



## Bone mineral density of ancient Anatolian populations

### Eski Anadolu toplumlarında kemik mineral yoğunluğu

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#### Objective

Bone mineral density (BMD) is strongly related to sex, age and genetics. Ancient Anatolian populations' BMD measurements may give insight into today's Mediterranean and European populations' BMD profiles. Therefore, BMD of ancient Anatolian populations was measured and age- and sex-related differences were assessed.

#### Materials and methods

Proximal femoral BMD of 149 males and 106 females of ages 15 to 65.5 years (mean age of 41±11 years) was measured using a dual energy X ray densitometer. The skeletons were excavated from six different regions of Anatolia (West, East, Mediterranean, Aegean, Marmara and Central Anatolia) and dated to Chalcolithic, Bronze, Iron, Hellenistic, Roman, Medieval ages and the nineteenth century.

#### Results

Bones of individuals of 45 years and above had significantly lower BMD. Female bones BMD declined significantly with age, where males BMD remained relatively constant and slightly higher than that of the females. The BMD of the left femur was greater than the right in both sexes.

#### Discussion

This study presented variations in BMD between the two sexes and with age. Females had lower BMD levels than males in ancient Anatolian populations. Differences were more obvious as age increased.

**Key words:** Bone mineral density, femur, Anatolia

#### Amaç

Kemik mineral yoğunluğu (KMY) cinsiyet, yaş ve genetik yapıyla doğrudan ilişkilidir. Eski Anadolu toplumlarında KMY örüntüsünün günümüz Akdeniz ve Avrupa toplumlarına ışık tutabileceği varsayılmaktadır. Çalışmanın amacı çift X-ışınlu yoğunluk ölçüm cihazıyla (DXA) farklı dönemlerdeki Anadolu toplumlarında yaş ve cinsiyete bağlı olarak KMY'ni ölçmektir.

#### Gereçler ve yöntem

15-65.5 yaşları arasında (ortalama yaş 41±11) toplam 149 erkek ve 106 kadının proksimal femurunda DXA ile KMY ölçülmüştür. Ölçülen kemikler, 6 farklı dönem (Kalkolitik, Bronz Demir, Helenistik, Roma, Orta çağ ve 19. yüz yıl) ve 6 farklı yöre (Batı, Doğu, Akdeniz, Ege, Marmara ve İç Anadolu) kazılarında elde edilmiştir.

#### Bulgular

Kemik mineral yoğunluğu 45 yaş ve üstü bireylerde daha düşüktür. Kadınlarda KMY yaş ile birlikte azalırken, erkeklerde daha duragan ve kadınlardan daha yüksek değerlerdedir. Her iki cinsiyette de sol femur KMY sağa göre daha yüksektir.

#### Çıkarımlar

Bu çalışma eski Anadolu toplumlarında KMY'nun cinsiyet ve yaş açısından varyasyon gösterdiğini ortaya koymuştur. Eski Anadolu toplumlarında, toplam kemik mineral yoğunluğu kadınlarda erkeklere göre daha az bulunmuştur. Kadınlarda yaş ilerledikçe KMY erkeklere oranla daha belirgin düşmektedir.

**Anahtar sözcükler:** Kemik mineral yoğunluğu, Anadolu, femur

Osteoporosis is a metabolic disease in which the bone mineral density (BMD) and quality of bone are reduced.<sup>[1]</sup> As BMD decreases silently and progressively, the risk of fracture increases significantly. In this respect, osteoporosis has become a growing health concern around the globe and is an extensive area of skeletal research. Today, osteoporosis is a major cause of morbidity and mortality,<sup>[2]</sup> but there is a marked interpopulation variation in the severity of the condition. Populations of European origin are among those most at risk for osteoporosis.<sup>[3]</sup> However, there is also considerable variation between different European groups. The reasons for these patterns are incompletely understood, but both genetic and environmental factors are likely to play important roles.<sup>[4, 5]</sup> The importance of osteoporosis today has stimulated interest in its occurrence in earlier populations. Two studies<sup>[6, 7]</sup> have been conducted on excavated skeletal remains around the world. Knowing about the health of human populations is important to understand their history and lifestyles.

