

## Morphometric, histometric and elemental profile of the metacarpal and metatarsal bones in adult Sanjabi sheep

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Article Info	Abstract
<b>Article history:</b> Received: 04 July 2024 Accepted: 05 November 2024 Available online: 15 May 2025	<p>Bone structure has been widely studied in mammals, however, osteon structure in sheep has received relatively little attention, especially in terms of its location on the forelimbs and hindlimbs. The aim of this study was to investigate the histometric characteristics and mineral composition of the metacarpus and metatarsus of adult Sanjabi sheep. Metacarpal and metatarsal bones were collected from five adult Sanjabi sheep (n = 10). Morphometric measurements were performed on computed tomographic scan images. Histometric parameters were measured on histological sections. The mineral composition of the bone samples was detected using the X-ray fluorescence method. The diameter of the Haversian canal in the right metatarsus was significantly greater than that in the other bones. The smallest diameter of the Haversian canal was observed for the right metacarpus. The diameter and area of the osteons in the right metacarpal were significantly greater than those in the other bones. The amount of essential mineral elements was not significantly different among bones. Aluminum and lead were significantly greater in the left metatarsus. The highest amount of copper was observed in the left metacarpus. These results indicated that there was a greater load on the right limb. This compensatory mechanism might be used to put more weight on the right forelimb and reduce the pressure caused by the weight of the rumen on the left forelimb. However, to prove this hypothesis, more detailed and extensive studies are needed in the future.</p>
<b>Keywords:</b> Mineral elements Osteon Sheep X-ray fluorescence	

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### Introduction

Sheep is one of the first herbivores to be domesticated on the Iranian Plateau approximately 11,000 years ago and at the end of the Neolithic period. Among the ancestors of domestic sheep, Mouflon (*Ovis orientalis*) and Ural (*Ovis vegnei*) are famous varieties.<sup>1</sup> In quadrupeds, the forelimbs essentially have a weight-bearing role, while the hindlimbs, due to their voluminous muscles, provide the energy needed for propelling the body forward. It has been reported that in cows, up to 60.00 and 40.00% of the body weight is borne by the forelimbs and hindlimbs, respectively.<sup>2</sup> In horses, these values were reported to be 58.00% for the forelimb and 42.00% for the hindlimb.<sup>3</sup> Other studies have shown that the forelimbs of cows bear 60.00 to 65.00% of their body weight.<sup>4</sup> It is noteworthy that the center of gravity of the body (as the point at which all the forces on the body are zero) plays an important role in the distribution of body weight among all four limbs. It

has been reported that the center of gravity of standing cows is usually near the shoulder area. Although the center of gravity varies from species to species, it can temporarily displace with age, physiological status such as pregnancy, and animal movement.

Currently, due to the importance of raising different breeds of domestic sheep in Iran and worldwide, it is very important to know the natural anatomy of different body structures of this animal for surgical procedures, clinical examinations and differential diagnosis of normal conditions from pathological conditions. Diagnostic imaging methods used for multiple purposes are among the best ways to evaluate body organs. Producing three-dimensional images and evaluating the morphometric parameters of the bones of the hand and foot, as well as the precise recognition of their tissue characteristics and constituent elements, considering the high probability of damage and fracture in these bones, is very useful for therapeutic, orthopedic, and surgical applications.

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The aim of this study was to measure and compare different histometrical parameters as well as the elemental composition of the metatarsal and metacarpal bones of Sanjabi sheep using computed tomography (CT) scan imaging of dense and spongy bone tissue, conventional histological methods, and the X-ray fluorescence (XRF) technique.

## Materials and Methods

**Bone sample collection.** For this study, 10 metacarpal bones and 10 metatarsal bones were obtained from five adult Sanjabi sheep. The samples were collected from a local slaughterhouse. Immediately after disarticulation, the limbs were washed and labeled according to the right/left and fore/hind limbs. The extra tissues were removed by washing under tap water; at least 5.00 g of tissue was removed from each bone for the XRF test and a smaller piece was fixed in 10.00% formalin for histomorphometric analysis.

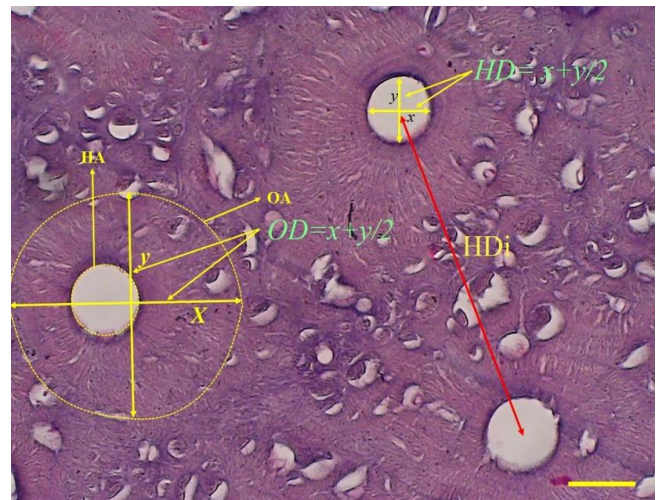
**Intraosseous anatomy study.** To examine the intraosseous anatomy, the specimens were transferred to the clinic. Computerized tomography was performed using a 16-slice scanner (Siemens Somatom 16-slice Multidetector; Siemens AG, Muenchen, Germany) under the following conditions: 0.40 sec tube rotation time, 110 kVp, 25.00 mA, 3.00 sec per slice, and 0.50 mm slice thickness. The compact (cortex) bone thickness was measured by CT scan software from different views. In transverse sections, four measurements were taken and in sagittal sections two measurements were taken and the mean values of the obtained data were calculated as shown in Figure 1.

