite useful in pharmaceutical and health research (11). XRF has been a frequently used method in bioarchaeological studies since the 1980s (12, 13). Furthermore, it has been shown that XRF elemental analysis can be used to identify or distinguish animal species (8).

With recent advancements in the technique, new applications are determining the isotopic accumulation of elements such as hydrogen (H), oxygen (O), and strontium (Sr) in bones and teeth (14). The technique provides information on the major components of various biological samples, including bones (8, 15), teeth (8, 16), and horns (17).

In recent years, the application of morphometric techniques in the biomedical field has increased and has been accepted as a new approach in morphological studies (18). Morphometric studies of the skull, in particular, are very important in terms of taxonomy, intra-species and interspecies comparative anatomy (19). In rodent taxonomy, morphometric analysis of skulls has become one of the fundamental techniques (20-23). Various morphometric studies of skulls have been performed on many domestic animals and rodents such as pigs (24), dogs, cats (25), guinea pig skulls (26), and African giant rats (27). In recent years, geometric morphometric studies (28, 29) have also become popular. Although studies on the mineral structure of long (medullary) bones in rats, based on adolescence and adulthood, have been conducted (30), this is the first time such studies have been performed on flat bones using the same criteria. This will also reveal whether there is a difference between medullary and flat bones based on age and sex.

The study was conducted to determine the rates of morphometric and mineral changes in the skull (flat) bones of female and male rats during the pre-adolescent, adolescent-adulthood, young adult, and older adult stages, and to investigate whether gender has an effect on these changes.

Materials and Methods

Research and Publication Ethics: This research ethics was obtained from the Firat University Local Ethics Committee for Animal Experiments (first approved no and date 2021/20 and 08.12.2021. Revised approved no. 2026/08-12, revised date 01/04/2026).

In this study, 40 (20 male, 20 female) Wistar Albino rats (*Rattus norvegicus*) obtained from the Elazığ Veterinary Control Institute of the Ministry of Agriculture and Forestry of the Republic of Turkey were used.

In our study, 4 groups were formed as pre-adolescent (1-1.5 months), adolescent –adulthood (3-4 months), young adult (8months) and older adult (after 20 months), 5 males and 5 females animals were used in each group. The formation of the groups was based on the reports in the literatures (31, 32). Following euthanasia performed at the Firat University Experimental Research Center, animal bones were dissected and macerated (by boiling). Skull bones from all groups were separated and metric measurements were taken using a digital caliper.

Metric measurements of the skull were made using a digital caliper based on 15 measurement points (TL; Total length, CBL; Condylbasal length, BL; Basal length, DL; Dental length, GLN; Greatest length of the nasals, PL; Parietal length, FL Frontal length, VL Viscerocranium length, GBOC Greatest breadth of the occipital condyles, GNB; Greatest neurocranium breadth, GNL; Greatest neurocranium length, BS; Breadth of skull, OZB; Oral zygomatic breadth, GBN; Greatest breadth of the nasals, PB; Palatal breadth). For the mandible, 5 osteometric measurement points were used (LA; Length from angle, LCR; Length of the cheektooth row, LD; Length of the diastema, L; Length, HVR; Height of the vertical ramus) (33).

For X-ray fluorescence (XRF) measurements, scanning and mapping were performed in 70 µm diameter areas using a PANALYTICAL brand AXIOS model XRF device located at the Bilecik Şeyh Edebali University Central Research Laboratory Application and Research Center (MARAL). Samples were ground to homogenize using the loose powder method for evaluation. They were filled into 4-micron mylar film-coated sample containers and analyzed using the Omnian semi-quantitative analysis program on a 2.4 kW wavelength XRF device (34). In the XRF analysis, the percentage concentrations of the elements Mg (MgO), Al (Al₂O₃), Si (SiO₂), P (P₂O₅), Cl (Cl), K (K₂O), Ca (CaO), Fe (Fe₂O₃), Ni (NiO), Zn (ZnO), and Sr (SrO) were determined.

Statistical analysis of the data obtained at the end of the study was performed using the IBM SPSS

Statistics Version 22.0 software package. The Shapiro-Wilk test was used to test whether the assumption of normal distribution of continuous measures was met. For cases where significant comparisons were found, Bonferroni, Scheffe, and Tamhane tests were used to determine whether the within-group variances were homogeneous in pairwise comparisons of groups, provided the assumptions were met.

In cases with more than one group variable, two-way analysis of variance was used for general comparisons of continuous measures. The statistical significance level was set at 0.05 for all tests (35).