Anatolia (Asia Minor), which is currently within the borders of Turkey, has been and still is a bridge connecting Asia to Europe. Archaeological populations of Anatolia include those that give roots to the current Mediterranean and European civilizations. Although there is much interest on osteoporosis in ancient societies, two studies<sup>[6, 7]</sup> have measured BMD in archaeological populations. These studies<sup>[6, 7]</sup> focused on osteoporotic bone changes in excavated skeletal remains around the world, however did not include Anatolia. Until now, archaeological bones have also not been studied using dual energy X-ray densitometer (DXA).

The aims of this study were (1) to measure the proximal femoral BMD among ancient Anatolian populations and to discern if there are differences between males and females using DXA, and (2) to compare different populations of different geographical areas of Anatolia. We expect that BMD in archaeological populations from Anatolia will present differences between males and females and will decrease with age, as is true in contemporary populations.

## MATERIALS AND METHODS

### *Skeletal Material*

Well-preserved human femoral bones from eleven sites of Anatolia (Figure 1) are part of the paleoanthropological collection of the Department of Anthropology, Faculty of Letters, University of Ankara (Dr. Gulec's collection). The study sample consisted of 149 males and 106 females ranging in age from 15 to 65.5 years. The Chalcolithic series included 5 femurs (4 males and 1 female) from the sites of Tilkitepe and Yümüktepe. Bronze Age samples include 3 femurs

from Babaköy (1 male and 2 females) and Zankhöyük (1 female) skeletal remains. Iron Age collections consisted of 25 complete adult femurs (16 males and 9 females) from Karagündüz, Gordion and Yazılıkaya. The Hellenistic series included materials from the Klazomenai, Börükçü, Muğla and Iaom sites (10 femurs, 8 males and 2 females) sites. Classical Roman period skeletal remains were excavated from Sardis and Datça (13 femurs, 7 males and 6 females). Medieval period collections included 184 adult femurs (104 males and 80 females) excavated from Karagündüz, Dilkaya, Panaztepe, Topaklı, Aşvankale and Vankalesi sites. Nineteenth century skeletal remains were excavated from Kelenderis, and consist of 15 femurs (11 males and 4 females). Only the adult femurs with closed epiphysis were studied. Sex of individuals was assessed using the conventional pelvic and skull morphological criteria.<sup>[8]</sup> Age categories included: Juveniles/Young Adults (15–24.9 years), Adults (25–44.9 years), and Mature Adults (45+ years). The femurs were chosen for examination because of their abundance and good preservation and each individual's right and left femurs were examined. Broken femurs and specimens that showed signs of trauma were excluded. Integrity of specimens was an important criterion in selection; significant loss of trabecular bone post-mortem affects the density of the sample. Poor storage technique may cause loss of cortical bone exposing the more delicate trabecular bone, which will subsequently crumble.



Figure 1. Location of investigated Anatolian sites.

### *Dual Energy X-ray Densitometry (DXA)*

Dual energy X-ray densitometry studies were performed at the Middle East Technical University Medical Center, Radiodiagnostic Unit, Ankara, Turkey. BMD of the femurs was measured in anatomic position by immersing them in water. The calibration medium for DXA was pure water. Density of water was calculated as 0 g/cm<sup>2</sup>. Anatomical neutral position of the femur during measurements was maintained by supporting the neck and head of the femur with a sponge that did not affect the BMD. The condyles were parallel to the table and the neck and head was at its neutral anteversion. All BMD measurements

were performed on the same Lunar DPX densitometer (Lunar, Madison, WI), using Lunar version 4.6d software. The densitometer calibration was performed daily using a phantom provided by the manufacturer. The national distributor and technical service of the device controlled the accuracy of the system using the peak test, air matrix test, limit switch test, machinery step control, static counter, beam distribution percentage, phantom measurements, and standard error, for both hardware and software, every three months. The setting of the system at measurements was 76 kVp and 150.0  $\mu$ A. The proximal femur (femoral neck, Ward's triangle, trochanter, total femur) was measured during the study. Only total femur BMD values were expressed as area BMD ( $\text{g}/\text{cm}^2$ ) as femoral neck, Ward's triangle and trochanter values were in line with the total femur values.

