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Determination of sex-related differences based on 3D reconstruction of the chinchilla (*Chinchilla lanigera*) vertebral column from MDCT scans

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ABSTRACT: This study was undertaken to obtain and analyse, on the basis of sex, three-dimensional (3D) reconstructions obtained by a 3D computer program from two-dimensional (2D) vertebral column sections taken by multidetector computed tomography (MDCT) images, in the chinchilla. A total of 16 adult chinchillas (*Chinchilla lanigera*) of both sexes were used. The MDCT images were taken under general anaesthesia, and were then transferred to a personal computer on which 3D reconstructions were carried out using a 3D modelling program (Mimics 13.1). The volume, surface area and vertebral body length of each vertebra (except caudal region) forming the vertebral column were measured from the 3D models created. The ratios (in percentage) of the measurements of each vertebra (except the sacral ones) forming the vertebral column region (cervical part, thoracic part, lumbar part) were determined for statistical analysis. We detected significant differences ($P < 0.05$) between sexes in all vertebrae forming the vertebral column of the chinchilla with respect to volume, surface area and vertebral body length, except for C6 and L1. This study is the first to carry out 3D reconstructions of data obtained from CT images in the chinchilla and the obtained results contribute to a more detailed understanding of the anatomy of this species. Our strategy may also be useful for the design of experiments exploring the vertebral column in domestic mammals and humans.

Keywords: morphometry; three-dimensional reconstruction; biometry; spine; anatomical structures

In anatomical studies, traditional methods cannot provide sufficient detail for the analysis of curved anatomical structures. 3D models obtained from 2D computed tomography (CT) images are more informative regarding morphological variations than 2D images. 3D images of the curved structure of the backbone also facilitate the diagnosis of vertebral column disorders (Athertya and Poonguzhali 2012). Each region of the 3D-reconstructed vertebral column can be rotated or changed in any direction (Qui and Zhu 2012). CT and 3D reconstruction play an important role in the diagnosis of a large number of diseases (Garland et al. 2002). Moreover, they are also important in forensic medicine and veterinary pathology (Thali et al. 2007).

The thoracic and lumbar regions in mammals are different from each other in terms of morphology and function (Jones and German 2014). 3D reconstruction from CT images of the cervical region in humans, based on measurements of the vertebral body and pedicle, is performed to reveal differences between sexes (Chen et al. 2013). In previous studies, measurements were taken from magnetic resonance (MR) images of the thoracic (Goh et al. 2000) and lumbar vertebrae (Sevinc et al. 2008) in humans, and differences in terms of age and sex were determined morphometrically. 3D reconstructions of MDCT images of the vertebral columns of elite weight-lifters were investigated to identify differences with a sedentary group, and the variations occurring in the vertebral column were also described (Ince 2010). Regional developments of the vertebral column in the rat (*Rattus norvegicus*) were studied by Bergman et al. (2006),

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and 3D reconstruction of CT images was used for morphological investigation in the Loggerhead sea turtle (*Caretta caretta*; Valente et al. 2007). Various morphometric measurements were also taken from the lumbar vertebrae of pigs using a digital calliper and these were compared with the values of the lumbar vertebrae in humans (Dath et al. 2007).

Rodents have been used as experimental animals both in human and veterinary medicine. Many medicinal devices and apparatuses are tested on experimental animals before being introduced in humans (Alfidi et al. 1975). The chinchilla, a member of the rodent family, is a popular laboratory animal used in many studies (Giebink 1999).

The anatomy of the hind limb skeleton of the chinchilla was investigated radiologically and using dissection methods (Cevik-Demirkan et al. 2007). Morphological changes in the maxillary and mandibular bones under pathological situations were studied and analysed radiologically (Sulik et al. 2007). The macro-anatomy of the vertebral column and thorax was also investigated using conventional methods (Ozdemir et al. 2007).