Results

The Ca/P ratio was higher in females than in males at every stage of life. The highest Ca/P ratio was observed in Group 1 in males and in Group 2 in females. The Zn ratio was higher in females than in males in all groups, but it was observed to decrease with age from Group 1 to Group 4 (Table 1).

In rat skulls, significant differences were found between genders in Mg, Al, Si, P, Cl, K, Ca, and Zn

($p < 0.001$). Significant differences were also found between groups and genders in Mg, Si, P, Cl, K, Ca, and Zn ($p < 0.001$). Similarly, the Ca/P ratio was statistically significant between groups and genders ($p < 0.001$).

Descriptive statistics and p -values for the rat animal group according to measurement points are given in Table 2. Accordingly, for the LA, L, and HVR measurement points, the group, sex, and group change within sex are statistically significant ($p < 0.050$). That is, developmentally (from group 1 to group 4), the average change is increasing in both females and males. The average measurement value of male rats is higher than that of females. In particular, while no significant difference was observed between females and males in mandibular morphometric measurements until adulthood (group 3), the difference widened in favor of male rats from group 3 onwards. Furthermore, the developmental change in the male animal group differs significantly from that in the female animal group, and this difference stems from the higher mean of males. For the LCR measurement point, both group and sex changes are significant ($p < 0.050$). For the LD variable, only group change is highly significant ($p < 0.001$).

Table 1. Concentration of rat mandibular elements by group (XRF results)

Elements	Gender	Group 1 (mean $\pm$ SD) (%)	Group 2 (mean $\pm$ SD) (%)	Group 3 (mean $\pm$ SD) (%)	Group 4 (mean $\pm$ SD) (%)	p value for group	p value for gender	p value for group * gender
Mg	Male	0.056 $\pm$ 0.006	0.067 $\pm$ 0.006	0.106 $\pm$ 0.005	0.102 $\pm$ 0.005	<0.001	<0.001	<0.001
	Female	0.044 $\pm$ 0.002	0.035 $\pm$ 0.003	0.054 $\pm$ 0.003	0.066 $\pm$ 0.002			
Al	Male	0.002 $\pm$ 0.001	0.005 $\pm$ 0.001	0.000 $\pm$ 0.000	0.002 $\pm$ 0.001	0.593	<0.001	0.002
	Female	0.004 $\pm$ 0.002	0.005 $\pm$ 0.002	0.000 $\pm$ 0.000	0.000 $\pm$ 0.000			
Si	Male	0.013 $\pm$ 0.002	0.024 $\pm$ 0.002	0.013 $\pm$ 0.002	0.008 $\pm$ 0.002	0.049	<0.001	<0.001
	Female	0.019 $\pm$ 0.002	0.019 $\pm$ 0.002	0.015 $\pm$ 0.002	0.009 $\pm$ 0.002			
P	Male	7.074 $\pm$ 0.012	8.377 $\pm$ 0.045	10.248 $\pm$ 0.017	9.708 $\pm$ 0.465	<0.001	<0.001	<0.001
	Female	6.628 $\pm$ 0.002	7.207 $\pm$ 0.003	7.973 $\pm$ 0.002	7.522 $\pm$ 0.002			
Cl	Male	0.160 $\pm$ 0.005	0.036 $\pm$ 0.004	0.058 $\pm$ 0.004	0.055 $\pm$ 0.004	<0.001	<0.001	<0.001
	Female	0.057 $\pm$ 0.002	0.035 $\pm$ 0.002	0.135 $\pm$ 0.002	0.054 $\pm$ 0.002			
K	Male	0.654 $\pm$ 0.007	0.180 $\pm$ 0.007	0.261 $\pm$ 0.007	0.295 $\pm$ 0.008	<0.001	<0.001	<0.001
	Female	0.283 $\pm$ 0.002	0.256 $\pm$ 0.002	0.622 $\pm$ 0.002	0.267 $\pm$ 0.001			
Ca	Male	23.285 $\pm$ 0.019	26.139 $\pm$ 0.009	31.635 $\pm$ 0.028	30.574 $\pm$ 0.018	<0.001	<0.001	<0.001
	Female	22.053 $\pm$ 0.002	24.095 $\pm$ 0.002	25.349 $\pm$ 0.007	24.402 $\pm$ 0.002			
Fe	Male	0.083 $\pm$ 0.003	0.073 $\pm$ 0.004	0.069 $\pm$ 0.047	0.044 $\pm$ 0.004	0.584	0.252	0.463
	Female	0.096 $\pm$ 0.045	0.084 $\pm$ 0.084	0.077 $\pm$ 0.002	0.076 $\pm$ 0.002			
Ni	Male	0.018 $\pm$ 0.003	0.014 $\pm$ 0.003	0.018 $\pm$ 0.003	0.036 $\pm$ 0.037	0.009	0.085	0.143
	Female	0.018 $\pm$ 0.002	0.010 $\pm$ 0.002	0.000 $\pm$ 0.000	0.011 $\pm$ 0.001			
Zn	Male	0.068 $\pm$ 0.003	0.067 $\pm$ 0.003	0.036 $\pm$ 0.051	0.044 $\pm$ 0.003	<0.001	<0.001	<0.001
	Female	1.192 $\pm$ 0.002	0.632 $\pm$ 0.314	0.244 $\pm$ 0.001	0.134 $\pm$ 0.002			
Sr	Male	0.019 $\pm$ 0.004	0.019 $\pm$ 0.002	0.018 $\pm$ 0.004	0.015 $\pm$ 0.002	0.376	0.278	0.286
	Female	0.054 $\pm$ 0.076	0.017 $\pm$ 0.002	0.015 $\pm$ 0.002	0.014 $\pm$ 0.002			
Ca/P	Male	3.296 $\pm$ 0.024	3.204 $\pm$ 0.032	3.172 $\pm$ 0.129	3.100 $\pm$ 0.016	<0.001	<0.001	<0.001
	Female	3.305 $\pm$ 0.047	3.613 $\pm$ 0.002	3.181 $\pm$ 0.001	3.261 $\pm$ 0.035			