**Histometric analysis.** Bone pieces (1.00 - 2.00 cm in width) were cross-sectioned at the mid-shaft point. The harvested tissues were fixed in 10.00% formalin for 48 hr and then decalcified using 10.00% nitric acid for 12 hr. The samples were divided into smaller pieces and subjected to tissue processing using conventional histological techniques. The paraffin blocks were cut into 5.00  $\mu$ m thick sections by a rotatory microtome (Leitz GmbH,

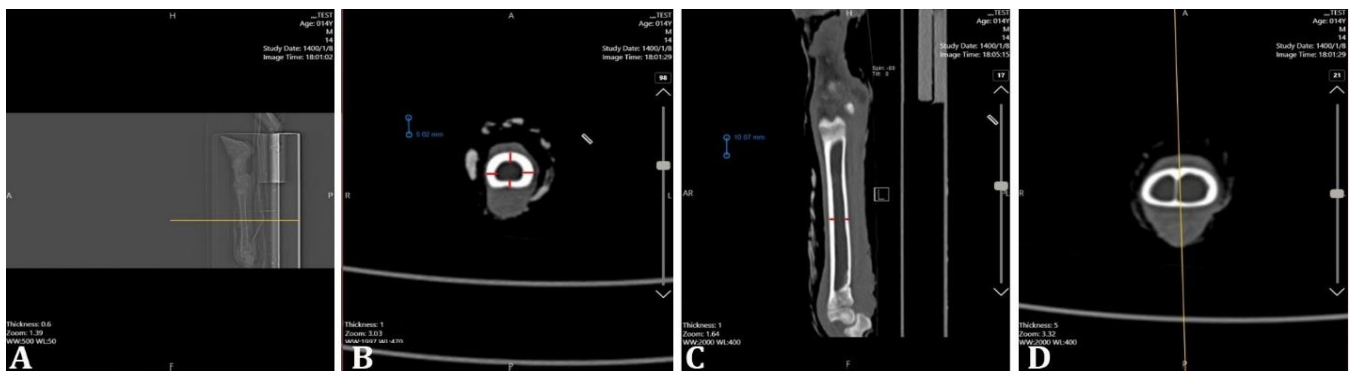
Wetzlar, Germany) and stained with Hematoxylin and Eosin. Each section was examined using a light microscope (Olympus, Tokyo, Japan) connected to a KECam (Kecam Technologies, Lagos, Nigeria), and the obtained images were transferred to ImageJ Software (National Institutes of Health, Bethesda, USA). At least 100 osteons were measured for each section. The following quantitative parameters were measured:

1. Diameters of the Haversian canals (Central canal of Haversian system which blood capillaries and nerve fibers),
2. Diameter of the osteon (a cylindrical unit of bone consisting of a central Haversian canal surrounded by concentric layers of lamella),
3. Area of the Haversian canal,
4. Area of the osteon,
5. Perimeter of the Haversian canal,
6. Perimeter of osteon, and
7. Distance between Haversian canals.

Since some of the Haversian canals and osteons were oval shaped rather than circular, their diameters were calculated as the maximum diameter + minimum diameter/2 (Fig. 2).



**Fig. 2.** Osteon and Haversian canal quantitative parameters studied in the metacarpal and metatarsal bones of sheep. HD: Haversian canal diameter, HA: Haversian canal area, OD: Osteon diameter, OA: Osteon area, HDi: Haversian canal distance (Scale bar = 20  $\mu$ m).



**Fig. 1.** Computed tomography of the sheep metacarpus for measuring the thickness of the cortex (Compact bone). **A and B)** Cross section at the mid-shaft level, **C and D)** Sagittal section at the longitudinal groove level. The red lines show the measurement sites.