We hypothesised that there are differences in the biometric ratios of individual bones constituting a similar functional skeleton group, between the sexes, and that 3D imaging would allow us to determine the actual morphometric properties of bones. We therefore sought to reveal sex-based differences on the basis of comparison of biometric ratios of individual vertebral bones in each vertebral segment. These were analysed in terms of the volume, surface area and length, by means of 3D reconstruction of MDCT images of chinchilla vertebral column, a part of the skeleton which has been suggested as most suitable for this type of study.

Since no studies applying this method to the vertebral column of the chinchilla are available, this aim of this study was to perform biometrical measurements of the vertebral column and assess possible differences between sexes based on 3D vertebral column reconstruction created from MDCT images in the chinchilla. Thus, a further goal of the study was to enrich the basic anatomical knowledge in this species.

MATERIAL AND METHODS

This study was approved by the Ethics Committee of the Veterinary Faculty of Selcuk University on December 14, 2011 (decision number: 2011/106).

A total of 16 adult chinchillas (*Chinchilla lanigera*) of both sexes (males, $n = 8$; females, $n = 8$) weighing between 500 and 600 g were studied. MDCT images of the vertebral column were used in order to obtain high-resolution pictures.

The animals were anaesthetised intravenously with a mixture of 60 mg/kg ketamine (ketalar) and 6 mg/kg xylazine (Rompun) (Poore et al. 1997). Under general anaesthesia, MDCT images of animals in prone position were obtained. The parameters of MDCT (Somatom Sensation 64; Siemens Medical Solutions, Germany) device are described in Table 1.

Dosage parameters and scans were performed on the basis of standard protocols and the literature (Prokop 2003; Kalra et al. 2004). In this way, we tried to obtain radiometric resolution at the lowest radiation level and with optimum image quality (MONOCHROME2; 16 bit). The axial images obtained in the DICOM format were transferred to a personal computer on which a 3D modelling program, Mimics 13.1 (Multimodal Immersive Motion Rehabilitation with Interactive Cognitive Systems), was installed.

In the first stage of automatic segmentation, the limits of the vertebrae were determined one by one and each vertebra was coloured differently. Following these procedures, 3D reconstruction of each vertebra was performed using the 3D translator component of the Mimics program. All reconstructed vertebrae were combined and the vertebral column was presented as a whole (Figure 1).

The volume, surface area and vertebral body length of the vertebrae (except the caudal ones) in the chinchilla were measured automatically using the 3D program by setting the grey level threshold to 226–1000 HU (Hounsfield unit). The vertebral body length was taken as the distance from the central vertebral head to the central vertebral fossa (Figure 2). Since individual biometrical measurements of the vertebrae forming the vertebral

Table 1. Parameters of the MDCT adjusted for this study

Physical detector collimation	32 × 0.6 mm
Final section collimation	64 × 0.6 mm
Section thickness	0.50 mm
Gantry rotation time	330 ms
kVp	120
mA	300
Resolution	512 × 512 pixels
Resolution range	0.92 × 0.92

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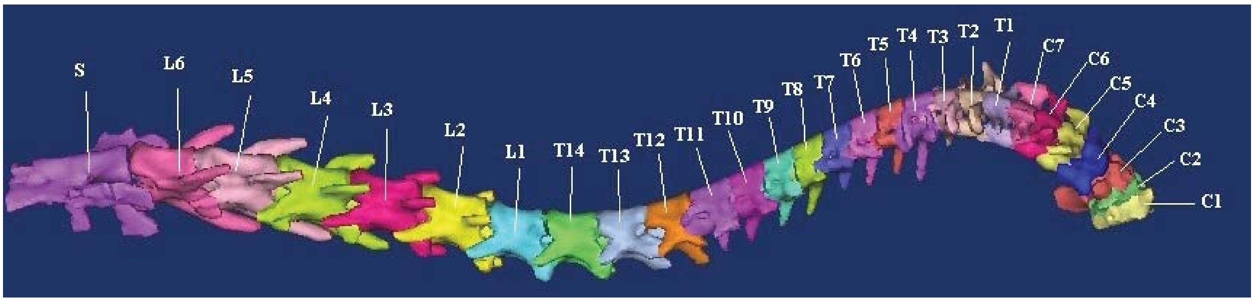

Figure 1. 3D reconstruction of the vertebral column

column were inherently different, their mean measurements were recorded as numerals, and no statistical analyses were performed on them. After all values of each vertebra were expressed as ratios (%; Tables 2–4) within each region (except the sacral one), they were analysed statistically to reveal differences between sexes. Calculation of percentage values for each sacral vertebra was not possible due to their complete ankylosis and, therefore, this region was not included in statistical analyses.