Table 2. Descriptive statistics and *p*-values for the rat mandible group based on measurement points

Metric measurements	Gender	Group 1 (mean $\pm$ SD) mm	Group 2 (mean $\pm$ SD) mm	Group 3 (mean $\pm$ SD) mm	Group 4 (mean $\pm$ SD) mm	<i>p</i> value for group	<i>p</i> value for gender	<i>p</i> value for group * gender
LA	Male	18.330 $\pm$ 0.310	21.356 $\pm$ 0.447	29.400 $\pm$ 2.307	31.828 $\pm$ 2.116	<0.001	<0.001	<0.001
	Female	16.956 $\pm$ 0.740	20.630 $\pm$ 0.676	23.642 $\pm$ 1.242	27.194 $\pm$ 0.405			
LCR	Male	7.610 $\pm$ 0.870	7.922 $\pm$ 0.270	7.406 $\pm$ 0.410	7.610 $\pm$ 0.150	0.020	0.002	0.093
	Female	7.302 $\pm$ 0.180	7.036 $\pm$ 0.460	6.476 $\pm$ 0.668	7.662 $\pm$ 0.467			
L	Male	12.296 $\pm$ 0.839	14.040 $\pm$ 1.321	15.654 $\pm$ 0.401	17.716 $\pm$ 1.526	<0.001	0.007	0.004
	Female	11.062 $\pm$ 0.335	12.692 $\pm$ 0.421	13.808 $\pm$ 0.429	15.782 $\pm$ 1.209			
LD	Male	5.562 $\pm$ 0.471	5.672 $\pm$ 0.395	6.226 $\pm$ 0.362	8.348 $\pm$ 1.171	<0.001	0.844	0.201
	Female	4.982 $\pm$ 0.268	5.490 $\pm$ 0.292	6.104 $\pm$ 0.433	8.102 $\pm$ 1.201			
HVR	Male	8.618 $\pm$ 0.205	10.550 $\pm$ 0.645	13.438 $\pm$ 0.513	15.308 $\pm$ 1.269	<0.001	<0.001	0.029
	Female	8.096 $\pm$ 0.261	8.588 $\pm$ 0.387	11.580 $\pm$ 0.320	13.426 $\pm$ 0.363			

Table 3. Descriptive statistics and *p*-values for group and sex based on head measurement points in rats