**Elemental measurements.** The elemental composition of metacarpal and metatarsal bones in Sanjabi sheep was detected using a handheld XRF analyzer (PW 1404; Philips, Eindhoven, The Netherlands). At least 5.00 g of each bone sample was turned into a fine powder and was used to detect elements from Mg through Bi on the periodic table. The collimator size was set at 0.30 mm for the analysis area diameter and operating voltages of 40.00 - 60.00 kV for different elements (20 - 30 sec) were used as the source of incident radiation. Elemental values are presented as percentages and ppm.<sup>5-7</sup>

**Statistical analysis.** The histomorphometric and mineral element data are expressed as the mean  $\pm$  standard deviation. The data were analyzed using SPSS Software (version 22.0; IBM Corp., Armonk, USA). A paired sample t test was used to compare the variables between the right and left metacarpus/metatarsus as well as between the forelimbs and hindlimbs. A significance level of  $p < 0.05$  was considered for all the patients.

## Results

**Intraosseous anatomy study.** The thickness of the compact bone is shown in Table 1. Although the thickness of the compact bone was greater in the right metatarsus than in the opposite metatarsus and metacarpi, this difference was not statistically significant ( $p > 0.05$ ). The lowest value was observed for the right metacarpus. The distance between the Haversian canal was significantly shorter in the left forelimb than in the other limbs ( $p < 0.05$ ; Table 1).

**Histometrical measurements.** The measurements of the Haversian canals and osteons in the metacarpus and metatarsus are presented in Tables 2 and 3. Comparing the forelimbs and hindlimbs, the Haversian canal diameter,

area and perimeter on the left and right forelimbs were lower than those on the left and right hindlimbs. The diameter of the Haversian canal in the right metatarsus was significantly greater than that of the other limbs ( $p < 0.05$ ). The lowest Haversian canal area was observed for the right forelimb ( $p < 0.05$ ). The Haversian canal perimeter measurements were not significantly different among the four limbs ( $p > 0.05$ ; Table 2). The osteon diameter and area in the right metacarpus were significantly greater than those in the other limbs ( $p < 0.05$ ). However, when comparing the right and left hindlimbs, the osteon diameter and area were greater in the left limb, however, this difference was not statistically significant ( $p > 0.05$ ). The osteon diameter in the right metacarpus was also greater than that in the other limbs, however, this difference was not significant ( $p > 0.05$ ). Although the osteon perimeter was greatest in the right forelimb, there was no significant difference between the limbs ( $p > 0.05$ ).

**Elemental profile.** The number and percentage of elements detected in the metacarpus and metatarsus of the Sanjabi sheep are depicted in Figures 3 and 4. The major compounds found were CaO, K<sub>2</sub>O, MgO, Na<sub>2</sub>O, P<sub>2</sub>O<sub>5</sub>, SO<sub>3</sub>, LOI and SiO<sub>2</sub>. The amounts of Cr, Ti, Ni, Cu, Mn, Fe, Al, Pb, and Zn are presented in ppm. The amount of Al and Pb in the left metatarsus was significantly greater than that in the other limbs ( $p < 0.05$ ). Although the amount of Fe in the left metatarsus and the amount of Zn in the right metatarsus were greater than those in the other limbs, the differences were not statistically significant ( $p > 0.05$ ). The greatest amount of Cu was detected in the left metacarpus, which was significantly greater than that in the other limbs ( $p < 0.05$ ; Fig. 3). Regarding the other trace mineral elements, there were no significant differences between the right/left bones or metacarpal/metatarsal bones ( $p > 0.05$ ; Fig. 4).

**Table 1.** Mean thickness of compact bones and distance between the Haversian canal in the sheep metacarpus and metatarsus bones.

Limbs	Cortex bone thickness (mm)	Haversian canals distance ( $\mu\text{m}$ )
Left metacarpus	2.31 $\pm$ 0.29 <sup>a</sup>	77.38 $\pm$ 26.63 <sup>b</sup>
Right metacarpus	2.09 $\pm$ 0.45 <sup>a</sup>	98.44 $\pm$ 14.85 <sup>a</sup>
Left metatarsus	2.18 $\pm$ 0.27 <sup>a</sup>	98.25 $\pm$ 33.71 <sup>a</sup>
Right metatarsus	2.37 $\pm$ 0.53 <sup>a</sup>	90.53 $\pm$ 7.03 <sup>a</sup>

<sup>ab</sup> Different superscripts in each column indicate significant differences ( $p < 0.05$ ).