### Statistics

Data as analyzed using the Statistical Package for the Social Sciences (SPSS) version 13.0. After calculating the standard descriptive statistics, t-test was applied. The differences in BMD among the three age groups identified were assessed with analysis of variance. The effects of sex, different population, and age on osteoporosis and the interaction between these factors were determined with two-way analysis of variance.

## RESULTS

The mean estimated age of the samples was  $41.0 \pm 11.0$  years. Males and females exhibited similar mean ages, 40 and 41 years respectively. Figure 2 shows the age distribution by sex. Many measured individuals consisted the 25-45 age group.

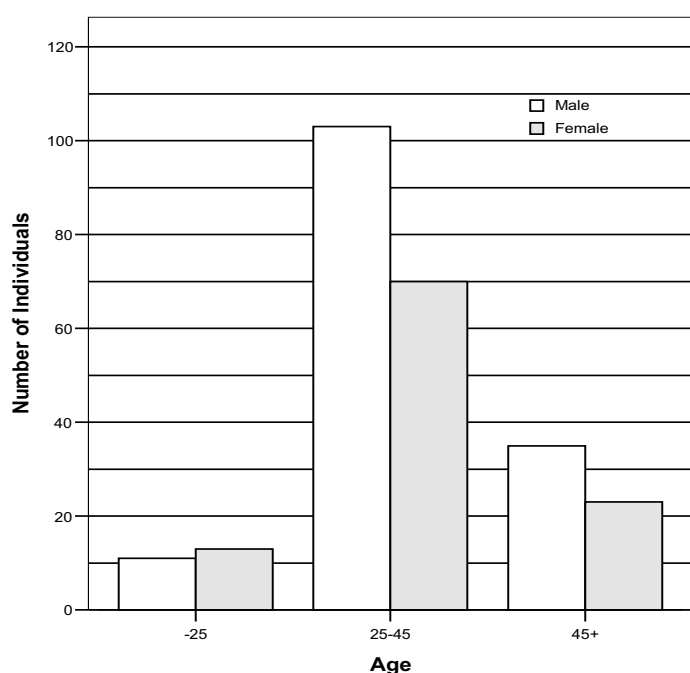


Figure 2. Age distribution by sex.

In the younger age group, there was no significant difference between genders in terms of bone mineral density in the proximal femur, although the difference increased with age. Overall, the highest BMD levels were observed in the 15-24.9 age groups for both genders. However, left femur TBMD levels peak in the 25-45 age group for both genders (Table 1).

In Table 2, results indicate that females presented a significant age-related decline in Right BMDN, Right BMDW, Right TBMD and Left BMDT where males did not present significant differences. In general, there was no left and right TBMD tendency in both sexes. Total BMD was different in different regions of Anatolia for both genders (Table 3).

In Figures 3 and 4, right and left TBMD are plotted in Juveniles/Young Adults and the regression lines are drawn for each gender. The right and left TBMD decreased with age. In general, the right and left TBMD values for males were higher than the values for females.

## DISCUSSION

This study shows that BMD decreased with age in ancient Anatolian civilizations in both genders. Average BMD of females was lower than that of males at all ages. A significant change between genders was only reported at ages above 45 years. Left TBMD values were generally greater than those of the right TBMD in both sexes. In today's clinical practice, femoral BMD is measured at the non-dominant side of the individual, which is usually the left hip region. For males that live in Anatolia today, BMD values present similar patterns;<sup>[9]</sup> they decrease with age. However, the differences between the left and right femur of today's young male and female individuals are not significant.<sup>[10]</sup> Further emphasis should be given to identify the dominant and non-dominant BMD differences in ancient populations.

Limitations of the current study included the small sample sizes of femoral bones when distributed across archaeological sites (particularly those within age categories), the fragmentary condition of samples and the low number of samples from earlier (Bronze age and Chalcolithic) populations. In this study, the sample sizes are large enough to assess general osteoporosis rates in the past Anatolian populations. One other limitation of this study was the possible corrosion of the macro and microanatomy of the measured bone specimens. However, as all measured specimens were excavated the chances of deterioration would not affect group comparisons. While comparing the BMD values of ancient populations to living individuals, the data should be taken cautiously. Finally, the mechanical strength of the specimens was not measured.