Metric measurements	Gender	Group 1 (mean $\pm$ SD) mm	Group 2 (mean $\pm$ SD) mm	Group 3 (mean $\pm$ SD) mm	Group 4 (mean $\pm$ SD) mm	<i>p</i> value for group	<i>p</i> value for gender	<i>p</i> value for group * gender
TL	Male	32.148 $\pm$ 1.174	37.078 $\pm$ 0.616	49.702 $\pm$ 2.527	50.504 $\pm$ 0.444	<0.001	<0.001	<0.001
	Female	32.632 $\pm$ 0.733	37.072 $\pm$ 0.339	42.004 $\pm$ 1.184	46.958 $\pm$ 0.988			
BL	Male	29.024 $\pm$ 0.915	33.756 $\pm$ 0.539	47.616 $\pm$ 2.226	49.058 $\pm$ 0.826	<0.001	<0.001	<0.001
	Female	29.320 $\pm$ 0.434	34.408 $\pm$ 0.809	39.486 $\pm$ 0.898	45.342 $\pm$ 1.786			
CBL	Male	31.272 $\pm$ 1.354	35.506 $\pm$ 0.653	48.942 $\pm$ 2.322	49.726 $\pm$ 1.006	<0.001	<0.001	<0.001
	Female	31.236 $\pm$ 0.804	36.430 $\pm$ 1.002	40.806 $\pm$ 0.386	45.570 $\pm$ 1.126			
DL	Male	17.694 $\pm$ 1.055	19.708 $\pm$ 0.286	25.636 $\pm$ 1.478	26.840 $\pm$ 0.610	<0.001	<0.001	<0.001
	Female	17.170 $\pm$ 0.611	20.340 $\pm$ 0.630	22.332 $\pm$ 0.437	24.630 $\pm$ 0.514			
GLN	Male	11.440 $\pm$ 0.462	15.284 $\pm$ 0.835	21.114 $\pm$ 1.086	20.592 $\pm$ 0.324	<0.001	<0.001	<0.001
	Female	12.208 $\pm$ 0.516	15.134 $\pm$ 1.040	16.930 $\pm$ 0.677	18.652 $\pm$ 1.875			
PL	Male	6.916 $\pm$ 0.565	7.462 $\pm$ 0.450	9.150 $\pm$ 1.490	8.552 $\pm$ 1.523	0.003	0.242	0.335
	Female	7.280 $\pm$ 0.457	7.472 $\pm$ 0.344	8.320 $\pm$ 0.838	7.594 $\pm$ 0.971			
FL	Male	11.458 $\pm$ 0.662	12.702 $\pm$ 0.470	15.906 $\pm$ 2.250	16.370 $\pm$ 0.382	<0.001	0.129	0.039
	Female	10.914 $\pm$ 0.504	12.652 $\pm$ 0.567	13.332 $\pm$ 0.649	17.068 $\pm$ 2.383			
VL	Male	10.298 $\pm$ 1.148	14.552 $\pm$ 0.389	20.232 $\pm$ 0.912	22.118 $\pm$ 1.479	<0.001	<0.001	<0.001
	Female	11.558 $\pm$ 0.741	14.306 $\pm$ 0.264	16.258 $\pm$ 0.696	18.772 $\pm$ 0.931			
GBOC	Male	6.330 $\pm$ 0.709	6.624 $\pm$ 0.693	6.186 $\pm$ 0.614	6.426 $\pm$ 0.542	0.101	0.066	0.139
	Female	6.008 $\pm$ 0.508	6.808 $\pm$ 0.471	6.962 $\pm$ 0.481	7.308 $\pm$ 0.910			
GBEAM	Male	13.636 $\pm$ 0.483	15.618 $\pm$ 0.162	18.572 $\pm$ 1.093	18.946 $\pm$ 1.205	<0.001	0.001	0.001
	Female	14.194 $\pm$ 0.390	15.246 $\pm$ 0.130	16.164 $\pm$ 0.877	17.608 $\pm$ 0.781			
GNB	Male	12.202 $\pm$ 0.399	13.936 $\pm$ 0.319	15.628 $\pm$ 1.452	17.010 $\pm$ 1.145	<0.001	0.041	0.070
	Female	12.670 $\pm$ 0.335	13.734 $\pm$ 0.684	14.486 $\pm$ 0.997	15.578 $\pm$ 0.794			
BS	Male	17.044 $\pm$ 0.325	18.488 $\pm$ 0.355	23.896 $\pm$ 1.228	24.758 $\pm$ 1.642	<0.001	<0.001	<0.001
	Female	17.036 $\pm$ 0.514	19.218 $\pm$ 0.816	20.800 $\pm$ 0.225	22.508 $\pm$ 1.024			
OZB	Male	6.276 $\pm$ 0.173	6.996 $\pm$ 0.482	10.336 $\pm$ 0.915	10.506 $\pm$ 0.929	<0.001	0.540	0.181
	Female	5.994 $\pm$ 0.237	7.330 $\pm$ 0.402	8.424 $\pm$ 0.027	11.262 $\pm$ 3.700			
GBN	Male	4.972 $\pm$ 0.307	5.592 $\pm$ 0.146	7.484 $\pm$ 0.330	7.828 $\pm$ 0.345	<0.001	0.053	0.027
	Female	4.938 $\pm$ 0.166	5.868 $\pm$ 0.800	6.610 $\pm$ 0.338	7.386 $\pm$ 0.548			
PB	Male	3.512 $\pm$ 0.320	3.610 $\pm$ 0.256	4.178 $\pm$ 0.644	5.504 $\pm$ 0.886	<0.001	0.995	0.372
	Female	3.526 $\pm$ 0.217	3.912 $\pm$ 0.430	4.286 $\pm$ 0.249	5.084 $\pm$ 0.259			