**Table 2.** Histometric parameters of the Haversian canals in the sheep metacarpus and metatarsus bones.

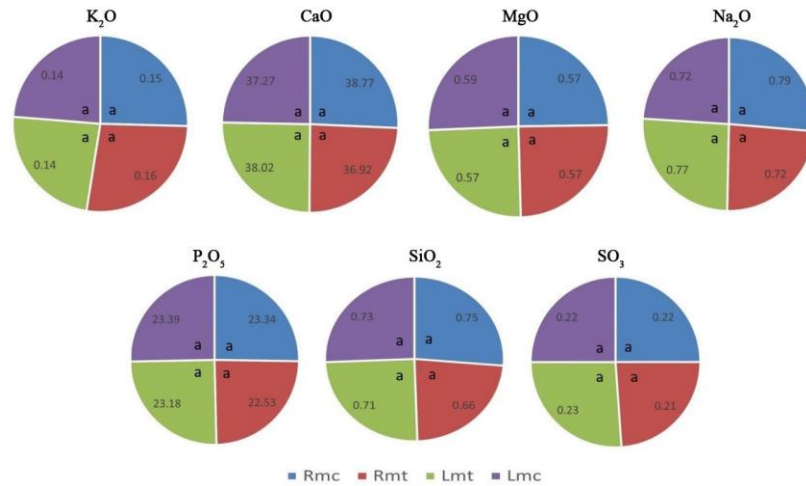
Limbs	Haversian canals diameter ( $\mu\text{m}$ )	Haversian canals area ( $\mu\text{m}^2$ )	Haversian canals perimeter ( $\mu\text{m}$ )
Left metacarpus	21.93 $\pm$ 4.64 <sup>a</sup>	555.71 $\pm$ 180.31 <sup>a</sup>	97.06 $\pm$ 16.38 <sup>a</sup>
Right metacarpus	20.43 $\pm$ 6.81 <sup>a</sup>	416.35 $\pm$ 289.70 <sup>b</sup>	93.83 $\pm$ 38.16 <sup>a</sup>
Left metatarsus	22.12 $\pm$ 9.58 <sup>a</sup>	600.35 $\pm$ 346.63 <sup>a</sup>	100.85 $\pm$ 26.65 <sup>a</sup>
Right metatarsus	31.97 $\pm$ 14.05 <sup>b</sup>	664.99 $\pm$ 273.24 <sup>a</sup>	114.57 $\pm$ 24.51 <sup>a</sup>

<sup>ab</sup> Different superscripts in each column indicate significant differences ( $p < 0.05$ ).

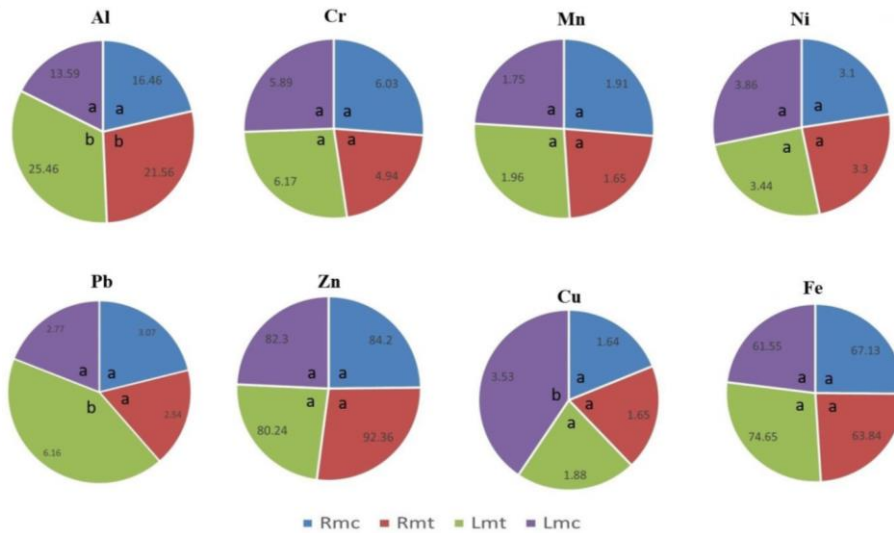
**Table 3.** Histometric parameters of the osteons in the sheep metacarpus and metatarsus bones.

Limbs	Osteon diameter ( $\mu\text{m}$ )	Osteon area ( $\mu\text{m}^2$ )	Osteon perimeter ( $\mu\text{m}$ )
Left metacarpus	86.89 $\pm$ 12.74 <sup>a</sup>	7,545.18 $\pm$ 751.64 <sup>a</sup>	346.48 $\pm$ 9.21 <sup>a</sup>
Right metacarpus	126.68 $\pm$ 60.82 <sup>b</sup>	8,720.76 $\pm$ 3,121.72 <sup>b</sup>	376.47 $\pm$ 67.68 <sup>a</sup>
Left metatarsus	83.75 $\pm$ 22.44 <sup>a</sup>	6,872.35 $\pm$ 2,481.62 <sup>a</sup>	332.28 $\pm$ 83.46 <sup>a</sup>
Right metatarsus	80.19 $\pm$ 24.02 <sup>a</sup>	6,281.53 $\pm$ 2,783.41 <sup>a</sup>	310.75 $\pm$ 71.37 <sup>a</sup>

<sup>ab</sup> Different superscripts in each column indicate significant differences ( $p < 0.05$ ).