Statistical analysis was carried out with the SPSS 15.0 Windows software package. An independent-samples *t*-test was performed and differences between males and females were revealed by calculation of mean values together with standard deviation values of measurements from the vertebral columns of male and female chinchillas. Statistical significance was set at $P < 0.05$.

RESULTS

Analysis of the 3D images of the chinchilla vertebral column (except the caudal region) revealed the presence of seven cervical, 14 thoracic, six lumbar and two sacral vertebrae (Figures 3–6).

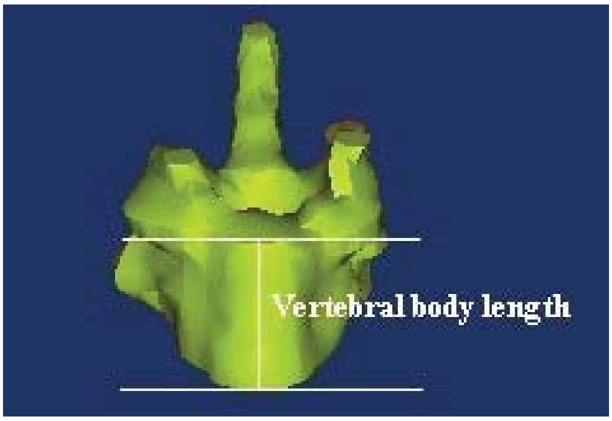

Figure 2. Measurement of the vertebral body length

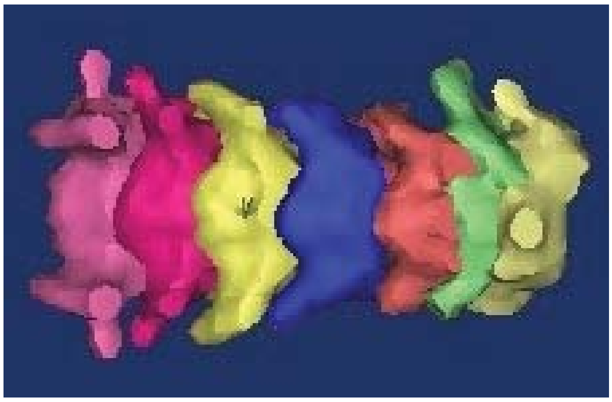

Figure 3. 3D reconstruction of cervical vertebrae

Table 2. Statistical analysis performed by taking percentage rates of volume, surface area and vertebral body length means obtained from 3D images of cervical vertebrae (mean ± SD; males: $n = 8$, females: $n = 8$)

Vertebrae	Volume ratio (%)			Surface area ratio (%)			Length ratio (%)		
	male	female	*P*	male	female	*P*	male	female	*P*
C1	14.31 ± 0.01	14.51 ± 0.03	0.001	13.27 ± 0.04	15.27 ± 0.02	0.001	15.25 ± 0.61	13.32 ± 0.36	0.001
C2	14.44 ± 0.08	14.54 ± 0.03	0.004	13.26 ± 0.08	15.54 ± 0.01	0.001	9.21 ± 0.52	10.12 ± 0.55	0.004
C3	15.16 ± 0.01	16.84 ± 0.06	0.001	14.90 ± 0.05	15.00 ± 0.01	0.001	13.30 ± 0.27	14.43 ± 1.16	0.017
C4	13.69 ± 0.01	12.04 ± 0.01	0.001	13.85 ± 0.07	12.42 ± 0.01	0.001	15.38 ± 0.58	13.99 ± 0.89	0.002
C5	14.17 ± 0.01	13.98 ± 0.01	0.001	14.73 ± 0.04	13.55 ± 0.01	0.001	16.11 ± 0.23	15.81 ± 0.31	0.045
C6	13.63 ± 0.01	13.94 ± 0.03	0.001	14.31 ± 0.03	14.10 ± 0.01	0.001	16.24 ± 0.46	16.47 ± 0.51	0.360
C7	14.58 ± 0.01	14.12 ± 0.07	0.001	15.64 ± 0.02	14.08 ± 0.01	0.001	14.49 ± 0.28	15.89 ± 0.36	0.001