According to Table 3, the measurement points TL, BL, CBL, DL, GLN, VL, and BS are statistically highly significant ($p < 0.001$) in terms of group, gender, and gender-based group variation. Considering all measurement points for these variables, the average developmental change (from group 1 to group 4) is increasing in both females and males.

Furthermore, while the mean developmental change was similar for male and female rats in groups 1 and 2, male rats in groups 3 and 4 showed a higher mean than female rats. The PL variable showed a significant difference only between groups ($p = 0.003$). In the GBN variable, the findings showed that male animals had a higher average value than females, and this difference was statistically significant ($p = 0.041$).

In some skull measurements (LD, BL, CBL, GBO, BS, OZB, GBN, PB), female rats in group 2 (between adolescence and adulthood) were found to have higher values. Morphometric measurements showed that while there was no significant difference in developmental changes between sexes in rats during the period up to puberty (group 1), some parameters showed faster development in females during the puberty-adulthood period (group 2), and from adulthood onwards, there was a faster increase in skull bone size in male rats.

Discussion

The results presented in this study provide valuable information regarding the morphometric and XRF characteristics of the skull bones of rats of different age groups and genders.

Although numerous morphometric and mineral structure studies have been conducted on the bones of laboratory animals, it is important to examine the effects of pre-puberty, puberty, and adulthood on bone development, and even how these effects differ between genders, by focusing on these specific life stages.

Regarding the effects of age and gender criteria on bone mineral levels; it has been reported that sex is a significant factor in bone element ratios (8, 37).

However, it was reported that there was no difference between genders in Fe and Zn ratios, and only Fe and Zn elements were common in the 48 bones examined (6). Ioannidou (37) reported that in sheep, males had denser bones, while in pigs, sex differences were age-dependent, with females showing higher density when they reached adulthood. Maciejewska et al. (38) also found that Zn and Sr were high in newborn rats and decreased with age, while Fe was slightly higher in 7-day-old rats compared to 14 and 28-day-old rats. Yilmaz et al. (30) reported that the levels of the main bone minerals, Ca and P, were higher in females until adulthood, and higher in males in adulthood; however, the elements Fe, Zn, and Sr were higher in young animals compared to adults. In addition, a study conducted by Baygeldi et al. (26) on guinea pig skulls reported that Zn and Sr elements were higher in young

animals, with a proportional decrease observed as age progressed. Yilmaz et al. (36) found that the Zn ratio in guinea pig femur bone was significantly higher in females than in males throughout all life periods.

In our study, no significant differences in Fe and Sr levels were observed based on sex or age; however, Zn levels were found to be higher in females than in males at every life stage, though they decreased proportionally with advancing age in both sexes. Similarly, while it has been reported that Fe, Zn, and Sr elements are higher in young animals than adults in the long bones of the same animals (30), in this study this characteristic was only observed for Zn mineral in the flat bones of the same animals.

Buddhachat et al. (7) examined the mineral content of humerus bones in 14 different species (cat, dog, elephant, horse, human, monkey, dolphin, hyena, lion, tapir, pig, sheep, buffalo, and tiger) using XRF analysis; they reported that the average humerus bone Ca percentages ranged from 16.083 (pigs) to 24.727 (horses), and the P percentages ranged from 3.917 (pigs) to 11.448 (cats). Nganvongpanit et al. (6) reported Ca as 21.05% and P as 8.05% in dogs.

Furthermore, Nganvongpanit et al. (8) reported Ca levels as 23.303%, 21.872%, and 21.512% in humans, elephants, and dogs, respectively, and P levels as 10.024%, 8.151%, and 8.885%. Yilmaz et al. (30) found the average percentage of Ca in the femur bone of adult rats to be 25.555 in males and 24.745 in females; they also found the average percentage of P to be 7.672 in males and 7.044 in females.