Bennike and Bohr<sup>[11]</sup> examined femoral bone

**Table 1.** Descriptive Statistical Results by Age and Sex.

Age Group		Male			Female			P
		N	Mean	Std. Deviation	N	Mean	Std. Deviation	
15-24.9	Right BMDN	6	1.062	0.131	8	0.882	0.126	0.575
	Right BMDW	6	0.954	0.192	8	0.839	0.202	0.505
	Right BMDT	6	0.925	0.255	8	0.799	0.179	0.513
	Right TBMD	6	1.152	0.182	8	0.956	0.169	0.958
	Left BMDN	7	0.894	0.398	9	0.849	0.166	0.295
	Left BMDW	7	0.827	0.436	9	0.730	0.166	0.131
	Left BMDT	7	0.804	0.474	9	0.736	0.149	0.121
	Left TBMD	7	1.041	0.337	9	0.917	0.146	0.188
25-44.9	Right BMDN	68	0.984	0.342	41	0.826	0.219	0.086
	Right BMDW	68	0.888	0.429	41	0.723	0.263	0.071
	Right BMDT	68	0.921	0.393	41	0.767	0.277	0.208
	Right TBMD	68	1.089	0.357	41	0.919	0.218	0.116
	Left BMDN	66	1.243	1.486	46	0.797	0.264	0.034
	Left BMDW	66	1.170	1.605	46	0.699	0.325	0.034
	Left BMDT	66	1.211	1.564	46	0.762	0.304	0.025
	Left TBMD	66	1.394	1.525	46	1.126	1.469	0.467
45+	Right BMDN	18	0.814	0.218	15	0.672	0.187	0.086
	Right BMDW	18	0.710	0.275	15	0.518	0.163	0.004
	Right BMDT	18	0.810	0.294	15	0.602	0.133	0.001
	Right TBMD	18	0.951	0.269	15	0.758	0.152	0.001
	Left BMDN	23	0.784	0.279	14	0.654	0.146	0.044
	Left BMDW	23	0.647	0.335	14	0.516	0.148	0.029
	Left BMDT	23	0.748	0.463	14	0.574	0.109	0.032
	Left TBMD	23	0.965	0.275	14	0.749	0.137	0.019

Significance at  $P \leq 0.05$ , BMDN, bone mineral density at femur neck; BMDW, bone mineral density at Ward's triangle; TBMD, Total bone mineral density; BMDT, bone mineral density at femur trochanter.

**Table 2.** Results of analysis of variance (ANOVA) for BMDN, BMDW, BMDT, TBMD, BMDN vs. age

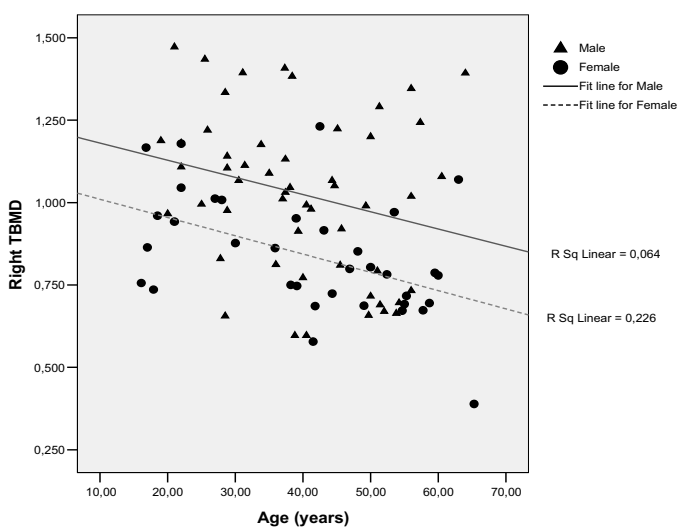
	Analysis of variance						
	Male			Female			
	Mean Square	F	Sig.	Mean Square	F	Sig.	
Right BMDN	0.237	2.444	0.093	0.161	3.836	0.027	
Right BMDW	0.249	1.613	0.205	0.336	5.903	0.005	
Right BMDT	0.087	0.643	0.528	0.167	2.833	0.067	
Right TBMD	0.156	1.413	0.249	0.166	4.108	0.021	
Left BMDN	1.936	1.244	0.293	0.135	2.432	0.096	
Left BMDW	2.320	1.265	0.287	0.226	3.093	0.052	
Left BMDT	1.938	1.094	0.339	0.222	3.697	0.030	
Left TBMD	1.705	1.042	0.357	0.824	0.549	0.580	

Significance at  $P \leq 0.05$ .