**Fig. 3.** Concentrations of trace elements, including: potassium oxide (K<sub>2</sub>O), calcium oxide (CaO), magnesium oxide (MgO), sodium oxide (Na<sub>2</sub>O), phosphorus pentoxide (P<sub>2</sub>O<sub>5</sub>), silicon dioxide (SiO<sub>2</sub>), and sulfur trioxide (SO<sub>3</sub>), in the metacarpus and metatarsus bones of sheep. Rmc: Right metacarpus, Rmt: Right metatarsus, Lmt: Left metatarsus, Lmc: Left metacarpus, “a” in each section indicate no significant differences ( $p > 0.05$ ).



**Fig. 4.** Concentrations of some metals, including: aluminum (Al), chromium (Cr), manganese (Mn), nickel (Ni), lead (Pb), zinc (ZN), copper (Cu), and iron (Fe), in the metacarpus and metatarsus bones of the sheep. Rmc: Right metacarpus, Rmt: Right metatarsus, Lmt: Left metatarsus, Lmc: Left metacarpus, “a, b” in related sections indicate significant differences ( $p < 0.05$ ).

**Discussion**

In the present study, the histometric characteristics of the metacarpal and metatarsal bones in sheep as well as the mineral elements of these bones were investigated. The main aim was to determine whether there was a significant difference between the microstructure of the metacarpal and metatarsal bones and their mineral contents, given that the forelimbs bear more body weight than the hindlimbs. The results indicated that the metacarpal and metatarsal bones exhibit slight differences in microscopic properties as well as in mineral composition. The histological differences were more pronounced in the right metacarpal bone, while, in terms of mineral content, the left metatarsal bone was more distinct from the other bones.

The measured parameters of the osteon structure, including the diameter, perimeter and area of the Haversian canal and osteon in the right metacarpal bone were lower than those in the opposite metacarpal and metatarsus bones, however, these parameters in the right metatarsus were greater than in the other limbs.

In biomechanics, a distinction is usually made between the mechanical behavior of bone tissue as a material and the mechanical behavior of a whole bone as a structure. This means that both the material composition and spatial arrangement of microstructural constituents play decisive roles in bone stiffness and toughness.<sup>8</sup> Several studies have reported that the structure and geometry of secondary osteons and Haversian canals depend on biological and environmental factors.<sup>9,10</sup>

One study found no significant difference in the osteon structure (e.g., osteon diameter and Haversian canal circumference) between adults aged 20 and 90 years. However, the location of the bone influenced the osteon structure, with the diameter of the osteon and the circumference of the Haversian canal being greater in the femur than in the rib.<sup>11</sup> Another study in dogs and deer reported no significant difference in the osteon structure between different anatomical regions of the humerus and femur bones.<sup>12</sup>

Individual factors affect the microstructure of bone tissue differently.<sup>13</sup> The size of secondary osteons decreases, and the activity increases with age.<sup>14</sup> Felder *et al.*, suggested that the minimum diameter of secondary osteons is limited by animal body weight and contributes relatively to red blood cell size.<sup>15</sup> Other researchers hypothesized that maximum amounts of secondary osteons are associated with nutrient transport.<sup>16</sup> On the other hand, Britz *et al.*, hypothesized that the effect of weight on the size of secondary osteons is related to the mechanical stress exerted on the bone.<sup>13</sup> However, van Oers *et al.*, reported that the size of secondary osteons indirectly decreases with increasing tension and pressure on the bone.<sup>17</sup> This issue has also been described by other researchers who have examined the types of bones in different species of animals.<sup>18</sup> In particular, the presence, density, distribution and size of secondary osteons are affected by different biomechanical stresses. For example, the osteon density is greater in wild boars than in domestic pigs<sup>19</sup> and in mofos and goats than in sheep.<sup>20</sup> The decrease in osteon density is caused by domestication and reduced physical and motor activities.<sup>21</sup>

According to the results of the abovementioned studies, the effects of pressure and mechanical stress on the size of bone microstructures have been confirmed. The results of the present study showed that the diameter as well as the area and perimeter of the Haversian canal in the right metacarpus were significantly smaller than those in the other limbs. Examining the morphometric characteristics of the osteons also revealed significant differences in the diameter, area and perimeter of the osteon in the right metacarpus compared to those of the other bones. Therefore, these results can indicate more biomechanical pressure and tension on the right forelimb of the sheep, although this difference in the amount of pressure on the limbs may not be very noticeable. Considering that in quadrupeds, more than half of the body weight is supported by the forelimbs, it seems reasonable to expect a difference between the histomorphometric characteristics of the forelimbs and hindlimbs. However, in comparing the left and right forelimbs, since the heavy rumen completely occupies the left half of the abdominal cavity in ruminants, it was expected that the left forelimb would bear more weight pressure than the right forelimb.