According to the results of this study, the percentage of calcium (Ca) in skull bones was determined to be 30.574% in adult males and 24.402% in adult females, while the percentage of phosphorus (P) was found to be 9.708% in adult males and 7.522% in adult females. These results show ratios consistent with literature reports. However, unlike the report given for long bones in the same subjects (30), the ratios of Ca and P in male skull bones appear to be higher than in long bones.

Yilmaz et al. (30) reported that calcium levels in the femurs of rats increased rapidly in females during puberty (group 2) and decreased rapidly in young adults (group 3), while the greatest increase in males was observed during young adults (group 3). In this study, it was observed that the Ca ratio reached its highest value in young adults (group 3) for both genders.

Although the Ca/P ratio in bones is not sufficient to definitively determine bone strength, it is an important parameter for osteoporosis (39, 40). Nganvongpanit et al. (6) found that among the 48 dog bones they examined, the highest Ca/P ratio was 4.09 in the patella, the lowest was 2.22 in the left carpal bone, and the average was 2.82. The Ca/P ratio has been reported as 2.21 in humans (41, 42), 1.69 in cattle (9), 2.17 in pigs (43), 1.28 in rabbits, and 2.12 in rats (44). On the other hand, Buddhachat et al. (7) found that the average Ca/P

ratio in 14 types of humerus bones was 2.66. In addition, Legros et al. (9) reported a Ca/P ratio of 2.18 in rats. Yılmaz et al. (30) reported values of 3.33 in adult male rats and 3.51 in females. However, in a study conducted by Baygeldi et al. (26) on the skulls of guinea pigs, it was noted that the Ca/P ratio in the guinea pigs ranged from 3.085 to 3.428 throughout the developmental period. In this study, the ratio was determined to be 3.10 in adult male rats and 3.26 in female rats in rat skull bones. These results are similar to those reported by Yılmaz et al. (30) and Baygeldi et al. (26), and higher than other reports. These differences in values may be due to differences in measurement techniques.

Legros et al. (9) also reported that the Ca/P ratio is lower in young rats, increases with age, and has ratios of 1.51 at birth, 1.60 at 30 days of age, and 1.65 at 1 year of age in rats. Our study results differ from those presented in this report.

Some authors (45, 46) have reported that, when examining the relationship between morphometric measurements of the bones of male and female rats, male rats exhibited higher measurements than females in both the control and experimental groups. Baygeldi et al. (26) reported that the measurement values of male guinea pigs were higher than those of female guinea pigs, that body development increased with age, and that gender change did not show a statistically significant difference for all measurement points ($p>0.05$). In the present study as well, morphometric measurements were found to be higher in males. Table 3 shows that the gender factor was significant ($p<0.001$) for the first five parameters.

In their study, Anbuhl et al. (47) examined the morphological development of the skull from birth to adulthood in 63 guinea pigs (*Cavia porcellus*) and noted that the skull grew significantly during the first 8 weeks of life. Baygeldi et al. (30) observed that the total skull length gradually increased from the first group to the fourth group. In this study, it was found that skull length (TL) in rats increased gradually with age in both sexes, but that this increase was more rapid in males as they reached adulthood.

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Cox and Jeffery (48) reported total skull measurements of adult squirrels, guinea pigs, and rats as 48.22 mm, 57.51 mm, and 43.40 mm, respectively. Baygeldi et al. (26) found the total skull measurement in adult guinea pigs to be 62.564 mm in males and 63.072 mm in females. In this study, rat skull length (TL) was found to be 50.50 mm in elderly males and 46.95 mm in elderly females.

Olude and Olopade (49) reported maximum mandibular height and length in African giant rats as 2.13 cm and 3.62 cm, respectively. Furthermore, they reported that there was a statistically significant relationship ($p<0.05$) between the two sexes in all four morphometric parameters. In the present study as well, a statistically significant association with gender was observed for the LA and HVR parameters ($p<0.001$).

In conclusion, morphometric measurements and mineral composition changes in rat skull bones were revealed in both sexes based on adolescence and adulthood.

It was determined that age periods and gender factors influenced the differences in morphometric and mineral composition of the skull bones. We believe this study will be an important resource for future age-related bone structure change studies and drug development research.

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Conflict of Interest Statement

The authors declare that there are no potential conflicts of interest regarding the research, authorship, and/or publication of this article.

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