**Table 3.** Mean values and comparison of individuals buried in the different regions.

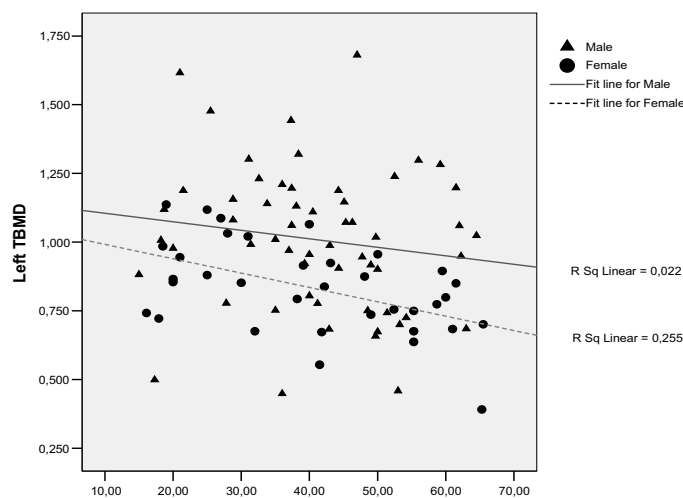
SEX		Chalcolithic	Bronze	Iron	Hellenistic	Roman	Middle	19 <sup>th</sup> C	F	Significance
MALE	Right TBMD	1.24	1.14	1.30	1.30	1.32	1.01	1.35	3.72	0.003
	Left TBMD	1.21	1.13	1.24	1.05	1.67	0.98	1.19	3.76	0.002
FEMALE	Right TBMD	-	1.18	1.05	1.13	1.20	0.84	1.04	4.31	0.002
	Left TBMD	1.12	1.23	1.02	-	1.20	0.82	0.97	4.88	0.001

Significance at  $P \leq 0.05$ .

**Figure 3.** The plot of Right TBMD by age and sex.

mineral content in Danish skeletons from three periods dating from 4200BC to 1536AD using a dual photon scanner. The authors found bone mineral content values to be higher in the earliest Neolithic Age (4200-1800 BC) group compared to the later populations. Lees et al.<sup>[12]</sup> also examined femoral BMD using DXA in female archaeological remains from Spitalfields, England dated between 1729 and 1852, but found no evidence of premenopausal bone loss and less severe postmenopausal loss compared to modern females. Another BMD study by Ekenman et al.<sup>[13]</sup> of medieval skeletons from Stockholm dated between 1300 and 1530 AD found an absence of low bone density in the older age groups, and a higher diaphyseal bone density in the lower extremities as compared to modern reference values. Findings of these studies were in line with our study as we found similar BMD values among young adults and adults.

In a recent study, cortical bone loss has been examined in the medieval British skeletal population of Wharram Percy using an identical method to ours.<sup>[14]</sup> Mays<sup>[14]</sup> found significantly lower values in females as compared to males, and again found a significant difference in bone loss between the young and old female age categories that is similar to bone loss

**Figure 4.** The plot of Left TBMD by age and sex.

reported in modern European subjects. The findings of that study were in line with the current study demonstrating a significant loss of BMD of female subjects with age. A subsequent study of cortical loss and assessment of BMD using DXA in the femur found a similar pattern of loss.<sup>[15]</sup> Mays<sup>[14, 15]</sup> suggested that these results indicate a modern lifestyle risk factors, which did not exist at Wharram Percy, may not be important in influencing the severity of bone loss.