Zedda and Babosova recently compared the histomorphometric differences in the humerus and femur between cattle and sheep and reported that the small histometric differences observed between these two bones in each species are due to more mechanical pressure and tension on the femur than on the arm.<sup>22</sup> In their study, the humerus had a larger Haversian canal diameter and a smaller osteon diameter than did the femur. A larger diameter of the Haversian canal indicates a thinner wall of the osteons and a lower force exerted on them. The result of the present study was inconsistent with the conclusion of these researchers. The reason for this inconsistency might be the different roles and positions of the bones of the limbs in supporting the body weight. Therefore, in one organ, the mechanical pressure exerted on each bone can differ by the position and location of the bone, the amount of muscular and tendinous attachments and the joint angle. In general, the bones of limbs in mammals exhibit regional differences in their tissue structure and even in the amount of minerals they ingest to withstand different pressures and tensions so that they can have sufficient resistance against fractures in different areas. In this regard, the results of the study conducted on the bones of the forelimbs and ribs of the deer showed that the amount of minerals from the humerus to the digital phalanx (proximal to distal) was decreased progressively and significantly, while the population and density of the osteons were increased from proximal to distal. They concluded that this unequal structural and mineral organization between different bones can be a regional adaptation in bones with different physical loads, which ultimately reduces the amount of microcracks.<sup>23</sup> Another study also showed that the bones of the limbs of mature sheep undergo rearrangement and remodeling (mostly in the distal bones), and the production of secondary osteons is a means for more adaptation against increased mechanical pressures.<sup>24</sup>

The largest osteon diameter in this study was related to the right metacarpus. Previous studies have shown that the diameter of the osteon is inversely proportional to the pressure exerted on the bone. Therefore, the osteons in the endosteal part of the cortex, which receives less pressure, are wider than the osteons in the periosteal part of the cortex (which receives more pressure).<sup>25</sup> The main mechanism of this relationship is attributed to the function of osteoblasts and osteoclasts. Mechanical stimulation leads to decreased activity of osteoclasts and increased activity of osteoblasts, and *vice versa*, immobility and reduced pressure on the bone increase the activity of osteoclasts and decrease the activity of osteoblasts. The effects of mechanical stress on osteoclasts and osteoblasts may be regulated by osteocytes. It is believed that osteocytes can sense changes in mechanical pressure on bones. In response, osteocytes can signal to inhibit osteoclast activity and stimulate osteoblast activity.<sup>26-28</sup>

A wide variety of techniques are being used to measure and study mineral elements in biological samples such as inductively coupled plasma–mass spectrometry,<sup>29</sup> atomic absorption spectroscopy<sup>30</sup> and XRF.<sup>31,32</sup> In this study, the XRF technique was used to analyze the elemental composition of metacarpal and metatarsal bones. This technique has been previously used on various bony organs such as bone, teeth, horns and antlers.<sup>5,6,31-33</sup>

In a study, it was shown that this technique has the ability to discriminate between old dental and bone samples obtained in archaeological excavations according to their calcium and phosphorus levels.<sup>5</sup> This technique can even be used to subtract and classify the bones of different species.<sup>33</sup> Others used this technique to determine the number of mineral elements in elephant teeth and compared the results with those of 15 different animal species.<sup>33</sup>

The composition of mineral elements in the bones of different species varies depending on genetic and environmental factors, especially their food habitat. In addition to the effect of aging on the reduction of bone strength and mineral content, which has been proven in several human studies,<sup>34-36</sup> other studies used of sheep as an animal model and found that improper nutrition (such as acidic diets and metabolic acidosis) and hormonal changes (ovariectomy) by increasing the excretion of calcium and phosphorus that lead to a decrease in the density of minerals and a decrease in the strength of the femur.<sup>37,38</sup> In the present study, there was no significant difference in the amount of the essential elements of the metacarpus and metatarsus. Regarding rare elements, there was a significant difference between the metacarpus and metatarsus only in the amount of Al, Cu and Pb. The Al content in the metatarsus was significantly greater than that in the metacarpus. The greatest amount of Cu was detected in the right metacarpus and the greatest amount of Pb was detected in the right metatarsus. Other measured trace element did not significantly differ between the forelimb/hindlimb or left limbs/right limbs. The presence of Pb in the bones, which was not expected, can be caused by environmental pollution. Environmental sources of lead can include lead-based fuels, spent ammunition and sewage. In previous studies, the presence of lead was reported for the first time in 1999 in teeth including elephant teeth.<sup>33</sup> Additionally, in a study, small amounts of lead were reported in the deciduous teeth of people living in southern Poland.<sup>30</sup>

Copper is an essential trace element that plays an important role in bone growth and strength, and its deficiency can lead to bone deformation and increased bone fracture probability.<sup>39</sup> Little is known about the differential distribution of copper among different bone types and specific pathways for copper have not been identified in sheep. Therefore, it is not clear whether copper is an essential trace element in the bones of this

animal. Fe and Mn, two essential elements for bone metabolism, are uniformly distributed in the bones of the forelimbs and hindlimbs. Fe in long bones plays an essential role in hematopoiesis and is mainly stored in the bone marrow, however, it is also found in the liver and spleen bound to ferritin protein. Although the amount of zinc was greater in the metatarsus, the difference was not statistically significant. In a study, Hidioglou *et al.*, reported zinc contents of 102 ppm and 108 ppm in sheep metacarpus and metatarsus, respectively.<sup>40</sup>

Calcium and phosphorus, which are the main constituents of hydroxyapatite crystals, did not differ significantly among the examined bones. However, Ca had the greatest amount among the other elements. Since hydroxyapatite crystals are mainly responsible for providing bone strength and hardness, it seems that due to the absence of significant differences in the essential elements of the metacarpus and metatarsus, there is no difference in their strength and hardness.