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Table 3. Statistical analysis performed by taking percentage rates of volume, surface area and vertebral body length means obtained from 3D images of thoracic vertebrae (mean ± SD; males: $n = 8$, females: $n = 8$)

Vertebrae	Volume ratio (%)			Surface area ratio (%)			Length ratio (%)		
	male	female	*P*	male	female	*P*	male	female	*P*
T1	5.18 ± 0.01	4.93 ± 0.01	0.001	5.63 ± 0.02	5.45 ± 0.01	0.001	5.39 ± 0.08	4.86 ± 0.15	0.001
T2	6.68 ± 0.01	7.01 ± 0.02	0.001	6.73 ± 0.01	6.28 ± 0.01	0.001	5.07 ± 0.09	6.02 ± 0.06	0.001
T3	6.60 ± 0.02	5.50 ± 0.01	0.001	6.83 ± 0.01	5.69 ± 0.01	0.001	4.77 ± 0.11	5.60 ± 0.07	0.001
T4	6.69 ± 0.01	6.03 ± 0.01	0.001	7.60 ± 0.01	6.43 ± 0.01	0.001	6.76 ± 0.03	6.71 ± 0.03	0.001
T5	5.13 ± 0.01	5.01 ± 0.01	0.001	5.77 ± 0.01	5.50 ± 0.01	0.001	5.54 ± 0.08	7.41 ± 0.02	0.001
T6	5.23 ± 0.01	5.18 ± 0.01	0.001	5.95 ± 0.01	5.64 ± 0.01	0.001	5.70 ± 0.08	4.52 ± 0.17	0.001
T7	5.07 ± 0.01	5.10 ± 0.01	0.001	5.54 ± 0.01	5.50 ± 0.01	0.001	6.01 ± 0.06	6.71 ± 0.03	0.001
T8	5.54 ± 0.01	6.94 ± 0.01	0.001	5.58 ± 0.02	6.83 ± 0.01	0.001	7.12 ± 0.07	6.97 ± 0.03	0.001
T9	7.26 ± 0.01	6.24 ± 0.01	0.001	7.00 ± 0.01	5.72 ± 0.01	0.001	7.39 ± 0.01	6.85 ± 0.02	0.001
T10	6.72 ± 0.01	6.43 ± 0.02	0.001	6.65 ± 0.01	7.10 ± 0.01	0.001	8.15 ± 0.04	6.97 ± 0.11	0,001
T11	8.32 ± 0.01	6.69 ± 0.01	0.001	8.32 ± 0.04	7.19 ± 0.01	0.001	8.77 ± 0.07	6.20 ± 0.03	0.001
T12	8.56 ± 0.01	10.24 ± 0.03	0.001	8.10 ± 0.01	10.02 ± 0.01	0.001	8.93 ± 0.07	9.36 ± 0.08	0.001
T13	11.06 ± 0.01	11.42 ± 0.03	0.001	9.81 ± 0.01	10.83 ± 0.01	0.001	10.15 ± 0.12	10.62 ± 0.12	0.001
T14	11.88 ± 0.01	13.22 ± 0.02	0.001	10.44 ± 0.01	11.77 ± 0.01	0.001	10.18 ± 0.27	11.15 ± 0.21	0.001

According to the 3D data obtained from male and female chinchillas, means of total volume, surface area and length of each region forming the vertebral column were measured as shown in Table 5.