Osteoporosis is defined by a reduction in the mass of bone per unit volume.<sup>[16, 17]</sup> The World Health Organization study group has also defined osteoporosis as a value for BMD that is 2.5 SD or more below the value in young adults.<sup>[18]</sup> A value within 1 SD of the reference value in young adults is considered to be normal, and a value between 1 and 2.5 SD below the reference value indicates osteopenia. Thus, the BMD of the Anatolian skeleton indicates normal bone mass (Table 1). For any individual, bone mass is a combination of peak bone density and subsequent bone loss. Both parameters are influenced by genetic, hormonal, and environmental factors.<sup>[19, 20]</sup> Peak bone mass is achieved during the first three decades of life.<sup>[21]</sup> In our study, at least 24 individuals passed away in the 15-24.9 age groups, 173 individuals

passed away between the 25-44.9, and 58 individuals passed away older than 45 years of age. In conclusion, some of the individuals passed away before having reached their peak bone mass. The low prevalence of osteoporosis in ancient Anatolian populations has been explained as a result of biases that occurred due to inaccurate age estimations at the time of death or low life expectancy in the past. The biggest problem in using archeological skeletal samples is age at death estimation.<sup>[22, 23]</sup> Although age estimation in juvenile skeletons is fairly accurate with an acceptable range of error, the estimation of age in older adults is more problematic.<sup>[23]</sup> It is evident that we cannot accurately estimate the age of skeletons older than 50 years of age, but that humans in the past did manage to live well beyond that age.

The mean right and left femur BMD values of the males from Anatolian archeological populations were significantly higher than those of the females in the all age groups. In the archeological material, as today, women were more affected by bone loss than men. As expected, older individuals presented significantly lower right and left total BMD values than the younger ones. Age is the main factor influencing total bone density among the archeological population of Anatolia. This confirms, on histological grounds in a prehistoric samples, that age surely plays a role in bone loss.<sup>[24, 25, 26]</sup> Environmental and genetic factors play a very important role in determining BMD. As for peak bone mass, it is influenced by dietary factors and physical activity.<sup>[27]</sup> Among dietary factors, calcium intake during growth is related to bone mass, a fact which is supported both by clinical<sup>[28, 29]</sup> and experimental<sup>[30]</sup> studies. Malnourished monkeys exhibit slower growth in midshaft femoral diameter than well-fed controls<sup>[31]</sup> and several studies have shown reduced long bone periosteal deposition in protein-deficient experimental animals.<sup>[32]</sup> Osteological evidence from archeological populations has also been used to support the hypothesis that nutritional factors play a large role in determining bone loss. For example, Ericksen<sup>[26]</sup> suggested that nutrition was an important factor in determining bone loss in her analysis of age related changes in Eskimo, Pueblo and Arikara archeological populations. While it remains possible that differences in nutritional factors have influenced the present study, it is an unlikely explanation given the pattern of variation observed among the Anatolian populations used in this study. The same is valid for people consuming macrobiotic diets.<sup>[33]</sup> Excess dietary fat inhibits calcium absorption and may adversely affect bone mass.<sup>[34]</sup> Genetic factors may also play a role in bone mass.<sup>[35, 36]</sup> Anatolia is a culturally and biologically heterogeneous region. It has been inhabited by a myriad of people with diverse origins

since the Paleolithic.<sup>[37, 38, 39, 40, 41, 42]</sup> In addition, the region has been central to several networks through which material culture,<sup>[39,40]</sup> languages<sup>[45, 46, 47, 41]</sup> and other cultural elements<sup>[33,39]</sup> have been transmitted. Consequently, the genetic and cultural origins of Anatolian populations, their affinities to each other, and their interactions with European and Asian populations are quite complex. It is mean that the observed changes and differences in bone density crosscut a lot of (probable) genetic variation in the archaeological sample.

This study illustrates variations in BMD differences in ancient Anatolian populations according to age and gender. The results of the current study suggest that both males and females in all periods presented age-related loss of BMD and that male BMD values were higher than those of females in all the age groups.

We have not found a high prevalence of clinical osteoporosis among the Anatolian populations. In fact there have been no findings of osteoporosis prevalence among the peoples of ancient Anatolia. In many cases the BMD of the ancient samples was better than that of the modern population and the reasons should be evaluated with further studies.

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