According to the obtained results, the right metacarpus had larger osteons and a smaller Haversian canal than the other limbs. These results show more pressure on the right limb, however, the results of examining the mineral elements did not show a significant difference in the number of essential elements. However, it seems that the difference in the histometric characteristics of the right metacarpus compared to other limbs can be explained by the presence of the rumen on the left side. The weight of the heavy rumen on the left side puts more pressure on the left forelimb and causes the animal to transfer more weight to the right limb via a compensatory mechanism to reduce the pressure on the left limb and distribute the weight pressure evenly between the front limbs.

This study has several limitations that should be acknowledged. The sample size was relatively small, consisting of only five adult Sanjabi sheep (n=10). This small sample size might limit the generalizability of the findings and the statistical power to detect subtle differences in bone structure and mineral composition. Future studies with larger sample sizes are needed to confirm these results. Furthermore, this study was cross-sectional, assessing bone structure at a single point in time. This limited the ability to evaluate how age, diet, or other environmental factors might affect the bone properties of Sanjabi sheep over time. Longitudinal studies are necessary to explore these aspects in more detail.

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## Conflict of interest

The authors declare no conflicts of interest.

## References

- Koocheki A, Ghorbani R. Traditional agriculture in Iran and development challenges for organic agriculture. *Int J Biodivers Sci Manag* 2005; 1(1): 52-57.
- Mori R, Kodaka T, Soeta S, et al. Preliminary study of histological comparison on the growth patterns of long-bone cortex in young calf, pig, and sheep. *J Vet Med Sci* 2005; 67(12): 1223-1229.
- Hood DM, Wanger IP, Taylor DD, et al. Voluntary limb-load distribution in horses with acute and chronic laminitis. *Am j Vet Res* 2001; 62(9): 1393-1398.
- König HE, Liebich HG. *Veterinary anatomy of domestic animals: textbook and color atlas*. 7<sup>th</sup> ed. New York, USA: Thieme Medical Publishing 2020; 183-263.
- Christensen AM, Smith MA, Thomas RM. Validation of X-ray fluorescence spectrometry for determining osseous or dental origin of unknown material. *J Forensic Sci* 2012; 57(1): 47-51.
- Kierdorf U, Stoffels D, Kierdorf H. Element concentrations and element ratios in antler and pedicle bone of yearling red deer (*Cervus elaphus*) stags-a quantitative X-ray fluorescence study. *Biol Trace Elem Res* 2014; 162(1-3):124-133.
- Gonzalez-Rodriguez J, Fowler G. A study on the discrimination of human skeletons using X-ray fluorescence and chemometric tools in chemical anthropology. *Forensic Sci Int* 2013; 231(1-3): 407. e1-e6.
- Augat P, Schorlemmer S. The role of cortical bone and its microstructure in bone strength. *Age Aging* 2006; 35(Suppl 2): ii27-ii31.
- Chang B, Liu X. Osteon: structure, turnover, and regeneration. *Tissue Eng Part B Rev* 2022; 28(2): 261-278.
- Hillier ML, Bell LS. Differentiating human bone from animal bone: a review of histological methods. *J Forensic Sci* 2007; 52(2): 249-263.
- Jowsey J. Studies of Haversian systems in man and some animals. *J Anat* 1966; 100(Pt 4): 857-864.
- Dominguez VM, Crowder CM. The utility of osteon shape and circularity for differentiating human and nonhuman Haversian bone. *Am J Phys Anthropol* 2012; 149(1): 84-91.
- Britz HM, Thomas CD, Clement JG, et al. The relation of femoral osteon geometry to age, sex, height and weight. *Bone* 2009; 45(1): 77-83.
- Martin RB, Pickett JC, Zinaich S. Studies of skeletal remodeling in aging men. *Clin Orthop Relat Res* 1980; (149): 268-282.
- Felder AA, Phillips C, Cornish H, et al. Secondary osteons scale allometrically in mammalian humerus and femur. *R Soc Open Sci* 2017; 4(11): 170431. doi: 10.1098/rsos.170431.
- Gibson VA, Stover SM, Gibeling JC, et al. Osteonal effects on elastic modulus and fatigue life in equine bone. *J Biomech* 2006; 39(2): 217-225.
- van Oers RF, Ruimerman R, van Rietbergen B, et al. Relating osteon diameter to strain. *Bone* 2008; 43(3): 476-482.
- Owerkowicz T, Crompton AW. Bone of contention in the evolution of endothermy. *J Vertebr Paleontol* 1995; 15: 47A-47A.
- Zedda M, Brits D, Giua S, et al. Distinguishing domestic pig femora and tibiae from wild boar through microscopic analyses. *Zoomorphology* 2019; 138: 159-170.
- Zedda M, Palombo MR, Brits D, et al. Differences in femoral morphology between sheep (*Ovis aries*) and goat (*Capra hircus*): macroscopic and microscopic observations. *Zoomorphology* 2017; 136: 145-158.
- Dittmann K. Histomorphometric study of the bone microstructure of primates and domestic animals with the goal of species identification with reference to the effects of domestication [German]. *Anthropol Anz* 2003; 61(2): 175-188.
- Zedda M, Babosova R. Does the osteon morphology depend on the body mass? A scaling study on macroscopic and histomorphometric differences between cow (*Bos taurus*) and sheep (*Ovis aries*). *Zoomorphology* 2021; 140: 169-181.
- Skedros JG, Sybrowsky CL, Parry TR, et al. Regional differences in cortical bone organization and microdamage prevalence in Rocky Mountain mule deer. *Anat Rec A Discov Mol Cell Evol Biol* 2003; 274(1): 837-850.
- Pearson OM, Lieberman DE. The aging of Wolff's "law": ontogeny and responses to mechanical loading in cortical bone. *Am J Phys Anthropol* 2004; Suppl 39: 63-99.
- Frost HM. Skeletal structural adaptations to mechanical usage (SATMU): 2. Redefining Wolff's law: the remodeling problem. *Anat Rec* 1990; 226(4): 414-422.
- You L, Cowin SC, Schaffler MB, et al. A model for strain amplification in the actin cytoskeleton of osteocytes due to fluid drag on pericellular matrix. *J Biomech* 2001; 34(11): 1375-1386.
- Huiskes R, Ruimerman R, van Lenthe GH, et al. Effects of mechanical forces on maintenance and adaptation of form in trabecular bone. *Nature* 2000; 405(6787): 704-706.
- Burger EH, Klein-Nulend J, Smit TH. Strain-derived canalicular fluid flow regulates osteoclast activity in a remodeling osteon--a proposal. *J Biomech* 2003; 36(10): 1453-1459.