When statistical results on the basis of the ratio of the mean values of each vertebra to the total mean vertebral values from that region were considered (Tables 2–4), a difference ($P < 0.05$) was observed for all vertebrae between the sexes in terms of volume and surface area. A statistically significant difference was also found for all vertebrae, except for C6 and L1, regarding vertebral body length in male and female chinchillas.

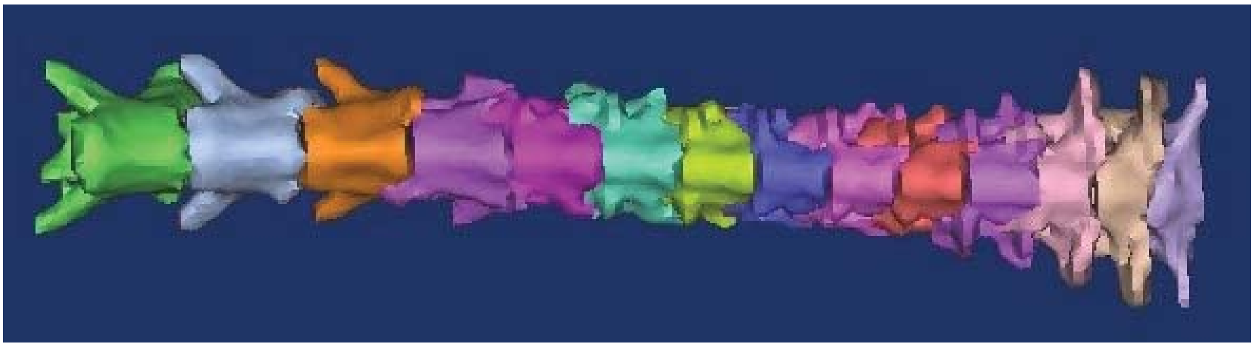

Figure 4. 3D reconstruction of thoracic vertebrae

Table 4. Statistical analysis performed by taking percentage rates of volume, surface area and vertebral body length means obtained from 3D images of lumbar vertebrae (mean ± SD; males: $n = 8$, females: $n = 8$)

Vertebrae	Volume ratio (%)			Surface area ratio (%)			Length ratio (%)		
	male	female	*P*	male	female	*P*	male	female	*P*
L1	11.68 ± 0.01	12.54 ± 0.03	0.001	11.86 ± 0.01	12.73 ± 0.01	0.001	13.40 ± 0.38	13.55 ± 0.04	0.296
L2	14.25 ± 0.01	14.07 ± 0.03	0.001	14.51 ± 0.02	14.64 ± 0.01	0.001	16.56 ± 0.17	14.03 ± 0.04	0.001
L3	16.49 ± 0.01	15.88 ± 0.03	0.001	16.78 ± 0.02	15.69 ± 0.01	0.001	17.18 ± 0.17	17.47 ± 0.04	0.001
L4	18.90 ± 0.01	19.17 ± 0.03	0.001	18.87 ± 0.04	19.11 ± 0.01	0.001	17.55 ± 0.26	18.49 ± 0.03	0.001
L5	20.29 ± 0.01	18.37 ± 0.04	0.001	20.17 ± 0.02	18.49 ± 0.01	0.001	19.24 ± 0.33	18.66 ± 0.03	0.001
L6	18.37 ± 0.01	19.94 ± 0.02	0.001	17.77 ± 0.01	19.31 ± 0.01	0.001	16.04 ± 0.27	17.78 ± 0.06	0.001

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Figure 5. 3D reconstruction of lumbar vertebrae

DISCUSSION

In a study performed on the vertebral columns of rats by Bergman et al. (2006), sex-based differences were determined in all vertebrae, except for S4, with respect to length. These researchers observed that the differences between the sexes gradually increased in the thoracic region and this increase continued into lumbar region; however, only minimal differences were observed in the sacral region. In this study, we determined statistically significant differences between males and females in all vertebrae forming the vertebral column of the chinchilla, with regard to volume and surface area. We also observed differences between the sexes in vertebral body length, with the exception of C6 and L1 (Tables 2–4).

As a result of measurements made on the basis of 3D reconstruction obtained from the CT images of the human cervical region, Chen et al. (2013) reported that the vertebral body length was differed significantly in C5, C6 and C7, between the sexes. In the chinchilla, on the other hand, a significant difference was observed in vertebrae in C1, C2, C3, C4, C5 and C7, with respect to vertebral body length.

Goh et al. (2000) determined that ratio of vertebral body length in T8, T9, T11 and T12 of the human thoracic region was lower in males than females. Thoracic vertebral body length related-differences between sexes in the chinchilla were observed in thoracic vertebrae.

In the study of Sevinc et al. (2008), it was stated that the ratio of the vertebral body length in L2 and L4 of the human lumbar region was higher in females than males, which is consistent with our findings.

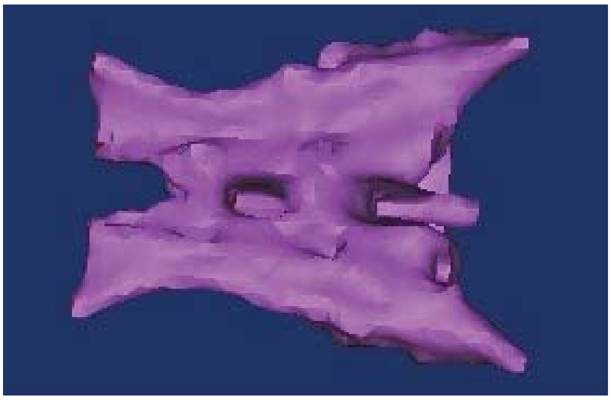

Figure 6. 3D reconstruction of sacral vertebrae

While the lumbar vertebral body length in pigs was constant from L1 to L5, this value decreased in L6 (Dath et al. 2007). In the chinchilla, we observed that the vertebral body length in the lumbar region increased both for females and males from L1 to L5, while it decreased in L6.

CT-related 3D reconstructive anatomical studies hold both advantages and disadvantages. The most important advantage is in terms of ethics; CT images of animals were taken under general anaesthesia without the need for killing them. The 3D models obtained from the CT images can be turned in whichever direction is wanted, thus enabling the measurement of desired values and a complete understanding of anatomical structures. The number of modern anatomical studies taking advantage of the use of cutting-edge technology and computer programs is increasing. The validity and reliability of these programs were proven by on-to-one comparison of biometric measurement values taken on skulls (Kim et al. 2012). The anatomy of many domestic lagomorphs and rodents, such as rabbits, guinea pigs, rats, mice, and hamsters, has been well described. Since chinchillas are gaining popularity as pets (Brenner et al. 2005), we believe that

Table 5. 3D data obtained from male (n = 8) and female (n = 8) chinchillas, means of total volume, surface area and length of each region forming the vertebral column

Region	Volume (mm^3)		Surface area (mm^2)		Length (mm)	
	male	female	male	female	male	female
Cervical	896.5	1003.81	1588.5	1937.16	21.7	21.2
Thoracic	1973.2	2280.3	3626.1	4144.8	65.1	71.7
Lumbar	2311.6	2795.1	3598.1	4336.82	53.0	61.35
Sacral	665.6	945.51	971.5	1327.5	12.7	17.8

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it is timely that their anatomical peculiarities be revealed to enable further clinical and anatomical research of their vertebral column. The need for expert staff who can evaluate the results of tomography in clinics, the lack of expensive technological devices such as CT, MR etc. in veterinary faculties or animal clinics, and the unwillingness of radiography experts to grant approval for taking CT images of animals in medical faculties or private hospitals, are among the disadvantages of this approach.

In conclusion, data obtained from the 3D reconstruction of the vertebral column in the chinchilla, revealed important statistically significant differences between the sexes. We hope that, in the future, genetic investigations are carried out to reveal the basis for these differences between male and female chinchillas. Both biometric perspectives of the vertebral column and the 3D reconstruction techniques used in this study add a new dimension to reconstruction studies. We also hope that this work making use of cutting-edge technology may add a modern element to anatomical education and believe that it contributes considerably to the present anatomical knowledge related to the chinchilla vertebral column.

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