29. Amr AM. Trace elements in Egyptian teeth. *Int J Phys Sci* 2011; 6(27): 6241-6245.
30. Fischer A, Wiechuła D, Przybyła-Misztela C. Changes of concentrations of elements in deciduous teeth with age. *Biol Trace Elem Res* 2013; 154(3): 427-432.
31. Buddhachat K, Klinhom S, Siengdee P, et al. Elemental analysis of bone, teeth, horn and antler in different animal species using non-invasive handheld X-ray fluorescence. *PloS One* 2016; 11(5): e0155458. doi: 10.1371/journal.pone.0155458.
32. Buddhachat K, Thitaram C, Brown JL, et al. Use of handheld X-ray fluorescence as a noninvasive method to distinguish between Asian and African elephant tusks. *Sci Rep* 2016b; 6: 24845. doi: 10.1038/srep24845.
33. Nganvongpanit K, Brown JL, Buddhachat K, et al. Elemental analysis of Asian elephant (*Elephas maximus*) teeth using X-ray fluorescence and a comparison to other species. *Biol Trace Elem Res* 2016; 170(1): 94-105.
34. Koester KJ, Barth HD, Ritchie RO. Effect of aging on the transverse toughness of human cortical bone: evaluation by R-curves. *J Mech Behav Biomed Mater* 2011; 4(7): 1504-1513.
35. Burr DB. Bone material properties and mineral matrix contributions to fracture risk or age in women and men. *J musculoskelet neuronal Interact* 2002; 2(3): 201-204.
36. Havaladar R, Pilli SC, Putti BB. Effects of aging on bone mineral composition and bone strength. *IOSR J Dent Med Sci* 2012; 1(3): 12-16.
37. Macleay JM, Olson JD, Turner AS. Effect of dietary-induced metabolic acidosis and ovariectomy on bone mineral density and markers of bone turnover. *J Bone Miner Metab* 2004; 22(6): 561-568.
38. West PA, MacLeay JM, Boskey AL. FT-IR imaging analysis of bone mineral changes in ovariectomized and dietary induced metabolic acidosis treated sheep. In *Proceedings: 51<sup>st</sup> Annual meeting of the orthopedic research society*. Washington, DC, USA 2005; 1131-1131.
39. Dollwet HH, Sorenson JR. Roles of copper in bone maintenance and healing. *Biol Trace Elem Res* 1988; 18: 39-48.
40. Hidiroglou M, Morris G, Ivan M. Chemical composition of sheep bones as influenced by molybdenum supplementation. *J Dairy Sci* 1982; 65(4): 619-624.